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Re: ***Preliminary Opinion on Potential Health Effects of Exposure to Electromagnetic Fields (EMF)***

Gentlemen,

The BioInitiative Working Group has reviewed the *Preliminary Opinion on Potential Health Effects of Exposure to Electromagnetic Fields (EMF)* dated November 29, 2013. We submit the following comments and suggested revisions. Thank you for providing this opportunity for comment. We hope these suggested revisions will be incorporated in the Final Opinion.

OVERALL COMMENTS

1. This Preliminary Opinion is an inadequate basis for updating the 2009 EU opinion on ‘*Health Effects of Electromagnetic Fields (EMF)*’ and should be sent back for major revisions. The conclusions drawn from the data presented are unreliable for judging possible health risks.
2. The Committee has not answered the question it was appointed to investigate. There is no conclusion in the Executive Summary on whether the Committee determined that possible health effects of EMF are established for childhood leukemia and exist for genotoxicity, for neurological effects, for brain tumors, male fertility, fetal and neonatal effects or other key areas of research. The title of the Opinion is ‘Preliminary Opinion on **Possible Effects** of Electromagnetic Fields (EMF) on Human Health’ (emphasis added). The Committee has given an answer to a different question, limiting its conclusions to whether certainty or causal effect is established. This was also the central failing of the SCENIHR 2009 Opinion on EMF. This Opinion is better titled ‘Preliminary Opinion on Scientific Certainty of Health Harm from Electromagnetic Fields (EMF)’.
3. The Opinion should be revised to clearly state whether the evidence supports a finding of **possible risk** for each type of evidence considered (each section). This report is not useful for the purpose intended due to the ambiguous basis for judging the sufficiency of the scientific evidence, which will eventually form a basis for concluding whether changes in the ICNIRP standards are warranted. The lack of a clear statement about the basis for judging what constitutes sufficient evidence of ‘Possible Effects’, and the embedded up-shifting language to instead require a demonstration of ‘conclusive or unequivocal evidence’ (Exhibit A).

4. Sections on brain tumors are flawed. The report consistently ignores or dismisses published scientific studies that report positive findings at exposure levels below ICNIRP standards (Exhibit B-Hardell). The SCENIHR conclusion that evidence for glioma is weaker now than in 2009 is unjustified, and can only be reached by excluding key scientific studies that reach the opposite conclusion. *There is a consistent pattern of increased risk for glioma (a malignant brain tumor) and acoustic neuroma with use of mobile and cordless phones* according to studies from Orebro University, Sweden released in 2012 and 2013.

5. Further, the Opinion misreads evidence of effects of some studies it does present when drawing conclusions (Exhibit C: Misreading Evidence - De Iuliis). In one example, statistically significant damage to sperm DNA and sperm motility and vitality was reported at cell phone radiation exposure of only 1 W/kg. The preliminary Opinion on page 77 wrongly characterizes the evidence to show that only very high SARs cause this effect. It says “(T)he authors claimed that their results clearly demonstrated that RF exposure can damage sperm function via mechanisms involving the leakage of electrons from the mitochondria and the induction of oxidative stress but **the employed SAR values are very high and not relevant to cell phone users.**” (emphasis added). Finally, the entire body of new evidence for risks to fertility and reproduction is dismissed in the Executive Summary with “*The previous SCENIHR opinion concluded that there were no adverse effects on reproduction and development from RF fields at exposure levels below existing limits. The inclusion of more recent human and animal data does not change that assessment*” and in Section 3.13.4 “(T)herefore, it is concluded that there is strong overall weight of evidence against an effect of low level RF fields on reproduction or development.” These conclusions are possible only by omitting key data, ignoring the conclusions of the authors, and dismantling the significance of the De Iuliis et al results by misreporting it. Critical evidence is misquoted, and then relied on by SCENIHR to dismiss the essential point.

6. Evidence for neurological effects (Exhibit D) should be incorporated into the analysis and conclusions of the Final Opinion. The involvement of oxidative stress on neurological/behavioral effects of ELF EMF and RFR were dismissed as “*not firmly identified*” in the Executive Summary. Exhibit D documents a significant number of overlooked studies of extremely-low frequency radiation that are reported to cause nervous system effects in 90% of the 105 studies available from 2007 to 2014. New neurological RFR studies report effects in 68% of studies on radiofrequency radiation (or 144 of 211 studies) in 2014. This has increased from 63% in 2012 (93 of 150 studies) in 2012. These studies should be included in the Final Opinion. They will likely change the Preliminary Opinion that now avoids making a judgment about whether neurological effects are sufficiently established as a cause of possible health effects.

7. Genetic effects (damage to DNA) from radiofrequency radiation are reported in 65% (or 74 of 114 studies); and 83% (or 49 of 59 studies) of extremely-low frequency studies (Exhibit E). These studies span the 2006/2007 to 2014 time period and many are overlooked. They should be included in the Final Opinion. They will likely change the conclusion of the Preliminary Opinion that skirt the issue of whether genotoxicity is sufficiently established as a cause of possible health effects (Sections 3.5.2.5, 3.7.2.5, and 3.11.3).

8. Evidence for Impacts of Physical and Biological Variables on Study Results (Exhibit F)
The main flaw of the preliminary Opinion is in neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies in Exhibit F, these effects depend on a variety of biological and physical parameters including polarization, frequency and environmental EMF. *In vitro* and *in vivo* negative studies have covered a negligible minority of

real cell phone signals, so the studies cannot provide evidence that the vast majority of other real cell phone signals are safe. Thus, the results of negative studies profiled in the Opinion cannot be extrapolated to the issue of the safety or lack of safety of cell phones in use today. **Well-conducted positive studies cannot be negated by poorly conducted negative studies.** The claim of "inconsistency" in *in vitro* and *in vivo* data and "conflicting results" has at least one simple explanation. The studies were performed under different conditions. Thus, results cannot be directly compared. **The SCENIHR report on inconsistency and conflicting results may rather reflect the level of superficial analysis of these studies.** Another fundamental flaw is in neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see for review (Belyaev 2010 in Exhibit F). In addition to laboratory studies, when brain cancer risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RF dose (for exposures 7 years or more in the past) (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of the most important Bradford Hill criteria which is dependence on dose.

Good epidemiological evidence for brain tumors from many other studies has been excluded (see Section 1 and Exhibits B and F). The SCENIHR preliminary Opinion is heavily biased in favor of the **Danish subscriber cohort study of mobile phone subscribers. This study has major flaws that have been substantially documented since it's publication.** It is not informative even according to the requirement of SCENIHR which says "(T)he minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body" (page 10).

None of the sections adequately address the literature on mitochondrial function and ELF-EMF and RFR. The studies in Table 7 are largely negative studies, and do not begin to address the central questions. This section needs to be revised to more comprehensively document existing literature as shown in Exhibit G.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network (Exhibit G). These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by ELF-EMF and RFR (i.e. a possible health effect that should be documented in the Final Opinion).

Electrophysiology: None of the sections adequately address the literature on changes in electrophysiology with exposure to ELF-EMF and RFR. This is a major area of importance and many papers are available for review. This section needs to be revised to more comprehensively document existing literature, especially in the context of blood-brain barrier changes and the propensity for seizures with disrupted electrophysiology (Exhibit G).

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if the blood-brain barrier (BBB) is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact suggesting that such exposures can

further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment, suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

All of these comments and criticisms argue most strongly for a conclusion in the SCENIHR Final Opinion on EMF that health effects are possible, and in some cases such effects are established.

EXHIBITS AND SPECIFIC FINDINGS AND CONCLUSIONS of The BioInitiative Working Group

Here the BioInitiative Working Group provides specific comments keyed to sections of the preliminary Opinion.

1. Evidence for Brain Tumors

The report consistently ignores or dismisses published scientific studies that report positive findings at exposure levels below ICNIRP standards (Exhibit A-Hardell). The SCENIHR conclusion that evidence for glioma is weaker now than in 2009 is unjustified, and can only be reached by excluding key scientific studies that reach the opposite conclusion. *There is a consistent pattern of increased risk for glioma (a malignant brain tumor) and acoustic neuroma with use of mobile and cordless phones* according to studies from Orebro University, Sweden released in 2012 and 2013.

Had the preliminary Opinion not excluded key papers by Hardell et al, there would be more evidence about the higher risks to adults (and children) of glioma with cell phone use starting early in life. It is another compelling reason to include these Hardell et al studies that have been ignored. Inclusion of the Hardell et al studies provides valuable evidence of possible risks to children from cell phone use. Excluding these key papers has allowed the SCENIHR Committee to avoid making the necessary judgment that evidence already exists that children were reported in at least one study to have higher rates of glioma with mobile phone use than adults. It would lead to the conclusion that “brain tumors are a possible health effect of use of a mobile phone in children, and that risk appears to be far higher than for adults”.

Because key studies are omitted, and because the standard for judging possible health effects has morphed into “unequivocal evidence” or “causal evidence”, then the Preliminary Opinion wrongly concludes that no risks are established. These sections highlight the problems (yellow highlight).

Health effects from Radiofrequency (RF) fields, Page 4

Epidemiological studies on RF EMF exposure do not unequivocally indicate an increased risk of brain tumours, and do not indicate an increased risk for other cancers of the head and neck region, or other

malignant diseases including childhood cancer. Earlier studies raised open questions regarding an increased risk of glioma and acoustic neuroma in heavy users of mobile phones. Based on the most recent cohort and incidence time trend studies, it appears that the evidence for an increased risk of glioma became weaker while the possibility of an association of RF EMF exposure with acoustic neuroma remains open.

Health effects from RF fields, Page 12

Epidemiological studies on RF exposure do not unequivocally indicate an increased risk of brain tumours, and do not indicate an increased risk for other cancers of the head and neck region, or other malignant diseases including childhood cancer. Earlier studies raised open questions regarding an increased risk of glioma and acoustic neuroma in heavy users of mobile phones. Based on the most recent cohort and incidence time trend studies, it appears that the evidence for glioma became weaker while the possibility of an association with acoustic neuroma remains open.

Discussion of brain tumours and other tumours of the head and neck area, Pages 65-66

Overall, there is little evidence that moderate mobile phone use is associated with any cancer in the head and neck region. This is supported by large-scale epidemiological studies of three different designs. Only one case-control study shows risk increases at moderate usage levels, but the results are incompatible with observed time trends in incidence rates in reality checks and can therefore not be used for hazard assessment. Evidence is more controversial for heavy users of mobile phones; "heavy use" is a qualitative characterisation and difficult to quantify as the users with the highest life-long use are compared to those with lesser use (combining years of use and amount of daily use), with various definitions and cut-points. For instance, in Interphone, "heavy users" were approximately 10% of life-long heaviest regular users (or about 5% of all study subjects). It corresponds to, for example, half an hour of daily use over 10 years or more (in the communication of the outcome of the IARC Monograph (IARC 2013)), but this figure must not be interpreted as any suggestion of a safety limit. For the segment of the heaviest users, the largest case-control study in particular observed about 40% increased risks for glioma and for acoustic neuroma. It cannot be concluded from the available studies whether this reflects a causal association. Limitations of the case-control studies, including selection bias and reporting bias, raise concern that the observed association in small subgroups could be attributable to methodological shortcomings. Time trend analysis in incidence rates and the two cohort studies show no evidence of any risk, but would not detect small risk increases after longer latencies in heavy users only.

RF Epidemiological Studies: Conclusions on epidemiology of neoplastic diseases, Page 67

Epidemiological studies do not unequivocally indicate an increased risk of brain tumors, other cancers of the head and neck region, or other malignant diseases including childhood cancer.

Page 172.

Further studies of the effects of RF fields associated with mobile phone use and brain tumours in children are recommended as a high priority [R19]. These should include children of a younger age than those that have been studied to date, and be of sufficient duration to include assessments of cancer risk later in life.

Inclusion of the Hardell et al studies provides valuable evidence of possible risks to children from cell phone use. Excluding it has allowed the SCENIHR Committee to avoid making the necessary judgment that evidence already exists that children have higher rates of glioma with mobile phone use. It would lead to the conclusion that 'brain tumors are a possible health effect of use of a mobile phone in children, and that risk appears to be far higher than for adults'.

2. Misreading Evidence - Evidence for Effects on Fertility and Reproduction

The section must be rewritten based on the following peer-reviewed studies, and their conclusions, but particularly because of the mishandling of the De Iullis et al (2009) study. This conclusion is also contradicted by a large number of new studies of RFR on sperm quality, motility and other male fertility parameters with very low-intensity cell phone radiation exposures; on pathological changes in the testes, and other serious health impacts that are reported by multiple laboratories around the world (see Exhibit B for references).

The De Iullis study reports that very LOW SARs of 1.0 W/kg (which are well below today's safety limits) significantly reduced sperm quality parameters, and not just the higher SARs of 27 W/kg and higher which were also reported to decrease motility.

De Iullis et al conclude that “(H)igh quality spermatozoa selected in discontinuous Percoll gradients displayed a decline in both vitality and motility after exposure to RF-EMR in a dose-dependent manner. The control populations maintained an average vitality of 89%; however, significant reductions in vitality were observed at exposure levels as low as 1.0 W/kg (p,0.01) (Figure 2A). Similarly, the control populations maintained motilities at an average of 86% over the incubation period, however after exposure to RF-EMR at levels of 1.0 W/kg, motility was observed to significantly decrease to 68% (p,0.05) and decreased still further at higher SAR exposures (Figure 2B).”

Further, “The research described in this article suggests that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high levels of ROS, might be excess exposure to RF-EMR from sources such as mobile phones.”

DeIullis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa *in vitro*. PLoS One 2009;4(7):e6446.

The SCENIHR preliminary Opinion mischaracterizes the fundamental exposure results the De Iullis et al, 2009 study and should be corrected. The preliminary Opinion on page 77 wrongly concludes that only very high SARs that are not relevant for cell phone users resulted in sperm damage. In fact, SAR levels as low as 1 W/kg can be common in men who keep a cell phone in their pants pocket, or use them near the genitals while sitting may experience such exposures.

Executive Summary, Page 13: Page 120: Conclusions on Reproduction and Developmental Effects: Page 172 – 173: and Page 177-179 Literature Identified but Not Cited.

It is quite stunning that the preliminary Opinion simply does not evaluate many key papers on RFR impacts to sperm and male fertility that it clearly knows to exist, because it lists them in Section 7 as “Literature Identified but Not Cited”, and still has the temerity to conclude that the evidence for potential effects of RF fields on male fertility is weak. It would not be weak if these papers were properly included in the review (see Exhibit B: Reference List for Important Fertility and Reproduction Papers).

3.13.4. RF fields

“The evidence suggesting that RF fields affect male fertility is weak and the existing *ex vivo* studies reporting positive effects have methodological problems. Cohort studies are recommended only if a study design is available that can overcome potential confounding and recall bias regarding phone use and the study has appropriate exposure assessment.”

“The previous SCENIHR opinion concluded that there were no adverse effects on reproduction and development from RF fields at non-thermal exposure levels. The inclusion of more recent human and animal data does not change this assessment. Therefore, it is concluded that there is strong overall weight of evidence against an effect of low level RF fields on reproduction or development.”

“De Iuliis et al, after 16 h exposure at 1800 MHz, SAR from 0.4 up to 27.5 W/kg also found an increase in ROS generation by the whole cell and mitochondria in a SAR-dependent manner, together with oxidative DNA damage (8-OHdG) and DNA fragmentation. Such effects translated to reduction in sperms motility and vitality. The authors claimed that their results clearly demonstrated that RF exposure can damage sperm function via mechanisms involving the leakage of electrons from the mitochondria and the induction of oxidative stress, but the employed SAR values are very high and not relevant to cell phone users.”

This kind of reporting misquotes the statistics, and thus wrongly dismisses the significance of the De Iuliis et al results by not pointing out that a) these important adverse effects occur at as low an SAR as 1.0 W/kg which is half of the ICNIRP safety limit of 2 W/kg (the FCC/IEEE safety limit is 1.6 W/kg). The reporting also does not differentiate between very high SAR exposures of up to 27.5 W/kg and lower SARs where DNA damage is reported as well. De Iuliis et al point directly to a threat from cell phone use but the preliminary Opinion misquotes the authors, saying the levels where such effects were seen are ‘not relevant to cell phone users.’ It directly misrepresents both data and conclusions of this important paper.

Human sperm are reported to be damaged by cell phone radiation at very low intensities in other studies, some reporting damage at exposure levels as low as 0.00034 – 0.07 $\mu\text{W}/\text{cm}^2$ (Exhibit B). There is a veritable flood of new studies reporting sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage (Exhibit C and Chart)

Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (See Section 18 for references - Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Eroglu et al, 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al (2012) report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster*. Gul et al (2009) reported rats exposed to stand-by level RFR (phones on but not transmitting calls) had a decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared ($\mu\text{W}/\text{cm}^2$). See Exhibit C for references.

Though causal evidence of one or more mechanism(s) are not yet fully refined, it is generally accepted that oxidative stress and free radical action may be responsible for the recorded genotoxic effects of EMFs which may lead to impairments in fertility and reproduction. Free radical action and/or hydrolytic enzymes like DNAase induced by exposure to EMFs may

constitute the biochemical actions leading to adverse changes in hormones essential in males and female reproduction, DNA damage, which in turn causes damage to sperm motility, viability, and sperm morphology. Such exposures are now common in men who use and who wear wireless devices on their body, or use wireless-mode laptop computers. It may also account for damage to ovarian cells and female fertility, and miscarriage in women (ELF-EMF at 16 mG intermittent exposure). Section 18: Fertility and Reproduction, BioInitiative 2012 Report at www.bioinitiative.org

3. Evidence for Neurological and Behavioral Effects (Effects on the Nervous System)

Executive Summary, Page 14, Section 3.5.2.5

Evidence for neurological effects from a more comprehensive review of relevant papers should be incorporated into the analysis and conclusions of the Final Opinion (Exhibit D). The involvement of oxidative stress on neurological/behavioral effects of ELF EMF and RFR were dismissed as “*not firmly identified*” in the Executive Summary on page 14, but clearly the evidence supports a finding of ‘possible health effect’ if not ‘probable effect’.

New neurological RFR studies to 2014 report effects in 68% of studies on radiofrequency radiation (or 144 of 211 studies) in 2014. This has increased from 63% in 2012 (93 of 150 studies) in 2012 (Exhibit D).

Studies of extremely-low frequency radiation are reported to cause nervous system effects in 90% of the 105 studies available in 2014.

These studies should be included in the Final Opinion. They will likely change the Preliminary Opinion that now avoids making a judgment about whether neurological effects are sufficiently established as a cause of possible health effects.

The Preliminary Opinion unnecessarily omits relevant studies on neurological effects (Exhibit D). Were they properly included, the Committee’s conclusions would be different, i.e., a finding of possible health effect would have to be the clear conclusion.

There are studies on the interaction of cell phone radiation on EEG during sleep. Changes in sleep EEG have been reported by Hung et al. (2007), Regel et al. (2007), Lowden et al (2011), Schmid et al. (2012), Loughran et al. (2012), Mohammed et al. (2013), and Pelletier et al. (2012), whereas no significant effect was reported by Fritzer et al (2007), Mohler et al. (2010, 2012) and Nakatani-Enomoto et al. (2013). Loughran et al. (2012) provided an interesting conclusion in their paper: “(T)hese results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that “previous negative results are not strong evidence for a lack of an effect...”

Considering the effects of neurological/behavioral effects of radiofrequency radiation published since 2007, there are 30 human study papers of which 11 showed effects. The effects studied included behavioral arousal, memory effects to cognitive functions. There are 34 animal studies, of which 32 showed effects. Effects studies included motor hyperactivity to cognitive behaviors. A difference between the humans and animal studies is that most of the animal studies deal with chronic/repeated exposure, whereas the human studies are mostly acute (one time) exposure. Effects of chronic/repeated exposure studies should play more weight in considering the risk effect. It must be pointed out that neurophysiological and behavioral changes have been reported

in both animals and humans after acute (one time) exposure to RFR, and most of the EEG studies are acute exposure experiments.

Behavioral effects of ELF EMF have been further substantiated in research since 2007. These included: changes in locomotor activity (9 studies), learning and memory functions (10 studies), anxiety (5 studies); depression-like behavior (2 studies), perception (1 study), cognitive dysfunction (1 study), emotional state (1 study), sleep onset (1 study), and comb building in hornets (1 study). Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.

The involvement of oxidative stress on neurological/behavioral effects of ELF EMF was not carefully considered. Oxidative changes (free radicals) seems to play a critical role (Akdag et al., 2010, 2013; Akpinar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Other physiological factors, e.g., sex, age, stress, etc, that can affect the effects of ELF EMF should be considered. A paper by Falone et al. (2008) reported the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Janac et al. (2012) reported age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo (in the egg) exposure to ELF EMF, chicks showed memory deficit only when they were under stress.

Effects have been reported after exposure to low (environmental) levels of ELF EMF. For example, Ross et al (2008) showed 'perception' alternation in human subjects exposed to magnetic field at 10 nT (0.00001 mT); a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and Stevens (2007) indicated changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 mT (0.005 mT).

Executive Summary, Page 14, Section 3.7.2.5.

A summary of the research literature on the neurological effects of ELF EMF published in 2007-2014 allows the SCENIHR Committee to survey the relevant literature more comprehensively. (In most studies, even only magnetic field was mentioned; there was no explicit statement that electric fields had been eliminated. In most ELF EMF exposure systems used in laboratory system, electric fields were also generated unless grounding was done. Thus, cells or animals were actually exposed to both magnetic and electric fields.)

- Neurotransmitters are chemicals that carry (transmit) signals from one nerve cell to another. Neurotransmitters are released from one nerve cell and react with molecules called receptors on another nerve cell. The reaction alters the activity of the second nerve cell. Activities in nerve cell could also change the properties of these receptors (mainly by changing the concentration or the affinity of the receptors to neurotransmitters). In the updated EMF literature, all the studies are on the effects of ELF EMF exposure on neurotransmitter receptors. Manikonda et al. (2007) reported effects of chronic ELF EMF exposure on NMDA receptors in the hippocampus of the rat. Salunke et al. (2013) reported that ELF EMF-induced anxiety in the rat involved NMDA receptors in the brain. There is a report on effects of magnetic field serotonin and dopamine receptors in the brain of the rat (Janac et al., 2009). Changes in subtypes of serotonin receptors 5HT(2A)

in the prefrontal cortex was reported. However, Masuda et al. (2011) reported that another type of serotonin receptor 5HT (1B) was not significantly affected after magnetic field exposure in an *in vitro* experiment. The researchers were trying to replicate two experiments carried out previously showing magnetic field exposure affected 5HT(1B) receptor. Some of the co-authors of the Masuda study were actually co-authors of one of these earlier studies. However, the 5HT(2A) receptors, particularly in the frontal cortex, are believed to be related to the psychiatric syndromes of depression in humans. Kitaoka et al. (2013) and Szemerszky et al. (2010) did report depression-like behavior in mice and rats, respectively, after chronic exposure to magnetic fields. There are two reports on dopamine receptors. Shin et al. (2007, 2011) reported an increase in D-1 dopamine receptors and activity in the striatum of the rat after magnetic field exposure. Dopamine in the striatum is involved in Parkinson's disease. Wang et al. (2008) reported that ELF magnetic fields potentiated morphine-induced decrease in D-2 dopamine receptors. The implication of these data is not readily clear. Both D-1 and D-2 dopamine receptors in the brain are involved in depression and drug addiction. There is one study on the cholinergic system. Ravera et al. (2010) reported changes in the enzyme acetylcholinesterase in cell membrane isolated from the cerebellum after magnetic field exposure. Interesting, these researchers also reported 'frequency window' effects in their experiment. Window effects, i.e., effects are observed at a certain range(s) of EMF frequency or intensity, were first reported by Ross Adey and Susan Bawin and Carl Blackman in the 1980s. A recently study by Fournier et al. (2012) reported an 'intensity window' effect of ELF magnetic field on neurodevelopment in the rat. The cholinergic systems in the brain play a major role in learning and memory functions.

- Behavioral effects of ELF EMF have been further substantiated in recent research. These included: changes in locomotor activity (Balassa et al., 2009; Dimitrijevic et al., 2014; Janac et al., 2012; Legros et al., 2012; Raus et al., 2012b; Shin et al., 2007, 2011; Todorovic et al., 2012), learning and memory functions (Che et al., 2007; Corbacio et al., 2011; Cui et al., 2012; Duan et al., 2013; Fournier et al., 2012; Fu et al., 2008; Harakawa et al., 2008; He et al., 2011; Liu et al., 2008b; Sun et al., 2010), anxiety (Balassa et al., 2009; He et al., 2011; Korpınar et al., 2012; Liu et al., 2008a; Salunke et al., 2013); depression-like behavior (Kitaoka et al., 2013; Szemerszky et al., 2011), perception (Ross et al., 2008), cognitive dysfunction (Davanipour et al., 2014), emotional state (Stevens, 2007), sleep onset (Hung et al., 2007), and comb building in hornets (Ishay et al., 2007). Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.

- In some of these observed neurological effects, oxidative changes (free radicals) again seemed to play a role (Akdag et al., 2010, 2013; Akpınar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Increase in free radicals causes cellular damages. Most of these effects are changes in enzymes involved in maintenance of oxidative balance in cells. A paper by Falone et al. (2008) reported an interesting finding. The researchers observed that, after magnetic field exposure, the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Thus, aging may make an individual more susceptible to the detrimental effects of ELF EMF. There are other factors that could affect an animal's response to ELF EMF. Janac et al. (2012) reported

age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo exposure to ELF EMF, chicks showed memory deficit only when they were under stress. Indeed, Lahijani et al. (2011) reported histological changes in the brain of chicks exposed to ELF EMF in ovo.

- The possible medical applications of ELF EMF should be given more attention. Several studies indicate that ELF EMF could enhance recovery of functions after nervous system damage and have protective effects against development of neurodegenerative diseases. Cuccurazzu et al. (2010) reported an ELF EMF-induced neurogenesis and repair of the nervous system after damage. Kumar et al. (2010) and Das et al. (2012) showed an enhanced restoration of functions after spinal injury in the rat. Kumar et al. (2013) further showed that ELF EMF exposure restored spinal cord injury-induced tonic pain and changes in neurotransmitter concentrations in the brain of the rat. Maestú et al. (2013) reported improvement in pain sensation in fibromyalgia patients after magnetic field stimulation. A possible beneficial effect on cerebral ischemia has been reported by Rauš Balind et al. (2014). Piacentini et al. (2008) reported a promotion of neural differentiation by ELF EMF. Kim et al. (2013) and Bai et al. (2013) reported stimulation by ELF EMF on neural differentiation of stem cells. Effects on stem cells and hippocampal neurogenesis also have been reported by Podda et al. (2013) and Leone et al. (2014). Protective effects of ELF EMF have been reported by Raus et al (2012a, b) after cerebral ischemia, Tassel et al. (2012a, b) on the development of Huntington's Disease, and Manjhi et al. (2013) on spinal cord injury induced osteoporosis. Furthermore, Cvetkovic et al. (2009) reported alteration of EEG by application of certain frequencies of magnetic fields. This may be useful in the treatment of certain neurological disorders such as sleep and psychiatric disorders. Static magnetic field has been shown by Wang et al. (2010) to act like an anti-Parkinson drug. Static magnetic field also has been shown to have anti-angiogenesis properties (Wang Z, Yang P, Xu H, Qian A, Hu L, Shang P. Inhibitory effects of a gradient static magnetic field on normal angiogenesis are reported in *Bioelectromagnetics* (6):446-453, 2009), which can be translated into an anticancer activity. Use of ELF EMF for cancer treatment has been extensively investigated. There is a study showing that pulsed electromagnetic fields turned on adenosine receptors in brain cancer cells that inhibit cancer growth (Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A₃ adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells is reported in *PLoS One* 7(6):e39317, 2012). Interesting, this effect was not observed when normal brain cells were exposed to magnetic field. The waveform of the fields may play an important role in the effect produced. There are several studies on pulsed (instead of sinusoidal) magnetic fields (Aldinucci et al., 2009; Capone et al., 2009; Cook et al. 2009; Glover et al., 2009) and complex fields (Ross et al., 2008). It has been speculated that intermittent EMF or fields that have a transient nature could be more biologically potent than constant fields. The conditions and parameters of the fields that could produce either detrimental or beneficial effects need further investigation. Furthermore, it is still not clear whether acute (one time) exposure would elicit effects different from chronic/repeated exposure. In the 2007-2014 literature, there are many studies reporting effects of chronic/repeated exposure. The study by Liu et al. (2008a) indicates that duration of exposure could be an important factor.

- The majority of the studies used magnetic fields above 0.1 mT (1 gauss; the highest was 8 mT). The intensities are much higher than those in the public environment. Thus,

caution should be taken in extrapolating the high-intensity cell and animal studies to environmental human exposure situation. Exposure to magnetic fields of 0.4 mT (0.0004 mT) has been implication in an increased risk of childhood leukemia. And, the recent report by Li et al. (Li DK, Ferber JR, Odouli R, Quesenberry CP Jr. A Prospective Study of In-utero Exposure to Magnetic Fields and the Risk of Childhood Obesity. *Sci Rep.* 2:540, 2012) on an increased risk of obesity of humans exposed prenatally to magnetic field at 0.25 mT (0.00025 mT). There is also a report of a blood pressure lowering effect in humans with mild-to-moderate hypertension after exposure to magnetic fields at 1 μ T (0.001mT) (Nishimura T, Tada H, Guo X, Murayama T, Teramukai S, Okano H, Yamada J, Mohri K, Fukushima M. A 1- μ T extremely low-frequency electromagnetic field vs. sham control for mild-to-moderate hypertension: a double-blind, randomized study. *Hypertens Res.* 34(3):372-377, 2011.) Apparently, humans are sensitive to magnetic field at level less than 1 mT. There is a study by Ross et al (2008) showing ‘perception’ alteration in human subjects exposed to magnetic field at 10 nT (0.00001 mT), a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and a study by Stevens (2007) indicating changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 mT (0.005 mT). These data do suggest magnetic fields at very low intensities could cause neurological effects in humans. In the 1990s, there were a series of more than 20 studies published by Reuven Sandyk showing that pulsed magnetic fields at pT (1 pT = 0.000000001 mT) levels could have therapeutic effects on Parkinson’s disease and multiple sclerosis (see e.g., Sandyk R. Reversal of cognitive impairment in an elderly Parkinsonian patient by transcranial application of picotesla electromagnetic fields. *Int J Neurosci.* 91(1-2):57-68, 1997, or, search for ‘Sandyk R’ in the PubMed.) However, Sandyk’s findings have never been independently confirmed.

- In summary, ELF EMF affects neurological functions and behavior in animals and humans. There is no definite data showing that these effects are detrimental to human health. However, since effects have been observed, it is advisable that one should limit one’s exposure to EMF.

Exhibit D is a summary of the research literature on the neurological effects of ELF EMF published in 2007-2014.

4. Evidence for Genotoxicity (Genetic Damage to DNA)

There are many more publications on genotoxicity of ELF-EMF and RFR since 2007 than the SCENIHR Working Group considered.

Genetic effects (damage to DNA) from radiofrequency radiation are reported in 65% (or 74 of 114 studies) (Exhibit E).

For ELF-EMF, genetic effects are reported to occur in 83% (or 49 of 59 studies) of extremely-low frequency studies (Exhibit E).

These studies should be included in the Final Opinion. They will likely change the conclusion of the Preliminary Opinion that skirt the issue of whether genotoxicity is sufficiently established as a cause of possible health effects (Sections 3.5.2.5, and 3.11.3).

Effects of EMF on oxidative status, a change of which disturbs all physiological functions is poorly analyzed because many relevant peer-reviewed papers are missing from the assessment.

- The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman ([Blank M, Goodman R](#). DNA is a fractal antenna in electromagnetic fields. [Int. J. Radiat. Biol.](#) 87(4):409-415, 2011) in which they stated that ‘...the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.’ However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. *Bioelectromagnetics* 13:237-246, 1992; Lai, H. and Carino, M.A.

Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. *Bioelectromagnetics* 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz magnetic field affects rats' performance in the water maze. *Bioelectromagnetics* 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. *Bioelectromagnetics* 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency. Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al., 2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonan et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, there are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF- Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

- An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplatin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonan et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Journi et al., 2012 Yoon et al., 2014); RFR- chemical mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent

electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

- Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroui et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010; Panagopoulous et al., 2013; Udroui et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 23(6):414-417, 2005; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. *Bioelectromagnetics.* 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. *Bioelectromagnetics.* 32(1):15-27, 2011.

- Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (mT) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. *Environ. Rev.* 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level. Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) ([de Bruyn L](#) and [de Jager L](#). Effect of long-term exposure to a randomly varied 50 Hz power frequency **magnetic field** on the fertility of the mouse. [Electromag. Biol. Med.](#) 29(1-2):52-61, 2010).

- Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas

a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found that neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et al. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in *E. coli* was *decreased* after exposure to a sinusoidal magnetic field and *increased* after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complexity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

- Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease ([Arendash GW](#), [Sanchez-Ramos J](#), [Mori T](#), [Mamcarz M](#), [Lin X](#), [Runfeldt M](#), [Wang L](#), [Zhang G](#), [Sava V](#), [Tan J](#), [Cao C](#). Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. [J Alzheimers Dis](#). 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. [PLoS One](#). 5(11):e13883, 2010); bone regeneration ([Lee HM](#), [Kwon UH](#), [Kim H](#), [Kim HJ](#), [Kim B](#), [Park JO](#), [Moon ES](#), [Moon SH](#). Pulsed electromagnetic field stimulates cellular proliferation in human intervertebral disc cells. [Yonsei Med. J](#). 51(6):954-959, 2010);

cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. *Br. J. Cancer.* 105(5):640-648, 2011), and tissue regeneration ([Gaetani R](#), [Ledda M](#), [Barile L](#), [Chimenti I](#), [De Carlo F](#), [Forte E](#), [Ionta V](#), [Giuliani L](#), [D'Emilia E](#), [Frati G](#), [Miraldi F](#), [Pozzi D](#), [Messina E](#), [Grimaldi S](#), [Giacomello A](#), [Lisi A](#). Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. *Cardiovasc. Res.* 82(3):411-420, 2009).

- It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

The genotoxicity assessment flaws lead to dismissal of the fertility implications of oxidative damage on sperm. Both genotoxicity (DNA damage to genes) in general and the consequence that genotoxicity from mechanisms related to free-radicals (oxidative damage to DNA) to sperm from cell phone radiation (RFR) mean that two promising lines of scientific evidence in SCENIHR's Opinion are compromised.

5. Evidence for Fetal and Neonatal Effects

Effects on the developing fetus from in-utero exposure to cell phone radiation have been observed in both human and animal studies since 2006. Sources of fetal and neonatal exposures of concern include cell phone radiation (both paternal use of wireless devices worn on the body and maternal use of wireless phones during pregnancy). Sources include exposure to whole-body RFR from base stations and WI-FI, use of wireless laptops, use of incubators for newborns with excessively high ELF-EMF levels resulting in altered heart rate variability and reduced melatonin levels in newborns, fetal exposures to MRI of the pregnant mother, and greater susceptibility to leukemia and asthma in the child where there have been maternal exposures to ELF-EMF. Divan et al (2008) found that children born to mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. Children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems (Divan et al, 2008). Aldad et al (2012) showed that cell phone radiation significantly altered fetal brain development and produced ADHD-like behavior in the offspring of pregnant mice. Exposed mice had a dose-dependent impaired glutamatergic synaptic transmission onto Layer V pyramidal neurons of the prefrontal cortex. The authors conclude the behavioral changes were the result of altered neuronal developmental programming in utero. Offspring mice were hyperactive and had impaired memory function and behavior problems, much like the human children in Divan et al (2008). Fetal (in-utero) and early childhood exposures to cell phone radiation and wireless technologies in general may be a risk factor for hyperactivity, learning disorders and behavioral problems in school.

See Herbert and Sage, Section 19: Fetal and Neonatal Effects of EMF and Section 20: Findings in Autism (ASC) Consistent with Electromagnetic Fields (EMF) and Radiofrequency Radiation (RFR) Exposure in the BioInitiative 2012 Report at www.bioinitiative.org for references and as published in *Pathophysiology*, Volume 20, Issue 3.

[Herbert M, Sage C (2013) Autism and EMF/RFR? Plausibility of a Pathophysiological Link-Part I. *Pathophysiology* [Volume 20, Issue 3](#), 191-209, June 2013]

[Herbert M, Sage C (2013) Autism and EMF/RFR? Plausibility of a Pathophysiological Link-Part II. Pathophysiology [Volume 20, Issue 3](#), 211-234, June 2013]

Fragopoulou et al (2012) reports that brain astrocyte development followed by proteomic studies is adversely affected by DECT (cordless phone radiation) and mobile phone radiation (Fragopoulou and Margaritis, Section 5: EMF Transcriptomics and Proteomics Research 2007-2012, BioInitiative 2012 Report at www.bioinitiative.org)

Common sense measures to limit both ELF-EMF and RF EMF in these populations is needed, especially with respect to avoidable exposures like incubators that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RF EMF are easily instituted. A precautionary approach may provide the frame for decision-making where remediation actions have to be realized to prevent high exposures of children and pregnant woman.

(Bellieni and Pinto, 2012 – Section 19, Fetal and Neonatal Effects, BioInitiative 2012 Report at www.bioinitiative.org)

6. Evidence for Heat Shock Protein Effects

3.5.1.4 Conclusions on neoplastic diseases from RF Exposure and

3.7.1.4 Conclusions on neoplastic diseases from ELF Exposure

SCENIHR emphasizes epidemiology studies of health effects such as cancers that generally affect a relatively small percentage of those exposed and take many years to develop. It does not include studies of the natural protective mechanisms in virtually all cells that protect against the immediate changes that lead to the long term health effects. Living cells synthesize stress proteins when exposed to potentially harmful stimuli that include electromagnetic fields (EMF) across a wide range of non-ionizing frequencies. Stress protein synthesis and oxidative damage to DNA stimulated by EMF are considered likely to lead to cancer and other diseases. Like the DNA damage, these effects occur at exposures well below levels that are now considered safe. Stress proteins can also be protective when induced prior to surgery, as in reducing oxidative damage following heart bypass surgery. Given the goals of SCENIHR, analysis of cell biology studies is essential. An EMF safety standard, based on the far more sensitive natural biological response, would not only be more realistic than the thermal criterion, but more protective as well.

7. Evidence for Impacts of Physical and Biological Variables on Study Results

The main flaw of the preliminary Opinion is in neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies in Exhibit F, these effects depend on variety of biological and physical parameters including polarization, frequency and environmental EMF. *In vitro* and *in vivo* negative studies have covered a negligible minority of real cell phone signals, so the studies cannot provide evidence that the vast majority of other real cell phone signals are safe. Thus, the results of negative studies profiled in the Opinion cannot be extrapolated to the issue of the safety or lack of safety of cell phones in use today. Well conducted positive studies cannot be negated by poorly conducted negative studies. The claimed of "inconsistency" in *in vitro* and *in vivo* data and "conflicting results" has at least one simple explanation. The studies were performed under different conditions. Thus, results cannot be directly compared. The SCENIHR report on inconsistency and conflicting results may rather reflect the level of superficial analysis of these studies. Another fundamental flaw is in

neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see for review (Belyaev 2010 in Exhibit F). In addition to laboratory studies, when brain cancer risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RF dose (for exposures 7 years or more in the past) (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of most important Bradford Hill criteria which is dependence on dose.

Good epidemiological evidence for brain tumors from many other studies has been excluded (see Section 1 and Exhibits B and F). The SCENIHR preliminary Opinion is heavily biased in favor of the Danish subscriber cohort study of mobile phone subscribers. This study has major flaws that have been substantially documented since its publication. It is not informative even according to the requirement of SCENIHR which says "(T)he minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body" (page 10).

ELF Carcinogenicity: Page 131 of the SCENIHR provides misleading and flawed conclusions on ELF and neoplastic diseases. As a matter of fact, the increased risk of childhood leukemia with daily average exposure above 0.3 to 0.4 μT is as strong as never before. All available studies from Europe, America and Asia consistently show such correlation. It has been further supported by recent meta-analysis by Zhao et al. (Zhao, Liu et al. 2014). The statement of lack of mechanisms for ELF effects is wrong. Recent studies provided more evidence for such mechanisms even if they have not been comprehensively studied, see below. Considerations of ELF carcinogenicity in the SCENIHR report did not use standard methods such as the Bradford Hill criteria which do not require complete knowledge of mechanisms in case when epidemiological evidence is overwhelming as in case of childhood leukemia (Zhao, Liu et al. 2014).

ELF affects cell proliferation: In line with many previous studies, new studies unmentioned in the SCENIHR report provide further evidence that ELF can affect cell proliferation under specific conditions of exposure (Segatore, Setacci et al. 2012; Bae, Do et al. 2013; Jadidi, Safari et al. 2013). Bai et al. investigated ELF effects on proliferation of epidermal stem cells (ESC) (Bai, Zhang et al. 2012). See additional comments in Exhibit F.

ELF induced ROS and genomic instability: Induction ROS and is generally considered as a candidate mechanism for carcinogenicity for EMF (IARC 2013). Several recent studies unmentioned in the SCENIHR report provided further evidence for this mechanism in case of ELF exposure (Duan, Wang et al. 2013; Khaki, Khaki et al. 2013). See additional comments in Exhibit F.

Mechanisms for effects of weak ELF: While all mechanisms of ELF effects are not known with certainty, new important data emerged about these mechanisms which were neglected by the SCENIHR report. For ELF fields, these mechanisms involve magnetoreception of fields in the μT -range which is observed in many studied animals including lizards (Nishimura, Okano et al. 2010). It should be stressed that the lack of precise knowledge for this mechanism (radical pairs and magnetite are mainly considered) does not preclude general acceptance of these phenomena. In analogy, and in accordance to the Bradford Hill criteria, lack of precise knowledge on mechanism for leukemogenesis of weak ELF $\geq 0.3 \mu\text{T}$, which was consistently shown in children

in multiple studies (Zhao, Liu et al. 2014) should not preclude classification of μ T-range ELF as an IARC carcinogen group 1. The SCENIHR report completely neglects variety of mechanisms based on ELF effects on ions (Halgamuge and Abeyrathne 2011; Foletti, Grimaldi et al. 2013). See additional comments in Exhibit F.

ELF section omits significant number of ELF positive studies: Except for aforementioned studies, ELF section of the SCENIHR report omits significant number of other ELF positive studies. These include but not limited to (Mariucci, Villarini et al. 2010; Nishimura, Okano et al. 2010; Ravera, Bianco et al. 2010; Severini, Bosco et al. 2010; Ulku, Akdag et al. 2011; Bai, Zhang et al. 2012; Ince, Akdag et al. 2012; Martirosyan 2012; Portelli, Madapatha et al. 2012; Balassa, Varro et al. 2013; Gang, Parker et al. 2013; Iorio, Bennato et al. 2013; Kang, Hong et al. 2013; Khaki, Khaki et al. 2013; Li, Zhang et al. 2013; Martirosyan, Baghdasaryan et al. 2013; Panagopoulos, Karabarounis et al. 2013; Shams Lahijani, Tehrani et al. 2013; Villarini, Ambrosini et al. 2013) See additional comments in Exhibit F.

8. Literature Identified but Not Cited (pages 217-219).

Entire bodies of relevant evidence are ignored, or key papers are not quoted (but they appear in the reference list as “literature identified but not cited”). This is not explained, and functionally disables scientific review of highly relevant emerging scientific studies. An explanation is needed. Further, revisions should be made to include many or most of them in the Final Opinion to include these and other relevant papers. These papers are included as ‘literature identified but not cited’ – as examples of the problem.

Blood-Brain Barrier Evidence

Nittby H, Brun A, Strömblad S, Moghadam MK, Sun W, Malmgren L, Eberhardt J, Persson BR, Salford LG (2011). Nonthermal GSM RF and ELF EMF/ELF MF effects upon rat BBB permeability. *Environmentalist*, 31(2), 140-8

Heat Shock Protein (Stress Protein) Evidence

Calabrò E, Condello S, Currò M, Ferlazzo N, Caccamo D, Magazù S, Ientile R. Modulation of heat shock protein response in SH-SY5Y by mobile phone microwaves. *World J Biol Chem.* 3 (2):34-40, 2012. (3.5)

Perez FP, Zhou X, Morisaki J, Jurivich D. Electromagnetic field therapy delays cellular senescence and death by enhancement of the heat shock response. *Exp Gerontol.* 2008 Apr;43(4):307-16. Epub 2008 Jan 29. (3.5 & 3.10)

Two other highly relevant papers on stress proteins that were ignored and should be incorporated. They are:

Blank M, Goodman R (2009) [Electromagnetic Fields Stress Living Cells. Pathophysiology 16:71-78.](#)

Blank M (2012) Evidence for Stress Response (Stress Proteins). In BioInitiative Report (2012) A Scientific Perspective on Health Risk of Electromagnetic Fields. Section 7, pp. 1-39. Published Online December 31, 2012
<http://www.bioinitiative.org/report/index.htm>

9. Mitochondrial Function and Disruptions in Electrophysiology

None of the sections adequately address the literature on mitochondrial function and ELF-EMF and RFR. The studies in Table 7 are largely negative studies, and do not begin to address the central questions. This section needs to be revised to more comprehensively document existing literature as shown in Exhibit G.

Mitochondria are broadly vulnerable, in part because the integrity of their membranes is vital to their optimal functioning – including channels and electrical gradients, and their membranes can be damaged by free radicals which can be generated in myriad ways including ELF-EMF and RFR exposure at environmental levels. Moreover, just about every step in their metabolic pathways can be targeted by environmental agents, including toxicants and drugs, as well as mutations.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network (Exhibit G). These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by ELF-EMF and RFR (i.e. a possible health effect that should be documented in the Final Opinion).

Other types of mitochondrial damage have been reported in at least some of the studies that have examined the impacts of EMF/RFR upon mitochondria. These include reduced or absent mitochondrial crista, mitochondrial DNA damage, swelling and crystallization, alterations and decreases in various lipids suggesting an increase in their use in cellular energetics, damage to mitochondrial DNA, and altered mobility and lipid peroxidation after exposures. Also noted has been enhancement of brain mitochondrial function in Alzheimer's transgenic mice and normal mice. The existence of positive as well as negative effects gives an indication of the high context dependence of exposure impacts, including physical factors such as frequency, duration, and tissue characteristics (Exhibit G).

Secondary mitochondrial dysfunction (i.e. environmentally triggered rather than rooted directly in genetic mutations) could result from EMF/RFR to damage channels, membranes and mitochondria themselves as well as from toxicant exposures and immune challenges. In a meta-analysis of studies of children with mitochondrial disorder and autism, the spectrum of severity varied, and 79% of the cases were identified by laboratory findings without associated genetic abnormalities.

Electrophysiology: None of the sections adequately address the literature on changes in electrophysiology with exposure to ELF-EMF and RFR. This is a major area of importance and many papers are available for review. This section needs to be revised to more comprehensively document existing literature, especially in the context of blood-brain barrier changes and the propensity for seizures with disrupted electrophysiology (Exhibit G).

Nervous system electrophysiology when disrupted by ELF-EMF and RFR can produce alterations in molecular, cellular and systems physiological function. It occurs in the brain as well as in the body, and impacts the transduction into the electrical signaling activities of the brain and nervous

system. If the cells responsible for generating synapses and oscillatory signaling are laboring under cellular and oxidative stress, lipid peroxidation, impaired calcium and other signaling system abnormalities, then mitochondrial metabolism will fall short, all the more so because of the challenges from the immune system which in turn can be triggered to a major extent by environment. How well will synaptic signals be generated? How well will immune-activated and thereby distracted glial cells be able to modulate synaptic and network activity? Microglial activation can impact excitatory neurotransmission mediated by astrocytes. Cortical innate immune response increases local neuronal excitability and can lead to seizures. Inflammation can play an important role in epilepsy.

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if the blood-brain barrier (BBB) is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact suggesting that such exposures can further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment, suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

One critical issue here is nonlinearity and context and parameter sensitivity of impact. In one study, rat brain slices exposed to EMF/RFR showed reduced synaptic activity and diminution of amplitude of evoked potentials, while whole body exposure to rats led to synaptic facilitation and increased seizure susceptibility in the subsequent analysis of neocortical slices. Another study unexpectedly identified enhanced rat pup post-seizure mortality after perinatal exposure to a specific frequency and intensity of exposure, and concluded that apparently innocuous exposures during early development might lead to vulnerability to stimuli presented later in development.

10. ELF Studies Support a Finding of ‘Probable’ or ‘Known’ Carcinogen

Overall, the ELF MF epidemiological evidence points consistently to an increased risk for childhood leukemia. In such circumstances, considering that no other interpretation (chance, bias, or confounding) could be substantiated in the past decade, the association became more credible and even in the absence of a mechanistic interpretation ELF MF should be upgraded to a 2A or even a group 1 carcinogen.

Many epidemiological studies of ELF MF and childhood leukemia were of high quality and there are no shortcomings that may prevent a causal interpretation. The WHO IARC panel was of the opinion that the studies allowed a causal interpretation; otherwise no classification into group 2B would have been possible. Only bias and confounding could not be ruled out with sufficient scientific certainty. This assessment was also supported by the lack of consistent support by *in vitro* and animal evidence.

3.7. Health effects from ELF fields (Page 123)

3.7.1. Neoplastic diseases

3.7.1.1. Epidemiological studies

Page 123, lines 24-31:

In summarizing the previous SCENIHR statement that endorsed the IARC classification of ELF magnetic fields as possibly carcinogenic to humans “due to consistently observed increased childhood leukemia risk in epidemiological studies” SCENIHR claimed that shortcomings in these studies prevented a causal interpretation.

In fact, the IARC panel was of the opinion that the studies allowed a causal interpretation; otherwise no classification into group 2B would have been possible. Only bias and confounding could not be ruled out with sufficient scientific certainty. This assessment was also supported by the lack of consistent support by *in vitro* and animal evidence.

Many epidemiological studies of ELF MF and childhood leukemia were of high quality and there are no shortcomings that may prevent a causal interpretation. Of course, the retrospective nature of most studies and the inevitable misclassification if measurements are done years after the assumed initiation of the disease introduce problems of interpretation. Considering the potential sources of bias IARC noted that although selection bias could have led to higher risk estimates, not the whole effect can be attributed to bias. This is corroborated by the fact that studies that relied on distance from power lines or wire-codes only and did not contact participants found the same effects. Misclassification bias, on the other hand, if non-differential would lead to reduced risk estimates. The same is true for the most likely scenarios of differential misclassification.

Overall, the epidemiological evidence points consistently to an increased risk. In such circumstances, considering that no other interpretation (chance, bias, or confounding) could be substantiated in the past decade, the association became more credible and even in the absence of a mechanistic interpretation ELF MF should be upgraded to a 2A or even a group 1 carcinogen.

In a recent study of distance from power lines and childhood cancer in Britain covering the period from 1962 through 2008 (Bunch et al. 2014) elevated childhood leukemia risks were reported for distances below 200 or 600 m from high-voltage power lines (400/275 kV) from the 1960s to the 1980s but no significant increases in more recent years. Authors interpreted this result as more likely due to changing population characteristics among those living near power lines than to physical factors. Indeed, the reported data speak for a change in population distribution around power lines. This study raises important questions about the importance of stability of residences for epidemiological studies of localized exposures, but overall speaks in favor of a relationship between ELF MF and childhood leukemia.

Page124-125:

SCENIHR mentioned in their previous report (2009) two studies that addressed the issue of survival from childhood leukemia and exposure to ELF MF. These studies reported poorer survival at increased levels of exposure (above 0.2/0.3 μT). In 2012 Schüz et al. reported results of a pooled study including data from 6 countries. This pooled analysis reported somewhat increased hazard ratios at moderately increased average exposure levels up to 0.3 μT but no increased hazard ratios above 0.3 μT . This study has been mentioned in the new SCENIHR report but without further discussion of its implications. Although the study included more than 3000 cases the small number of children at elevated exposure levels and the lack of follow-up data on post diagnostic exposure for most of the cases prohibit far reaching conclusions.

Page 126-131:

SCENIHR provides a brief overview of *in vitro* and *in vivo* animal studies of ELF MF exposure and endpoints relevant for the issue of potential mechanisms of a relationship between ELF-MF and neoplastic diseases. While the presentation encompasses all publications of relevance since the last report it again lacks a discussion of the difficulties of such studies and the very small likelihood to detect an effect of exposure due to the lack of a profound biophysical mechanism as a starting point.

In conclusion, the preliminary opinion of SCENIHR concerning ELF MF covers the relevant literature and no essential omission has been detected. However, it is recommended to not separate the findings from previous reports but to assess the evidence as a whole. Furthermore, it appears that SCENIHR does not sufficiently challenge the validity especially of studies that did not find an effect of exposure.

Contributed by Prof. Michael Kundi, PhD med habil Institute of Environmental Health, Medical University of Vienna, Vienna, Austria

11. RFR Studies Support a Finding of ‘Probable’ or ‘Known’ Human Carcinogen

A recent publication by Hardell and Carlberg reports that “(F)urther research has thus strengthened the evidence in support of an increased risk of malignant brain tumours and acoustic neuroma associated with use of mobile phones. Based on the latest findings and using the so called Hill viewpoints from the 1960’s exposure to RF-EMF from mobile phones may now be classified as a human cancer causing agent, Group 1, according to the definitions used by IARC.”

Hardell L, Carlberg M. Using the Hill viewpoints from 1965 for evaluating strengths of evidence of the risk for brain tumors associated with use of mobile and cordless phones. *Rev Environ Health* 2013;38:97-106. doi: 10.1515/reveh-2013-0006.

There is credible scientific evidence that RF exposures cause changes in cell membrane function, metabolism and cellular signal communication, as well as activation of proto-oncogenes and triggering of the production of stress proteins at exposure levels thousands of times below current regulatory limits. There is also generation of reactive oxygen species, which cause single- and double-strand DNA damage, chromosomal aberrations and nerve cell death. A number of different effects on the central nervous system have also been documented, including activation of the endogenous opioid systems, changes in brain function including memory loss, slowed learning, motor dysfunction and performance impairment in children, and increased frequency of headaches, fatigue and sleep disorders. Melatonin secretion is reduced, resulting in altered circadian rhythms and disruption of several physiological functions. See Chapters 1, 5–12 of the 2007 BioInitiative Report [1], [2-6] and Chapters 1, 5-24 of the 2012 BioInitiative Report [7]. These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable [1,19]. The young are also largely unable to remove themselves from such environments. Second-hand non-ionizing radiation, like second-hand smoke may be considered a public health concern based on the evidence at hand.

Exposure to electromagnetic fields (both extremely low-frequency ELF-EMF from power frequency sources like power lines and appliances; and radiofrequency radiation or RFR) has

been linked to a variety of adverse health outcomes that may have significant public health consequences. The most serious health endpoints that have been reported to be associated with extremely low frequency (ELF) and/or radiofrequency radiation (RFR) include childhood and adult leukemia, childhood and adult brain tumors, and increased risk of the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS). In addition, there are reports of increased risk of breast cancer in both men and women, genotoxic effects (DNA damage, chromatin condensation, micronucleation, impaired repair of DNA damage in human stem cells), pathological leakage of the blood-brain barrier, altered immune function including increased allergic and inflammatory responses, miscarriage and some cardiovascular effects. Insomnia (sleep disruption) is reported in studies of people living in very low-intensity RF environments with WI-FI and cell tower-level exposures. Short-term effects on cognition, memory and learning, behavior, reaction time, attention and concentration, and altered brainwave activity (altered EEG) are also reported in the scientific literature. Biophysical mechanisms that may account for such effects can be found in various articles and reviews. [2-7]

The BioInitiative Working Group concluded in 2007 that existing public safety limits were inadequate to protect public health, and agreed that new, biologically-based public safety limits were needed more than five years ago. The 2007 BioInitiative Report was prepared by more than a dozen world-recognized experts in science and public health policy; and outside reviewers also contributed valuable content and perspective.

From a public health standpoint, experts reasoned that it was not in the public interest to wait. In 2007, the evidence at hand coupled with the enormous populations placed at possible risk was argued as sufficient to warrant strong precautionary measures for RFR, and lowered safety limits for ELF-EMF. The ELF recommendations were biologically-based and reflected the ELF levels consistently associated with increased risk of childhood cancer, and further incorporated a safety factor that is proportionate to others used in similar circumstances. The public health cost of doing nothing was judged to be unacceptable in 2007.

12. Plausible Biological Mechanisms are Known

Oxidative stress through the action of free radical damage to DNA is a plausible biological mechanism for cancer and diseases that involve damage from ELF to the central nervous system.

Plausible biological mechanisms are already identified that can reasonably account for most biological effects reported for exposure to RF and ELF at low-intensity levels (oxidative stress and DNA damage from free radicals leading to genotoxicity; molecular mechanisms at very low energies are plausible links to disease, e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation). It is also important to remember that traditional public health and epidemiological determinations do not require a proven mechanism before inferring a causal link between EMFs exposure and disease. Many times, proof of mechanism is not known before wise public health responses are implemented.

“Obviously, melatonin’s ability to protect DNA from oxidative damage has implications for many types of cancer, including leukemia, considering that DNA damage due to free radicals is believed to be the initial oncogenic event in a majority of human cancers [Cerutti et al., 1994]. In addition to cancer, free radical damage to the central nervous system is a significant component of a variety of neurodegenerative diseases of the aged including Alzheimer’s disease and Parkinsonism. In experimental animal models of both of these conditions, melatonin has proven highly effective in forestalling their onset, and reducing their severity.” [9]

The De Iuliis et al study which is quoted by the SCENIHR committee with respect to both genotoxicity and oxidative stress, and to sperm motility damage discusses that oxidative damage is a plausible mechanism for these effects.

“Oxidative stress has been known for some time to limit the fertilizing potential of human spermatozoa through the induction of peroxidative damage to the sperm plasma membrane [13,20]. Oxidative stress is also known to be associated with DNA damage in human spermatozoa [21]. Furthermore, the source of the free radicals responsible for generating such stress appears to be the mitochondria [15]. However, the factors responsible for inducing the mitochondria to leak electrons and propagate the production of ROS have not been elucidated. The research described in this article suggests that one of the key environmental factors involved in the stimulation of sperm mitochondria to produce high levels of ROS, might be excess exposure to RF-EMR from sources such as mobile phones.”

See also Exhibit C: Reference List for Important Fertility and Reproduction Papers

13. Consistent Failure to Identify the Potential for Health Effects (Opinion-wide)

The evaluative language quoted below indicates the disparity between what was asked of the authors (to identify Possible Effects of EMF) and what they eventually chose to use as a basis for their analysis process that no change in the ICNIRP standards is warranted at this time (see Exhibit A).

SIXTEEN (16) instances of “no causal evidence” or “prevents a causal interpretation” or “is not causally linked” or “not informative for causal linkage”.

THREE (3) instances of “does not provide convincing evidence”.

THREE (3) instances of “not definitive”.

SEVEN (7) instances of “do not unequivocally indicate”.

These criteria are inconsistent with a review that is titled “Possible Effects”. Further, the approach in judging the emerging evidence is inconsistent with the charter of the Scientific Committee* to give advice needed for “*consumer safety, public health and the environment on new or emerging problems.*” Some statements acknowledge important new evidence of effect; yet then shift the burden of proof to a higher level requiring that adverse health effect, a known mechanism, a causal level of evidence be conclusively demonstrated, or physical evidence of harm be demonstrated. There is nothing in the report that says the authors were directed to provide proof of effect (or consistent indications, or consistent demonstration of effect; or consistent support for, or certainty of effects) at levels below ICNIRP limits. With the same flawed approach in drawing conclusions from emerging science as demonstrated by the SCENIHR, hardly any environmental or occupational condition would be qualified as an emerging or newly identified health risk*.

*Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention

to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the **Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)** and are made up of external experts.

Preparation of this review has been completed with generous donations of the time and resources of authors of the BioInitiative Working Group.

Qualifications of the BioInitiative 2012 Working Group

The 2012 BioInitiative Report was prepared by 29 authors from ten countries, ten holding medical degrees (MDs), 21 PhDs, and three MsC, MA or MPHs. Among the authors are three former Presidents of the Bioelectromagnetics Society and five full members of BEMS. One distinguished author is the Chair of the Russian National Committee on Non-Ionizing Radiation. Three were members of the 2011 IARC Working Group that established RFR as a Group 2B Possible Human Carcinogen (Hardell, Belyaev and Blackman). Another was until recently a Senior Advisor on Science, Policy, Emerging Issues, Integrated Environmental Assessment to the European Environmental Agency. Full titles and affiliations of authors is in Section 25 of the BioInitiative Report at www.bioinitiative.org. See specific conclusions and findings of the BioInitiative 2012 Report at www.bioinitiative.org. It is incorporated by reference in this comment.

In twenty-four technical chapters, the BioInitiative Working Group authors discuss the content and implications of about 1800 new studies since 2007. Overall, these new studies report abnormal gene transcription (Section 5); genotoxicity and single-and double-strand DNA damage (Section 6); stress proteins because of the fractal RF-antenna like nature of DNA (Section 7); chromatin condensation and loss of DNA repair capacity in human stem cells (Sections 6 and 15); reduction in free-radical scavengers - particularly melatonin (Sections 5, 9, 13, 14, 15, 16 and 17); neurotoxicity in humans and animals (Section 9); carcinogenicity in humans (Sections 11, 12, 13, 14, 15, 16 and 17); serious impacts on human and animal sperm morphology and function (Section 18); effects on the fetus, neonate and offspring (Section 18 and 19); effects on brain and cranial bone development in the offspring of animals that are exposed to cell phone radiation during pregnancy (Sections 5 and 18); and findings in autism spectrum disorders consistent with EMF/RFR exposure effects. Global precautionary actions that have been taken in countries around the world, and recommended by medical and research experts are documented in Section 22. Use of the Precautionary Principal and it's relevance are presented in Section 23. Key scientific evidence and public health policy recommendations are in Section 24.

Respectfully submitted on behalf of the BioInitiative Working Group by:

Cindy Sage, MA and David O. Carpenter, MD
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Exhibit A: Consistent Failure to Identify the Potential for Health Effects (Opinion-wide)

The evaluative language quoted below indicates the disparity between what was asked of the authors (to identify Possible Effects of EMF) and what they eventually chose to use as a basis for their analysis process that no change in the ICNIRP standards is warranted at this time.

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These criteria are inconsistent with a review that is titled “Possible Effects”. Further, the approach in judging the emerging evidence is inconsistent with the charter of the Scientific Committee* to give advice needed for “*consumer safety, public health and the environment on new or emerging problems.*” Some statements acknowledge important new evidence of effect; yet then shift the burden of proof to a higher level requiring that adverse health effect, a known mechanism, a causal level of evidence be conclusively demonstrated, or physical evidence of harm be demonstrated. There is nothing in the report that says the authors were directed to provide proof of effect (or consistent indications, or consistent demonstration of effect; or consistent support for, or certainty of effects) at levels below ICNIRP limits. With the same flawed approach in drawing conclusions from emerging science as demonstrated by the SCENIHR, hardly any environmental or occupational condition would be qualified as an emerging or newly identified health risk*.

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All of the areas **highlighted in yellow** in the preliminary Opinion indicate problems (omissions, mischaracterizations of exposure data leading to erroneous conclusions about possible public health risks, misreading of original study results, dismissal of important findings, need for a known mechanism, and failure to use proper criteria for judging potential for health effects as opposed to causal effects).

There is a serious consequence which comes from dismissing effects linking EMF/RFR exposures reported in scientific studies to an ‘all or none’ finding by using embedded criteria that demand ‘causal’ or ‘conclusive’ or ‘definitive’ or ‘consistent demonstration of effect’. It is clear that such erasing possible impacts of great global health consequence will chill public health responses that would otherwise occur if the correct standards for judging the evidence were used in this Opinion. Public health activities hinge on not causality but sufficiency of evidence to warrant a proportionate preventative action in line with established precautionary principles. This draft Opinion provides no guidance in this area.

Since the charge to the Scientific Committee is to evaluate the possible health effects (not to prove beyond a shadow of a scientific doubt the causality of such exposures to health harm), the Opinion needs complete re-working. It may be also that the Committee needs new membership capable of a different, and more appropriate approach to the important assessment that SCENIHR is charged to prepare. Page and line numbers are included to key to the Opinion sections.

Page 5: Health effects from Extremely Low Frequency (ELF) fields

12 The new epidemiological studies are consistent with earlier findings of an increased risk
13 of childhood leukemia with long-term average exposure to magnetic fields above 0.3 to
14 0.4 μ T. However, as stated in the previous opinions, no mechanisms have been identified
15 that could explain these findings. The lack of experimental support and shortcomings
16 identified for the epidemiological studies **prevent a causal interpretation.**

Page 12-13: Health effects from RF fields

42 Epidemiological studies on RF exposure **do not unequivocally indicate** an increased risk of
43 brain tumours

20-22 However, research conducted since the previous SCENIHR opinion adds weight to the conclusion
that **RF exposure is not causally linked** to these symptoms,

Page 13 Health effects from ELF fields

49 The new epidemiological studies are consistent with earlier findings of an increased risk
50 of childhood leukemia with daily average exposure above 0.3 to 0.4 μ T. As stated in the
51 previous SCENIHR opinions, no mechanisms have been identified in experimental studies
52 that could explain these findings. Due to lack of support from experimental data and
shortcomings in the epidemiological studies, **evidence remains weak that the observed
association reflects a causal effect.**

For symptoms associated with longer-term exposures (measured in days to months), the
30 evidence from observational studies **against a causative association** with RF exposure is
31 broadly consistent but has gaps, most notably in terms of the objective monitoring of
32 exposure.

Page 58

24-28 They reported higher incidence rates of brain cancers in countries with the most frequent mobile
phone subscriptions. The study is **not informative for causal inference**, as popular use of mobile phones can
also reflect standard of living, which is also associated with, for example, availability of diagnostic
services.

Page 65-66 Discussion of brain tumours and other tumours of the head and neck area

5-7 For the segment of the heaviest users, the largest case-control study in particular observed about
40% increased risks for glioma and for acoustic neuroma. It cannot be concluded from the available
studies **whether this reflects a causal association.**

Here, the conclusion that there might legitimately be causal evidence for increased risk for brain tumors
with cell phone use but it no longer matters, because, indeed, technologies might change in the future. This
is a preposterous statement. It has the impact of trivializing the issue, minimizing identified risks and
leaping to an irrational conclusion that negates any need for the Scientific Committee to advise caution.

19-25 Therefore, the increased risks seen in heavy users in the case-control studies, mainly
driven by technologies not in operation anymore or operating more efficiently today, could perhaps not be
due to methodological shortcomings **but indeed reflect a causal association.** This finding might be
irrelevant for any future cancer prevention activities since those relevant cumulative RF exposure levels

are not reached anymore, not even among those using mobile phones for longer duration or much more often than the users of the 1980s or 1990s.

Pages 114-115 *Provocation Studies*

The fact that these effects disappear once blinding is used and the participant is therefore unaware of the exposure suggests first, that **no casual (causal) effect of RF exposure** exists and second, that believing RF 48 to be present is sufficient to induce symptoms via a placebo effect. While further work using this paradigm would be beneficial, at present these studies suggest **there is no causal link between exposure and symptoms.**

Page 123 3.7. Health effects from ELF fields

22 3.7.1. Neoplastic diseases

23 3.7.1.1. Epidemiological studies

24 What was already known on this subject?

25 The previous SCENIHR statement endorsed the IARC assessment of classifying ELF
26 magnetic fields as possibly carcinogenic to humans due to consistently observed
27 increased childhood leukaemia risk in epidemiological studies (SCENIHR, 2009); the
28 latter stems mainly from two pooled analyses based on studies completed before the
29 year 2000, showing a two-fold risk increase with ELF magnetic fields above 0.3-0.4 μT
30 (time-weighted average) but raising concerns about shortcomings of those studies
31 **preventing a causal interpretation** (Ahlbom et al., 2000; Greenland et al., 2000).

Page 125 *Discussion on epidemiological studies*

27 Pooled analyses of the more recent studies on ELF magnetic fields and childhood
28 leukaemia confirm those of earlier studies, however, the new generation of studies shows
29 little methodological advancement compared to the ones conducted before 2000.
30 Therefore **it remains difficult to judge whether the apparently quite robust empirical**
31 **association is likely to be causal** or a result of methodological shortcomings of the
32 studies.

Page 125 *Conclusions on epidemiological studies*

42 The previous assessment of the 2009 SCENIHR statement of a possible association
43 between long term exposure to ELF magnetic fields and an increased risk of childhood
44 leukaemia remains valid. From an epidemiological point of view, the association appears
45 to be robust, having been observed in multiple studies in different settings at different
46 points in time. Unfortunately, little progress has been made in explaining the finding,
47 **both in terms of finding a plausible mechanism for a causal association** or in identifying
48 alternative explanations.

Page 131 3.7.1.4. *Conclusions on neoplastic diseases*

18 The new epidemiological studies are consistent with earlier findings of an increased risk
19 of childhood leukemia with daily average exposure above 0.3 to 0.4 μT . As stated in the
20 previous opinions, no mechanisms have been identified in experimental studies that
21 could explain these findings. Lack of support from experimental studies and shortcomings
22 of the epidemiological studies **prevent a causal interpretation.**

Page 141 3.7.3.1 *Conclusions on Symptoms*

The 2009 opinion concluded that **no consistent relationship** had been demonstrated between ELF exposure and symptoms, neither in the general public nor in people with IEI-EMF.

Page 142 *Conclusions on symptoms* 3.7.3 *Other Health Effects*

49 The studies published since the 2009 opinion show discordant results. However,
50 observational studies suffered from weaknesses and **do not provide convincing evidence**
51 of an effect of ELF exposure on symptoms in the general population and most
52 experimental evidence also points to the **absence of any causal effect.**

Page 144-145 *Neoplastic diseases*

48 The new epidemiological studies are consistent with earlier findings of an increased risk
49 of childhood leukemia with daily average exposures above 0.3 to 0.4 μT . As stated in the
50 previous opinions, no mechanisms have been identified in experimental studies that
1 could explain these findings. Lack of support from experimental studies and shortcomings
2 of the epidemiological studies prevent a causal interpretation.

Page 170 3.13. *Research recommendations*

44 Research to date has not been able to identify with any certainty any adverse health
45 effect resulting from exposure to EMFs at any frequency or intensity typically found in the
46 workplace or everyday environment. Epidemiological studies have reported associations
47 between EMF exposure and certain diseases, most notably for an increased risk of
48 childhood leukaemia with exposure to low frequency magnetic fields, but none of these
49 associations can be considered causal

Page 176. 3.14. *Guidance on research methods*

Indeed, organ-specific dosimetry is considered necessary to help to establish causality.

Page 177 - 178

To give particular attention to issues affected by important gaps in knowledge in the previous opinions, especially:

35 2a. the potential adverse effects of EMF on the nervous system, including neurobehavioural disorders and on the risk of neo-plastic diseases;

37 *RF fields*

38 Previous studies suggesting that RF exposure may affect brain activities as reflected by
39 changes in the EEG during wake and sleep are further substantiated by the results of
40 more recent studies. However, given the variety of applied fields, duration of exposure,
41 number of considered leads, and statistical methods it is difficult to derive firm
42 conclusions. For event-related potentials and slow brain oscillations results are
43 inconsistent. Likewise, studies on cognitive functions in humans lack consistency. The
44 biological relevance of reported small physiological EEG changes remains unclear, and
45 mechanistic explanation is still lacking.
46 A reasonable body of experimental evidence now suggests that exposure to RF does not
47 trigger symptoms, at least in the short-term. While additional observational studies are
48 required to assess whether longer-term exposure could be associated with symptoms,
49 the evidence to date weighs against a causal effect.

2 Studies on neurological diseases and symptoms show no clear effect, but the evidence is
3 limited. Human studies on child development and behavioural problems provide only
4 weak evidence because of conflicting results and methodological limitations. Direct
5 effects of exposure from mother's mobile phone use during pregnancy are not plausible
6 owing to extremely low fetal exposure to mobile phone EMF.

7 Epidemiological studies on RF exposure do not unequivocally indicate an increased risk of
8 brain tumours, and do not indicate an increased risk for other cancers of the head and
9 neck region, or other malignant diseases including childhood cancer. Earlier studies
10 raised open questions regarding an increased risk of glioma and acoustic neuroma in
11 heavy long-term users of mobile phones. Based on the most recent cohort and incidence
12 time trend studies, the evidence for glioma became weaker while the possibility of an
13 association with acoustic neuroma remains open.

14 A considerable number of well-performed in vivo studies using a wide variety of animal
15 models have been mostly negative in outcome. These studies are considered to provide
16 evidence for the absence of a carcinogenic effect.

17 A large number of in vitro studies pertaining to genotoxic as well as non-genotoxic end
18 points have been published since the last opinion. In most of the studies, no effects of

*19 exposure at levels below exposure limits were recorded, although in some cases DNA
20 strand breaks and spindle disturbances were observed.*

Page 178 [ELF fields](#)

*The new epidemiological studies are consistent with earlier findings of an increased risk
41 of childhood leukemia with long-term daily average exposures above 0.3 to 0.4 μ T. As
42 stated in the previous opinions, **no mechanisms have been identified** and no support is
43 existing from experimental studies that could explain these findings, which, together
44 with shortcomings of the epidemiological studies **prevent a causal interpretation.***

Exhibit B : Comment by Drs. Lennart Hardell, Fredrik Soderqvist, PhD and Michael Carlberg, MSc.

Section 3.5.1.1 Epidemiological Studies, RF fields epidemiology, Pages 57-68

We have read the SCENIHR 2013 Preliminary opinion on Potential health effects of exposure to electromagnetic fields (EMF), especially relating to epidemiological studies on neoplastic diseases. It is concluded at page 4 in the abstract that “*Based on the most recent cohort and incidence time trend studies, it appears that the evidence for glioma became weaker while the possibility of an association with acoustic neuroma remains open*”.

This statement is not based on facts but on selective inclusion of studies with omission of the most recent publications, e.g. from our research group (the Hardell group). Our studies were well known to the Expert group since Dr Kjell Hansson Mild was one of these experts and also a co-author in most of the Hardell group studies. In fact he communicated our studies to the SCENIHR expert group obviously without response. If these studies had been included it would be apparent that the final conclusions on brain tumour risk in SCENIHR are not based on scientific facts. In contrast the evidence for glioma and acoustic neuroma would become stronger if recent publications had been included.

In the Terms of Reference (page 16) it is stated that the Committee is requested e.g.:

1. To update its opinions of 2009 in the light of newly available information
2. To give particular attention to issues affected by important gaps in knowledge in the previous opinions, especially:
 - The potential adverse effects of EMF on the nervous system, including neurobehavioral disorders, and on the risk of neo-plastic diseases;

It seems as if the Committee has been anxious to include ‘newly available information’ at least regarding some studies, e.g. **Benson VS, Pirie K, Schüz J, Reeves GK, Beral V, Green J. Int J Epidemiol 2013, Sep 27**, see page 64, not included in reference list. On the contrary our studies were excluded. In the following a summary is given.

Background:

The carcinogenic effect of RF-EMF on humans was evaluated at a meeting during 24 – 31 May 2011 at the International Agency for Research on Cancer (IARC) at WHO in Lyon, France. The Working Group consisted of 30 scientists representing four areas: ‘animal cancer studies’, ‘epidemiology’, ‘exposure’ and ‘mechanistic and other relevant data’ (<http://monographs.iarc.fr/ENG/Meetings/vol102-participants.pdf>). One of us, LH, was invited as an expert in the epidemiology group. On 31 May 2011 IARC categorised RF-EMFs from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields, as a Group 2B, i.e. a ‘possible’, human carcinogen. The decision was almost unanimous.

The IARC decision on mobile phones was based mainly on two sets of case-control human studies on brain tumour risk; our studies from Sweden (the Hardell group) and the IARC Interphone study. Both provided complementary and supportive results on positive associations between two types of brain tumours; glioma and acoustic neuroma, and exposure to RF-EMF from mobile phones. No consistent evidence was found for

meningioma, a benign type of brain tumour. After the IARC meeting we have published further studies with new data, both overview of studies with meta-analysis (number 1 below) and our case-control study including brain tumour cases diagnosed during 2007-2009 (number 2-4 below). Furthermore we applied the Hill viewpoints on the risk for brain tumours associated with use of mobile and cordless phones (number 5 below). These criteria were developed in the 1960's during the height of the tobacco and lung cancer controversy.

Recent studies from the Hardell group not included in SCENIHR 2013:

1. **Hardell L, Carlberg M, Hansson Mild K. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. *Pathophysiology* 2013;20:85-110. Epub 2012 Dec 21.**

Abstract

The International Agency for Research on Cancer (IARC) at WHO evaluation of the carcinogenic effect of RF-EMF on humans took place during a 24-31 May 2011 meeting at Lyon in France. The Working Group consisted of 30 scientists and categorised the radiofrequency electromagnetic fields from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields (RF-EMF), as Group 2B, i.e., a 'possible', human carcinogen. The decision on mobile phones was based mainly on the Hardell group of studies from Sweden and the IARC Interphone study. We give an overview of current epidemiological evidence for an increased risk for brain tumours including a meta-analysis of the Hardell group and Interphone results for mobile phone use. Results for cordless phones are lacking in Interphone. *The meta-analysis gave for glioma in the most exposed part of the brain, the temporal lobe, odds ratio (OR)=1.71, 95% confidence interval (CI)=1.04-2.81 in the ≥10 years (>10 years in the Hardell group) latency group. Ipsilateral mobile phone use ≥1640h in total gave OR=2.29, 95% CI=1.56-3.37. The results for meningioma were OR=1.25, 95% CI=0.31-4.98 and OR=1.35, 95% CI=0.81-2.23, respectively. Regarding acoustic neuroma ipsilateral mobile phone use in the latency group ≥10 years gave OR=1.81, 95% CI=0.73-4.45. For ipsilateral cumulative use ≥1640h OR=2.55, 95% CI=1.50-4.40 was obtained.* Also use of cordless phones increased the risk for glioma and acoustic neuroma in the Hardell group studies. Survival of patients with glioma was analysed in the Hardell group studies yielding in the >10 years latency period hazard ratio (HR)=1.2, 95% CI=1.002-1.5 for use of wireless phones. This increased HR was based on results for astrocytoma WHO grade IV (glioblastoma multiforme). Decreased HR was found for low-grade astrocytoma, WHO grades I-II, which might be caused by RF-EMF exposure leading to tumour-associated symptoms and earlier detection and surgery with better prognosis. *Some studies show increasing incidence of brain tumours whereas other studies do not. It is concluded that one should be careful using incidence data to dismiss results in analytical epidemiology.* The IARC carcinogenic classification does not seem to have had any significant impact on governments' perceptions of their responsibilities to protect public health from this widespread source of radiation.

2. **Carlberg M, Söderqvist F, Hansson Mild K, Hardell L. Meningioma patients diagnosed 2007-2009 and the association with use of mobile and cordless phones, *Environ. Health* 2013;12:60, doi:10.1186/1476-069X-12-60. Epub Jul 19, 2013**

Abstract

BACKGROUND: To study the association between use of wireless phones and meningioma.

METHODS: We performed a case-control study on brain tumour cases of both genders aged 18--75 years and diagnosed during 2007--2009. One population-based control matched on gender and age was used to each case. Here we report on meningioma cases including all available controls. Exposures were assessed by a questionnaire. Unconditional logistic regression analysis was performed.

RESULTS: In total 709 meningioma cases and 1,368 control subjects answered the questionnaire. *Mobile phone use in total produced odds ratio (OR) = 1.0, 95% confidence interval (CI) = 0.7-1.4 and cordless phone use gave OR = 1.1, 95% CI = 0.8-1.5.* The risk increased statistically significant per 100 h of cumulative use and highest OR was found in the fourth quartile (>2,376 hours) of cumulative use for all studied phone types. There was no statistically significant increased risk for ipsilateral mobile or cordless phone use, for meningioma in the temporal lobe or per year of latency. Tumour volume was not related to latency or cumulative use in hours of wireless phones.

CONCLUSIONS: *No conclusive evidence of an association between use of mobile and cordless phones and meningioma was found.* An indication of increased risk was seen in the group with highest cumulative use but

was not supported by statistically significant increasing risk with latency. Results for even longer latency periods of wireless phone use than in this study are desirable.

- 3. Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Pooled analysis of case-control studies on acoustic neuroma diagnosed 1997-2003 and 2007-2009 and use of mobile and cordless phones. *Int J Oncol.* 2013;43:1036-1044. Epub 2013 Jul 22.**

Abstract

We previously conducted a case-control study of acoustic neuroma. Subjects of both genders aged 20-80 years, diagnosed during 1997-2003 in parts of Sweden, were included, and the results were published. We have since made a further study for the time period 2007-2009 including both men and women aged 18-75 years selected from throughout the country. These new results for acoustic neuroma have not been published to date. Similar methods were used for both study periods. In each, one population-based control, matched on gender and age (within five years), was identified from the Swedish Population Registry. Exposures were assessed by a self-administered questionnaire supplemented by a phone interview. Since the number of acoustic neuroma cases in the new study was low we now present pooled results from both study periods based on 316 participating cases and 3,530 controls. Unconditional logistic regression analysis was performed, adjusting for age, gender, year of diagnosis and socio-economic index (SEI). Use of mobile phones of the analogue type gave odds ratio (OR) = 2.9, 95% confidence interval (CI) = 2.0-4.3, increasing with >20 years latency (time since first exposure) to OR = 7.7, 95% CI = 2.8-21. Digital 2G mobile phone use gave OR = 1.5, 95% CI = 1.1-2.1, increasing with latency >15 years to an OR = 1.8, 95% CI = 0.8-4.2. The results for cordless phone use were OR = 1.5, 95% CI = 1.1-2.1, and, for latency of >20 years, OR = 6.5, 95% CI = 1.7-26. Digital type wireless phones (2G and 3G mobile phones and cordless phones) gave OR = 1.5, 95% CI = 1.1-2.0 increasing to OR = 8.1, 95% CI = 2.0-32 with latency >20 years. *For total wireless phone use, the highest risk was calculated for the longest latency time >20 years: OR = 4.4, 95% CI = 2.2-9.0.* Several of the calculations in the long latency category were based on low numbers of exposed cases. Ipsilateral use resulted in a higher risk than contralateral for both mobile and cordless phones. OR increased per 100 h cumulative use and per year of latency for mobile phones and cordless phones, though the increase was not statistically significant for cordless phones. *The percentage tumour volume increased per year of latency and per 100 h of cumulative use, statistically significant for analogue phones. This study confirmed previous results demonstrating an association between mobile and cordless phone use and acoustic neuroma.*

- 4. Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24**

Abstract

Previous studies have shown a consistent association between long-term use of mobile and cordless phones and glioma and acoustic neuroma, but not for meningioma. When used these phones emit radiofrequency electromagnetic fields (RF-EMFs) and the brain is the main target organ for the handheld phone. The International Agency for Research on Cancer (IARC) classified in May, 2011 RF-EMF as a group 2B, i.e. a 'possible' human carcinogen. The aim of this study was to further explore the relationship between especially long-term (>10 years) use of wireless phones and the development of malignant brain tumours. We conducted a new case-control study of brain tumour cases of both genders aged 18-75 years and diagnosed during 2007-2009. One population-based control matched on gender and age (within 5 years) was used to each case. Here, we report on malignant cases including all available controls. Exposures on e.g. use of mobile phones and cordless phones were assessed by a self-administered questionnaire. Unconditional logistic regression analysis was performed, adjusting for age, gender, year of diagnosis and socio-economic index using the whole control sample. Of the cases with a malignant brain tumour, 87% (n=593) participated, and 85% (n=1,368) of controls in the whole study answered the questionnaire. *The odds ratio (OR) for mobile phone use of the analogue type was 1.8, 95% confidence interval (CI)=1.04-3.3, increasing with >25 years of latency (time since first exposure) to an OR=3.3, 95% CI=1.6-6.9. Digital 2G mobile phone use rendered an OR=1.6, 95% CI=0.996-2.7, increasing with latency >15-20 years to an OR=2.1, 95% CI=1.2-3.6. The results for cordless phone use were OR=1.7, 95% CI=1.1-2.9, and, for latency of 15-20 years, the OR=2.1, 95% CI=1.2-3.8. Few participants had used a cordless phone for >20-25 years.* Digital type of wireless phones (2G and 3G mobile phones, cordless phones) gave increased risk with latency >1-5 years, then a lower risk in the following latency groups, but again increasing risk with latency >15-20 years. Ipsilateral use resulted in a higher risk than contralateral mobile and cordless phone use. Higher ORs were calculated for tumours in the temporal and overlapping lobes. Using the meningioma cases in the same study as reference entity gave somewhat higher ORs indicating that the results were unlikely to be explained by recall or observational bias. This study confirmed previous results of an association between mobile and cordless phone use and malignant brain tumours. These findings provide support for the hypothesis that RF-EMFs play a role both in the initiation and promotion stages of carcinogenesis.

5. **Hardell L, Carlberg M. Using the Hill viewpoints from 1965 for evaluating strengths of evidence of the risk for brain tumors associated with use of mobile and cordless phones. *Rev Environ Health* 2013;38:97-106. doi: 10.1515/reveh-2013-0006.**

Abstract

BACKGROUND: Wireless phones, i.e., mobile phones and cordless phones, emit radiofrequency electromagnetic fields (RF-EMF) when used. An increased risk of brain tumors is a major concern. The International Agency for Research on Cancer (IARC) at the World Health Organization (WHO) evaluated the carcinogenic effect to humans from RF-EMF in May 2011. It was concluded that RF-EMF is a group 2B, i.e., a "possible", human carcinogen. Bradford Hill gave a presidential address at the British Royal Society of Medicine in 1965 on the association or causation that provides a helpful framework for evaluation of the brain tumor risk from RF-EMF.

METHODS: All nine issues on causation according to Hill were evaluated. Regarding wireless phones, only studies with long-term use were included. In addition, laboratory studies and data on the incidence of brain tumors were considered.

RESULTS: *The criteria on strength, consistency, specificity, temporality, and biologic gradient for evidence of increased risk for glioma and acoustic neuroma were fulfilled. Additional evidence came from plausibility and analogy based on laboratory studies. Regarding coherence, several studies show increasing incidence of brain tumors, especially in the most exposed area. Support for the experiment came from antioxidants that can alleviate the generation of reactive oxygen species involved in biologic effects, although a direct mechanism for brain tumor carcinogenesis has not been shown. In addition, the finding of no increased risk for brain tumors in subjects using the mobile phone only in a car with an external antenna is supportive evidence. Hill did not consider all the needed nine viewpoints to be essential requirements.*

CONCLUSION: Based on the Hill criteria, *glioma and acoustic neuroma should be considered to be caused by RF-EMF emissions from wireless phones and regarded as carcinogenic to humans, classifying it as group 1 according to the IARC classification. Current guidelines for exposure need to be urgently revised.*

SUMMARY

During 2013 our research group has published results from further studies on brain tumour risk associated with use of mobile and/or cordless desktop phones. We published data on tumour risk for use of these devices during 20 years or more. Clearly we find again an increased risk for malignant brain tumours including the most common type glioma ('brain cancer'). We find also increased risk of acoustic neuroma, a benign tumour of the hearing nerve (number VIII). These tumours usually lead to hearing problems (deafness), tinnitus and dizziness although rarely lethal. Still we find no clear increased risk for meningioma, even after 20 years use of the mobile phone.

Especially worrying is that we find highest risk for glioma and acoustic neuroma in subjects who started use of the wireless phone before the age of 20 years. We have also found that the prognosis of glioma (astrocytoma grade IV) is worse the longer time one has used the wireless phone. That means that long-term use shortens the survival.

Further research has thus strengthened the evidence in support of an increased risk of malignant brain tumours and acoustic neuroma associated with use of mobile phones. Based on the latest findings and using the so called Hill viewpoints from the 1960's exposure to RF-EMF from mobile phones may now be classified as a human cancer causing agent, Group 1, according to the definitions used by IARC.

It is unfortunate that SCENIHR has disregarded these findings and instead relies heavily on the much criticised Danish cohort study on mobile phone users with poor exposure data. We have discussed the many shortcomings in that study, see **Söderqvist F, Carlberg M, Hardell L. Review of four publications on the Danish cohort study on mobile phone subscribers and risk of brain tumors. *Reviews Environmental Health*. 2012; 27: 51-58.** SCENIHR lacks reference to our publication and accordingly also critical comments on the

Danish cohort study. The same lack of critical review applies to the study by Benson et al, included in SCENIHR, but without acknowledge of the limitations in that study.

The CEFALO study on brain tumour risk in children is included in SCENIHR, however without a critical review of the study. For example use of cordless phones was assessed only during the 3 first years of use, a most peculiar definition. Our review of that study is omitted from SCENIHR, see **Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. Childhood brain tumour risk and its association with wireless phones: a commentary. *Environmental Health*. 2011; 10: 106.**

In addition to the Danish cohort study and the UK study by Benson et al SCENIHR relies heavily on time trend analyses. However the conclusion by IARC in the 2011 evaluation was that: "*Time-trend analyses did not show an increased rate of brain tumours after the increase in mobile phone use. However, these studies have substantial limitations because most of the analyses examined trends until the early 2000s only. Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins, or if phone use only affects a small proportion of cases—eg, the most heavily exposed, or a subset of brain tumours.*" See **Baan R, Grosse Y, Lauby-Secretan B, et al. Carcinogenicity of radiofrequency electromagnetic fields. *Lancet Oncology*. 2011; 12: 624-626.**

In our publication number 4 above, we presented restricted cubic spline plot of the relationship between latency of wireless phone use and malignant brain tumours, see figure below. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Obviously the latency is 20+ years for malignant brain tumours according to these results. Thus, it confirms the conclusion by IARC on incidence data that "*Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins*"; in fact it may even be two decades based on our data. Our results are also in agreement with de Vocht et al "*According to these ecological results the latency period is at least 11-12 years, but probably more than 20 years.*" See **de Vocht F, Hannam K, Buchan I. Environmental risk factors for cancers of the brain and nervous system: the use of ecological data to generate hypotheses. *Occup Environ Med* 2013; 70: 349-356.**

In summary, the preliminary SCENIHR conclusion that glioma risk is weaker now is not scientifically justified. The only way that conclusion could be reached by SCENIHR is to exclude critical studies that present evidence to the contrary, i.e. studies that report the risk of glioma (and acoustic neuroma) is stronger now than in 2009. Including our studies would give different conclusions supported by critical review of the limitations in cohort studies and incidence data. The Preliminary Opinion should be sent back to the Committee for new evaluation of the scientific data, and should integrate the results of these published data.

1) Hardell L, Carlberg M, Hansson Mild K. Use of mobile phones and cordless phones is associated with increased risk for glioma and acoustic neuroma. *Pathophysiology* 2013;20:85-110. Epub 2012 Dec 21.

2. Carlberg M, Söderqvist F, Hansson Mild K, Hardell L. Meningioma patients diagnosed 2007-2009 and the association with use of mobile and cordless phones, *Environ. Health* 2013;12:60, doi:10.1186/1476-069X-12-60. Epub Jul 19, 2013

3. Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Pooled analysis of case-control studies on acoustic neuroma diagnosed 1997-2003 and 2007-2009 and use of mobile and cordless phones. *Int J Oncol*. 2013;43:1036-1044. Epub 2013 Jul 22.

4. Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24
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Respectfully submitted

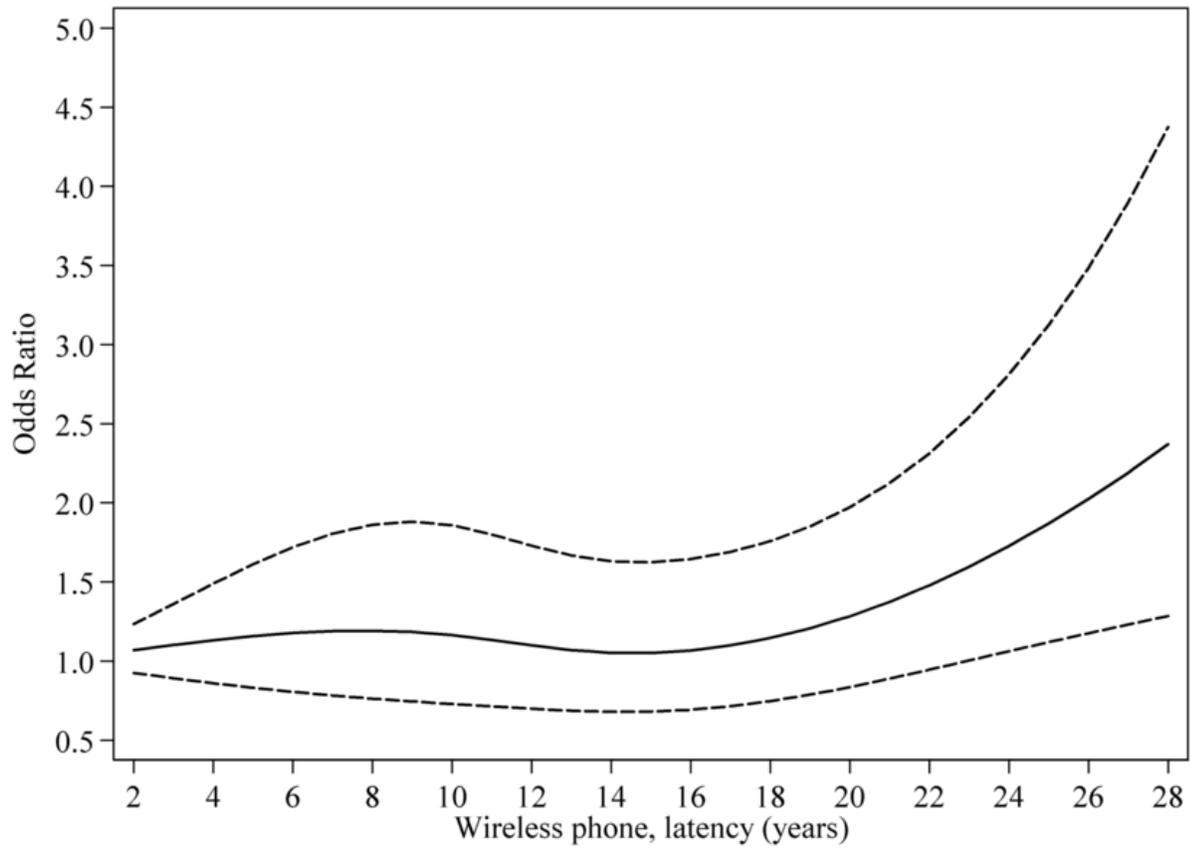
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Attached Figure

In our publication **Hardell L, Carlberg M, Söderqvist F, Hansson Mild K. Case-control study of the association between malignant brain tumors diagnosed 2007-2009 and mobile and cordless phone use. *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24**, we presented restricted cubic spline plot (see figure below) of the relationship between latency of wireless phone use and malignant brain tumours, see figure below. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Obviously the latency is 20+ years for malignant brain tumours according to these results. Thus, it confirms the conclusion by IARC on incidence data that *“Such analyses are uninformative if excess risk only manifests more than a decade after phone use begins”*; in fact it may even be two decades based on our data. Our results are also in agreement with de Vocht et al *“According to these ecological results the latency period is at least 11-12 years, but probably more than 20 years.”*



Restricted cubic spline plot of the relationship between latency of wireless phones and malignant brain tumours. The solid line indicates the OR estimate and the broken lines represent the 95% CI. Adjustment was made for age at diagnosis, gender, SEI-code and year of diagnosis. Population based controls were used. (Hardell et al *Int J Oncol.* 2013;43:1833-1845. Epub 2013 Sep 24)

Exhibit C: Reference List for Important Fertility and Reproduction Papers

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Exhibit B-1
RF Color Charts for Fertility and Reproduction Studies with Exposures
PUBLISHED STUDIES RELEVANT TO SCENIHR REVIEW OF EMF –
FERTILITY AND REPRODUCTION
C. SAGE, BIOINITIATIVE WORKING GROUP, APRIL 2014

Agarwal A, Deepinder F, Sharma RK, Ranga G, Li J. 2008. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. *Fertil Steril.* 89(1):124-8.

1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human males) in dose-dependent manner.	Agarwal, 2008
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Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. 2009. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertil Steril.* 92(4) 1318-1325.

1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human males) in dose-dependent manner.	Agarwal, 2008
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Aitken RJ, Bennetts LE, Sawyer D, Wiklendt AM, King BV. 2005 Impact of radio frequency electromagnetic radiation on DNA integrity in the male germline 28:171-179.

0.09 W/Kg	900 MHz study of mice for 7 days, 12-hr per day (whole-body) resulted in significant effect on mitochondria and genome stability	Aitken, 2005
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Aldad TS Gan G Gao XB Taylor HS. 2012. Fetal radiofrequency radiation exposure from 800-1900 MHz rated cellular telephones affects neurodevelopment and behavior in mice. *Sci. Rep.* 2, 312. DOI: 10.1038/srep00312

0.0003 - 0.06 W/Kg	Neurobehavioral disorders in offspring of pregnant mice exposed in utero to cell phones - dose-response impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. Hyperactivity and impaired memory function in offspring. Altered brain development.	Aldad, 2012
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Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. 2012 Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. *J Pediatr Urol.* [Epub ahead of print]

0.091 W/Kg	Wireless internet 2400 MHz, 24-hrs per day/20 weeks increased DNA damage and reduced DNA repair; levels below 802.11 g Authors say "findings raise questions about safety of radiofrequency exposure from Wi-Fi internet access devices for growing organisms of reproductive age, with a potential effect on fertility and integrity of germ cells" (male germ cells are the reproductive cells=sperm)	Atasoy, 2012
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Avendano C, Mata A, Sanchez Sarmiento CA, Doncei GF. 2012. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. *Fertility and Sterility.* American Society for Reproductive Medicine, Published by Elsevier Inc. doi:10.1016/j.fertnstert.2011.10.012.

0.5 - 1.0 $\mu\text{W}/\text{cm}^2$	Wi-Fi level laptop exposure for 4-hr resulted in decrease in sperm viability, DNA fragmentation with sperm samples placed in petri dishes under a laptop connected via WI-FI to the internet.	Avendano, 2012
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Behari J, Kesari KK 2006. Effects of microwave radiations on reproductive system of male rats. Embryo Talk 1 (Suppl.1):81-5.

0.00034 $\mu\text{W}/\text{cm}^2$	Chronic exposure to mobile phone pulsed RF significantly reduced sperm count,	Behari, 2006
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Dasdag, S et al, 1999. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. Urological Research 27(3):219-223.

0.141 W/Kg	Structural changes in testes - smaller diameter of seminiferous	Dasdag, 1999
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De Iuliis GN, Newey RJ, King BV, Aitken RJ. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4(7):e6446.

0.4 - 1.0 W/Kg	One 6-hr exposure to 1800 MHz cell phone radiation in human sperm cells caused a significant dose response and reduced sperm motility and viability; reactive oxygen species levels were significantly increased after exposure to 1.0 W/Kg; study confirms detrimental effects of RF/MW to human sperm. The authors conclude "(T)hese findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring."	De Iuliis, 2009
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De Iuliis GN, Newey RJ, King BV, Aitken RJ. 2009. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4(7):e6446.

1.0 W/Kg	Human semen degraded by exposure to cell phone frequency RF increased free-radical damage.	De Iuliis, 2009
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Forgács Z, Somosy Z, Kubinyi G, Bakos J, Hudák A, Surján A, Thuróczy G. Effect of whole-body 1800 MHz GSM-like microwave exposure on testicular steroidogenesis and histology in mice. Reprod Toxicol. 2006; Jul;22(1):111-7.

45 $\mu\text{W}/\text{cm}^2$	Pulsed RFR affected serum testosterone levels in mice	Forgacs, 2006
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A.F. Fragopoulou, et al., Cranial and postcranial skeletal variations induced in mouse embryos by mobile phone radiation, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.10.002

0.6 - 0.9 W/Kg	Mouse embryos develop fragile cranial bones from in utero 900 MHz The authors say "(O)ur results clearly show that even modest exposure (e.g., 6 min daily for 21 days" is sufficient to interfere with the normal mouse developmental process"	Fragopoulou, 2009
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Gul A, Celebi H, Ugras S. The effects of microwaves emitted by cellular phones on ovarian follicles in rats. Archives of Gynecology

< 1.0 W/Kg	Rats exposed to mobile phone radiation on STANDBY ONLY for 11-hr 45-min plus 15-min TRANSMIT mode; 2 times per day for 21 days showed decreased number of ovarian follicles in pups born to these pregnant rats. The authors conclude "the decreased number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries."	Gul, 2009
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Kumar S Behari J Sisodia R. 2012. Impact of Microwave at X-Band in the aetiology of male infertility. *Electromagnetic Biology and Medicine*, 31(3): 223–232. online DOI: 10.3109/15368378.2012.700293.

0.014 W/Kg	Sperm damage from oxidative stress and lowered melatonin levels resulted from 2-hr per day/45 days exposure to 10 GHz.	Kumar, 2012
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Magras, IN & Zenos, TD, 1997. RF Radiation-induced changes in the prenatal development of mice. *Bioelectromagnetics* 18:455-461.

0.168 - 1.053 uW/cm2	Irreversible infertility in mice after 5 generations of exposure to RFR from an 'antenna park'	Magras & Zenos, 1997
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Navakatikian, MA & Tomashevskaya, LA, 1994 Phasic behavioral and endocrine effects of microwaves of nonthermal intensity. In: *Biological Effects of Electric and Magnetic Fields*, Volume 1, Carpenter, DO, (Ed.) Academic Press, Inc., San Diego, CA., pp. 333-342.

100 uW/cm2	A 24.3% drop in testosterone after 6 hours of CW RFR exposure	Navakatikian, 1994
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Otitoloju AA, Obe IA, Adewale OA, Otubanjo OA, Osunkalu VO. 2010. Preliminary study on the induction of sperm head abnormalities in mice, *Mus musculus*, exposed to radiofrequency radiations from global system for mobile communication base stations. *Bulletin of Environmental Contamination and Toxicology* 84(1):51-4.

0.07 - 0.1 uW/cm2	Sperm head abnormalities in mice exposed for 6-months to base station level RF/MW. Sperm head abnormalities occurred in 39% to 46% exposed mice (only 2% in controls) abnormalities was also found to be dose dependent. The implications of the pin-head and banana-shaped sperm head. The occurrence of sperm head observed increase occurrence of sperm head abnormalities on the reproductive health of humans living in close proximity to GSM base stations were discussed."	Otitoloju, 2010
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Panagopoulos DJ. 2012. Effect of microwave exposure on the ovarian development of *Drosophila melanogaster*. *Cell Biochem Biophys*. 63(2):121-132.

0.795 W/Kg	GSM 900 MHz, 217 Hz significantly decreases ovarian development and size of ovaries, due to DNA damage and premature cell death of nurse cells and follicles in ovaries (that nourish egg cells)	Panagopoulos, 2012
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Salama N, Kishimoto T, Kanayama HO. Effects of exposure to a mobile phone on testicular function and structure in adult rabbit. *International Journal of Andrology* 2010;33(1):88-94.

0.43 W/Kg	Significant decrease in sperm mobility; drop in sperm concentration; and decrease in seminiferous tubules at 800 MHz, 8-hr/day, 12 weeks, with mobile phone radiation level on STANDBY ONLY (in rabbits)	Salama, 2008
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Somozy, Z et al, 1993. Effects of modulated and continuous microwave irradiation on pyroantimonate precipitable calcium content junctional complex of mouse small intestine. *Scanning Microsc* 7(4): 1255-1261

60 uW/cm²

RFR caused structural changes in cells of mouse embryos

Somozy, 1991

Chart References

Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R (2009). Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. *Fertil Steril*, 92(4), 1318-25.

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March 24, 2014

**Exhibit D: An Update on Neurological Effects of Nonionizing
Electromagnetic Fields by Prof. Henry Lai, PhD,
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Introduction

Neurological effects are caused by changes in the nervous system. Factors that act directly or indirectly on the nervous system causing morphological, chemical, or electrical changes in the nervous system can lead to neurological effects. The final manifestation of these effects can be seen in psychological changes, e.g., memory, learning and perception. The nervous system is an electrical organ. Thus, it should not be surprising that exposure to electromagnetic fields could lead to neurological changes. Morphological, chemical, electrical, and behavioral changes have been reported in animals and cells after exposure to nonionizing electromagnetic fields (EMF) across a range of frequencies. The consequences of physiological changes in the nervous system are very difficult to assess. We don't quite understand how the nervous system functions and reacts to external perturbations. The highly flexible nervous system could easily compensate for external disturbances. On the other hand, the consequence of neural perturbation is also situation-dependent. An EMF-induced change in brain electrical activity, for instance, could lead to different consequences depending on whether a person is watching TV or driving a car.

The following is a summary of the research literature on the neurological effects of EMF exposure published between 2007-2014. The literature on radiofrequency and extremely-low frequency EMFs are placed in two separate sections. Each section has a discussion and a list of publications with abstracts. Summary sentences in the abstracts are underlined for reader convenience. Where additional information is relevant, some earlier papers, or papers not specifically related to neurological effects, are also included with citations contained within the discussion.

In this paper, as in the update paper on genetic effects, analyses show that there are more publications showing effects than no effects with the recent neurological literature. With E representing a biological effect, and NE representing no biological effects, the recent literature finds RFR-neurological effects at: E=144 publications (68%); NE=67 publications (32%); and ELF-neurological effects at: E=95 (90%); NE=10 (10%).

Section 1: Neurological effects of Radiofrequency Radiation (RFR) (2007-2012)

Discussion

- (1) There are many new studies on human subjects. Many of them are on changes in brain electrical activities after acute exposure to cell phone radiation. Bak et al (2010) reported effects on event-related potentials. Maganioti et al. (2010) further reported that RFR affected the gender-specific components of event-related potentials (see also Hountala et al., 2008). Croft et al (2008) reported changes of the alpha-wave power of EEG. The same authors (Croft et al., 2010) further reported that effects differed between various new cell phone transmission systems, which have different signaling characteristics. They observed effects after exposure to second generation (2G), but not third generation (3G) radiation, whereas Leung et al. (2011) found similar EEG effects with both 2G and 3G radiations. Lustenberger et al. (2013) found increased slow-wave activity in humans during exposure to pulse-modulated RF EMF toward the end of the sleep period. Vecchio and associates reported that cell phone RFR affected EEG and the spread of neural synchronization conveyed by interhemispherical functional coupling of EEG rhythms (Vecchio et al., 2007) and enhanced human cortical neural efficiency (Vecchio et al., 2012a). An interesting finding is that RFR could interact with the activity of brain epileptic foci in epileptic patients (Tombini et al., 2012; Vecchio et al., 2012b). However, no significant effect on EEG was reported by Parentos et al. (2007) or Trunk et al. (2012), and Kleinlogel et al. (2008 a, b) also reported no significant effects on resting EEG and event-related potentials in humans after exposure to cell phone RFR. Furthermore, Krause et al. (2007) reported no significant effect of cell phone radiation on brain oscillatory activity, and Inomata-Terada et al. (2007) concluded that cell phone radiation does not affect the electrical activity of the motor cortex.

- (2) There are studies on the interaction of cell phone radiation on EEG during sleep. Changes in sleep EEG have been reported by Hung et al. (2007), Regel et al. (2007), Lowden et al (2011), Schmid et al. (2012), and Loughran et al. (2012), whereas, no significant effect was reported by Fritzer et al (2007), Mohler et al. (2010, 2012) and Nakatani-Enomoto et al. (2013). Loughran et al. (2012) provided an interesting conclusion in their paper: "These results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that "previous negative results are not strong evidence for a lack of an effect..." Increase in REM sleep was reported by Pelletier et al. (2012) in developing rats after chronic exposure.

Mohammed et al. (2013) reported a disturbance in REM sleep EEG in the rat after long term exposure (1 hr/day for 1 month) to a 900-MHz modulated RFR.

(3) With these electrophysiological changes in the brain, what behavioral effects have been reported? The outcomes are summarized in the tables below. The animal studies are mostly studies on rodents (i.e., rat and mouse).

Human studies that showed behavioral effects:

	Behavior studies/results	Exposure duration
de Tommaso et al. (2009)	Reduction in behavioral arousal	10 min
Hung et al. (2007)	Sleep latency	30 min
Leung et al. (2011)	Cognitive functions	10 min
Luria et al. (2009)	Spatial working memory (In a subsequent study (Hareuveny et al., 2011), the authors indicated that some of the effects observed may not be related to RFR exposure.)	60 min
Lustenberger et al. (2013)	Sleep-dependent motor-task performance improvement	All-night
Redmayne et al. (2013)	Well-being	Use of cellphone and cordless phone
Regel et al. (2007)	Cognitive functions	30 min
Thomas et al. (2010b)	Overall behavioral problems in adolescents	
Vecchio et al. (2012b)	Enhanced cognitive-motor processes	45 min
Vecsei et al. (2013)	Thermal pain threshold	30 min
Wiholm et al. (2009)	'Virtual' spatial navigation	150 min

	task	
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Human studies that did not show behavioral effects:

	Behavior studies/results	Exposure duration
Cinel et al. (2007)	Order threshold task	40 min
Cinel et al. (2008)	Subjective symptoms	40 min
Curcio et al. (2008)	Reaction time task, sequential figure tapping task	3 x 15 min
Curcio et al. (2012)	Somatosensory task	40 min
Danker-Hopfe et al. (2011)	Effect on sleep	
Eltiti et al. (2009)	Cognitive functions	50 min
Fritzer et al. (2007)	Sleep and cognitive functions	During sleep
Haarala et al. (2007)	Cognitive functions	90 min
Irlenbusch et al. (2007)	Visual discrimination threshold	30 min
Kleinlogel et al. (2008a)	Well being	30 min
Loughran et al. (2013)	Cognitive effects and EEG	30-60 min
Mohler et al. (2010, 2012)	Effect on sleep	
Nakatani-Enomoto et al. (2013)	Effect on sleep	3 hr
Riddervold et al. (2008)	Trail making B test	45 min
Sauter et al. (2011)	Cognitive functions	7 hr 15 min in two episodes
Schmid et al. (2012a)	Cognitive functions	30 min
Schmid et al. (2012b)	Cognitive functions	30 min
Unterlechner et al. (2008)	attention	90 min
Wallace et al. (2012)	Cognitive functions	10- 50 min (whole body)

		exposure)
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Animal studies that showed behavioral effects:

	Behavior studies/results	Exposure duration
Aldad et al. (2012)	Hyperactive, impaired memory	In utero
Arendash et al. (2010, 2012)	Improved cognitive behavior	Daily, 2-6 months
Bouji et al. (2012)	Contextual emotional behavior deficit	15 min
Cammaerts et al. (2012)	Olfactory and/or visual memory deficit in ants	
Cammaerts et al. (2013)	Food collection behavior of ants	180 hr
Daniels et al. (2009)	Decreased motor activity	
Deshmukh et al. (2013)	Cognitive functions	2 hr/day, 30 days
Fragopoulou et al. (2010)	Spatial memory deficit	2 hr/day, 4 days
Hao et al. (2012)	Learning and memory deficit	6 hr/day, 5 days/wk, 10 wk
İkinci et al. (2013)	Learning behavior deficit	Prenatal exposure
Júnior et al. (2014)	Stress behavioral patterns	25 sec every 2 min for 3 days
Kumar et al. (2009)	hypoactivity	50 missed call/day, 4 wk
Kumlin et al. (2007)	Improved learning and memory	2 hr/day, 5 days/wk, 5 wk
Lu et al. (2012)	Spatial memory deficit	3 hr/day, 30 days
Maaroufi et al. (2013)	Spatial learning and memory deficit	1 hr/day, 21 days
Mathur (2008)	Analgesic effect	2 hr/day, 45 days

Megha et al. (2012)	Cognitive functions	2 hr/day, 30 days
Narayanan et al. (2009)	Learning deficit	50 missed call/day, 4 wk
Narayanan et al. (2010)	Passive avoidance deficit	50 missed call/day, 4 wk
Narayanan et al. (2012)	Elevated plus maze-emotionality test	28 days
Nittby et al. (2008)	Reduced memory functions	2 hr/wk, 55 wk
Ntzouni et al. (2011)	Non-spatial memory deficit	90 min/day, 17 days
Ntzouni et al. (2013)	Spatial and non-spatial memory deficit	90 min/day, 66-148 days
Odacı et al. (2013)	Motor function	Prenatal exposure
Pelletier et al. (2012)	Food intake increase	5 weeks
Qin et al. (2014)	Learning and memory deficits	2 hr/day, 30 days
Razavinasab et al. (2014)	Learning and memory deficits	In utero
Sarapultseva et al. (2013)	Motor activity in protozoa	0.05-10 hr
Sharma et al. (2013)	Spatial memory deficit	2 hr/day, 30 days
Sokolovic et al. (2012)	Anxiety-related behavior	4 hr/day for 20, 40, 60 days
Vácha et al. (2009)	Magnetoreception in cockroach	
Wang et al. (2013)	Spatial memory deficit	6 min

Animal studies that did not show behavioral effects:

	Behavior studies/results	Exposure duration
Ammari et al. (2008c)	spatial memory	15 min/day, 8 or 24 wk
Haghani et al. (2013)	Motor function	6 hr/day during gestation period

Almost all the animal studies reported effects, whereas more human studies reported no effects than effects. This may be caused by several possible factors: (a) Humans are less susceptible to the effects of RFR than are rodents. (b) It may be more difficult to do human than animal experiments, since it is, in general, easier to control the variables and confounding factors in an animal experiment. (c) In the animal studies, the cumulative exposure duration was generally longer and studies were carried out after exposure, whereas in the human studies, the exposure was generally one time and testing was done during exposure. This raises the question of whether the effects of RFR are cumulative. This consideration could have very important implication on real life human exposure to EMF. However, it must be pointed out that neurophysiological and behavioral changes have been reported in both animals and humans after acute (one time) exposure to RFR, and most of the EEG studies mentioned above are acute exposure experiments. (In the 2007-2013 papers listed below, see those marked '(E)' and not classified as 'CE'). (d) In the animal studies, the effects studies were mostly learning and memory functions. The hippocampus in the brain, particularly the cholinergic system, plays a major role in learning and memory functions. Various studies (2007-2013) indicated that RFR affected the activities/morphology/chemistry of the hippocampus in animals (Aboul Ezz et al., 2013; Ammari et al., 2010; Barcal et al., 2007; Baş et al., 2009, 2013; Carballo-Quintas et al., 2011; Fragopoulous et al., 2012; Hao et al., 2012; İkinci et al., 2013; Kesari et al., 2011; Lopez-Martin et al., 2009; Lu et al., 2012; Maskey et al., 2010 a,b, 2012; Narayanan et al., 2010; Ning et al., 2007; Nittby et al., 2008; Odaci et al., 2008; Razavinasab et al., 2014; Tong et al., 2013; Wang et al., 2013; Yang et al., 2012). (Reports on effects of the hippocampus can also be found in the ELF section below). As early as 1987, we have reported that RFR affected cholinergic system in the hippocampus of the rat (Lai H, Horita A, Chou CK, Guy AW. Low-level microwave irradiation affects central cholinergic activity in the rat. *J Neurochem.* 48:40-45, 1987). Thus, it is not surprising that 'learning and memory' functions are affected in the rodents by RFR. In the human studies listed above, the most common effect studied was cognitive function. Since the exposure in most of these human studies was localized in the brain, particularly in the temporal cortical area, it is questionable whether the psychological tests used were appropriate.

- (4) There are studies on the effects of cell phone radiation and the auditory system. Most research (Kwon 2009, 2010a, b; Parazzini et al., 2009; Stefanics et al., 2007, 2008) reported no effects, which seems to agree with the pre-2007 studies in this area. However, there are two reports by Kaprana et al. (2011) and Khullar et al (2013) showing effects on auditory brainstem response, two papers by Panda et al (2010, 2011) that concluded: "Long-term and intensive GSM and CDMA mobile phone use may cause damage to cochlea as well as the auditory cortex.", and a paper (Mandala et al., 2013) reporting effect on auditory-evoked cochlear

- nerve response. Maskey et al. (2013) reported chemical changes in the superior olivary complex, a neural component of the auditory system, in mice after chronic exposure to RFR. Velayutham et al. (2014) reported hearing loss in cell phone users and Sudan et al. (2013) observed weak associations between cell phone use and hearing loss in children at age 7. These effects may not be caused by the radiation.
- (5) There are several studies that showed neurological changes in humans after use of wireless devices, but those changes apparently were not caused by exposure to the radiation. Abramson et al. (2009) reported changes in cognitive functions in young adolescents. (“The accuracy of working memory was poorer, reaction time for a simple learning task shorter, associative learning response time shorter and accuracy poorer in children reporting more mobile phone voice calls”). Arns et al. (2007) observed more focused attention in frequent cell phone users, which was probably a “cognitive training effect”. Yuan et al. (2011) reported morphological changes in the brain of adolescents with “internet addiction disorder”.
- (6) There are several studies showing differential effects of different waveforms. This is an important consideration in understanding how EMF interacts with living organisms and nonthermal effects. Croft et al. (2010) reported that 2G, but not 3G, cell phone radiation affected resting EEG. Hung et al. (2007) showed that 2, 8, 217 Hz-modulated RFR differentially affected sleep. Lopez-Martin et al. (2009) reported that modulated and non-modulated RFR had different effects on gene expression in the brain. Nylund et al. (2010) found that different carrier-frequencies (900 MHz versus 1800 MHz) had different effects on protein expression. Schmid et al. (2012) concluded that “modulation frequency components (of a RFR) within a physiological range may be sufficient to induce changes in sleep EEG”. Zhang et al. (2008) reported that an intermittent exposure to RFR had a more potent effect on gene expression in the brain than a continuous exposure. Apparently, ELF-modulation plays a role on determining the biological effects of RFR. Indeed, in the following section on the neurological effects of ELF EMF, one can find many studies showing EEG and behavioral effects in animals after exposure to ELF fields (Capone et al., 2009; Carrubba et al., 2007, 2010; Cook et al., 2009; Corbacio et al., 2011; Cvetkovic and Cosic, 2009; Legros et al., 2012; Perentos et al., 2008; Ross et al., 2008; Shafiei et al.,

- 2012; Shin et al., 2007, 2011; Stevens, 2007). This is of considerable importance, since all cell phone signals are modulated by low frequency components.
- (7) In the 2007-2014 literature below on the neurological effects of RFR, there are several papers indicating that oxidative stress played a role in the effects observed: Cetin et al., 2014; Dasdag et al., 2009, 2012; Del Vecchio et al., 2009; Deshmukh et al., 2013a; Dragicevic et al., 2011; Eser et al., 2013; Gao et al., 2013; Imge et al., 2010; Jing et al., 2012; Kesari et al., 2011; Liu et al., 2011; Maaroufi et al., 2013; Megha et al., 2012; Meral et al., 2007; Nazıroğlu et al., 2012; Qin et al., 2014; Sokolovic et al., 2009; Xu et al., 2010. (Dragicevic et al. (2011) reported a decrease in mitochondrial free radical production in the hippocampus and cerebral cortex of the mouse after RFR exposure.) There was one study (Poullétier de Gannes et al, 2011) that found no significant oxidative stress in brain cells after exposure to Enhanced Data rate for GSM Evolution (EDGE) signal. Kang et al (2013) reported that “neither combined RF radiation alone nor combined RF radiation with menadione or H₂O₂ influences the intracellular ROS level in neuronal cells.” The mediating roles of cellular free radicals and oxidative status on the biological effects of EMF are worth looking into.
- (8) An important issue that has been extensively debated in the media is whether children are more vulnerable to the effect of cell phone radiation than adults? The claim that children have thinner skulls and thus absorb more energy is not valid. And the claim that a child’s head absorbs more energy from a cell phone is also debatable. It is quite possible that the pattern of energy distribution of cell phone energy absorption in the head is significantly different between a child and an adult (cf. Christ A, Kuster N. Differences in RF energy absorption in the heads of adults and children. *Bioelectromagnetics*. Suppl 7:S31-44. 2005; Christ A, Gosselin MC, Christopoulou M, Kühn S, Kuster N. Age-dependent tissue-specific exposure of cell phone users. *Phys. Med. Biol.* 55(7):1767-1783, 2010; Gandhi OP, Morgan LL, de Salles AA, Han YY, Herberman RB, Davis DL. Exposure limits: the underestimation of absorbed cell phone radiation, especially in children. *Electromagn. Biol. Med.* 31(1):34-51, 2012.). Scientific data on whether a child is biologically more vulnerable to cell phone radiation is sparse. In the 2007-2014 literature that I surveyed, there are several studies that indicate that animals (including humans) of different ages respond differently to cell phone radiation. Bouji et al. (2012) reported differences in neuro-immunity, stress, and

behavioral responses to GSM signals between 'young adult' (6 weeks-old) and 'middle age' (12 month-old) rats. Croft et al. (2010) showed that GSM signals affected certain electrical activities of the brain in young human adults (19-40 years old) but not in adolescents (13-15 years old) or elderly (55-70 years old) subjects. Leung et al. (2011) reported that performance in a cognitive test was affected by GSM signal in adolescents but not in young or old human subjects. Noor et al. (2011) reported differences in neurochemical responses to 900-MHz RFR between adult and young rats. And, Vecchio et al. (2010) found differences in brain electric activities between young and elderly human subjects responding to GSM signals. It must be pointed out that although these studies reported an age-dependent effect of cell phone radiation, they do not necessarily imply that children are more vulnerable to cell phone radiation than adults. (See also: Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. *Ecotoxicol Environ Saf.* 80:140-144, 2012.) There are several papers showing effects of exposure to RFR during perinatal periods on the development and functions of the nervous system (Aldad et al., 2012; Bas et al., 2013; Cetin et al., 2014; Divan et al., 2008, 2011, 2012; Gao et al., 2013; Haghani et al., 2013; İkinçi et al., 2013; Jing et al., 2012; Kokturk et al., 2013; Odaci et al., 2008, 2013; Ragbetli e al., 2010; Razavinasab et al., 2014; Zareen et a., 2009). The cerebellum seems to be a structure especially vulnerable to the exposure (Eser et al. 2013; Haghani et al., 2013; Kokturk et al., 2013; Ragbetli e al., 2010).

(9) In many of these studies, a cell phone was used in the exposure of animals and humans. But information on how the cell phone was activated, in many instances, was not provided. Thus, the amount of energy deposited in the body was not known. Some studies used the phone in 'stand-by' mode. Kjell Mild and his associates reported that when a stationary cell phone is on 'stand-by' mode, it actually infrequently emits a very small amount of energy (Mild KH, Andersen JB, Pedersen GF. Is there any exposure from a mobile phone in stand-by mode? *Electromagn Biol Med.* 31(1):52-56, 2012).

(10) I think that a few words should be said about 'thermal' and 'nonthermal' effects. It is not easy to conclude that an RFR effect is 'nonthermal', because of the uneven distribution of the energy in the body. On the other hand, it is also not easy to prove that an effect is 'thermal'. There is an important criterion for the proof of 'nonthermal' effect. It is 'modulation effect'. If you expose an animal

or cells at the same frequency and SAR (thus, the same distribution and amount of energy) but at different modulations (i.e., energy is delivered with different time sequences) and produce different effects, then it is good proof of a nonthermal effect. Most studies do not include different modulations. Thus, the effects reported by these studies cannot be concluded as 'nonthermal'. There are some studies, however, that reported different biological effects with RFRs of the same frequency and intensity but different modulations (see point #6 above and the section on 'genetic effects', and some of my earlier papers). From these; I would conclude that nonthermal effects probably exist. Another important argument for EMF nonthermal effects is that low-level ELF-EMF can produce biological effects. The energy carried by ELF-EMF is very small and thermal effect is unlikely. (High intensity ELF-EMF can produce electric currents in the body and possibly heating.) The 'thermal/nonthermal' distinction is purely a scientific question. In public exposure policy, we only need to know at what level of exposure an effect occurs. Exposure guideline should be set based on it, and it doesn't matter whether the effect is thermal or nonthermal.

Section 2: Neurological effects of extremely low frequency electromagnetic fields (ELF EMF) (2007-2012)

Discussion

The following is a summary of the research literature on the neurological effects of ELF EMF published in 2007-2014. (In most studies, even only magnetic field was mentioned; there was no explicit statement that electric fields had been eliminated. In most ELF EMF exposure systems used in laboratory system, electric fields were also generated unless grounding was done. Thus, cells or animals were actually exposed to both magnetic and electric fields.)

1. Neurotransmitters are chemicals that carry (transmit) signals from one nerve cell to another. Neurotransmitters are released from one nerve cell and react with molecules called receptors on another nerve cell. The reaction alters the activity of the second nerve cell. Activities in nerve cell could also change the properties of these receptors (mainly by changing the concentration or the affinity of the receptors to neurotransmitters). In the updated EMF literature, all the studies are on the effects of ELF EMF exposure on neurotransmitter receptors. Manikonda et al. (2007) reported effects of chronic ELF EMF exposure on NMDA receptors in the hippocampus of the rat. Salunke et al. (2013) reported that ELF EMF-induced anxiety in the rat involved NMDA receptors in the brain. There is a report on effects of magnetic field serotonin and dopamine receptors in the

brain of the rat (Janac et al., 2009). Changes in a subtypes of serotonin receptors 5HT(2A) in the prefrontal cortex was reported. However, Masuda et al. (2011) reported that another types of serotonin receptor 5HT (1B) was not significantly affected after magnetic field exposure in an in vitro experiment. The research were trying to replicate two experiments carried out previously showing magnetic field exposure affected 5HT(1B) receptor. Some of the co-authors of the Musuda study were actually co-authors of one of these earlier studies. However, the 5HT(2A) receptors , particularly in the frontal cortex, are believed to be related to the psychiatric syndromes of depression in humans. Kitaoka et al. (2013) and Szemerszky et al. (2010) did report depression-like behavior in mice and rats, respectively, after chronic exposure to magnetic fields. There are two reports on dopamine receptors. Shin et al. (2007, 2011) reported an increase in D-1 dopamine receptors and activity in the striatum of the rat after magnetic field exposure. Dopamine in the striatum is involved in Parkinson's disease. Wang et al. (2008) reported that ELF magnetic fields potentiated morphine-induced decrease in D-2 dopamine receptors. The implication of these data is not readily clear. Both D-1 and D-2 dopamine receptors in the brain are involved in depression and drug addiction. There is one study on the cholinergic system. Ravera et al. (2010) reported changes in the enzyme acetylcholinesterase in cell membrane isolated from the cerebellum after magnetic field exposure. Interesting, these researchers also reported 'frequency window' effects in their experiment. Window effects, i.e., effects are observed at a certain range(s) of EMF frequency or intensity, were first reported by Ross Adey and Susan Bawin and Carl Blackman in the 1980s. A recently study by Fournier et al. (2012) reported an 'intensity window' effect of ELF magnetic field on neurodevelopment in the rat. The cholinergic systems in the brain play a major role in learning and memory functions. There were a series of studies carried out more than a decade ago showing effects of ELF magnetic field on the cholinergic systems, e.g., Lai and Carino (1999) (60-Hz magnetic field and central cholinergic activity: effects of exposure intensity and duration. *Bioelectromagnetics* 20:284-289, 1999). Not many studies have been carried out in recent years to further investigate the effects of EMF on this important neurological function.

2. Behavioral effects of ELF EMF have been further substantiated in recent research. These included: changes in locomotor activity (Balassa et al., 2009; Dimitrijevic et al., 2014; Janac et al., 2012; Legros et al., 2012; Raus et al., 2012b; Shin et al., 2007, 2011; Todorovic et al., 2012), learning and memory functions (Che et al., 2007; Corbacio et al., 2011; Cui et al., 2012; Duan et al., 2013;

- Fournier et al., 2012; Fu et al., 2008; Harakawa et al., 2008; He et al., 2011; Liu et al., 2008b; Sun et al., 2010), anxiety (Balassa et al., 2009; He et al., 2011; Korpinar et al., 2012; Liu et al., 2008a; Salunke et al., 2013); depression-like behavior (Kitaoka et al., 2013; Szemerszky et al., 2011), perception (Ross et al., 2008), cognitive dysfunction (Davanipour et al., 2014), emotional state (Stevens, 2007), sleep onset (Hung et al., 2007), and comb building in hornets (Ishay et al., 2007). Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.
3. In some of these observed neurological effects, oxidative changes (free radicals) again seemed to play a role (Akdag et al., 2010, 2013; Akpinar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Increase in free radicals causes cellular damages. Most of these effects are changes in enzymes involved in maintenance of oxidative balance in cells. A paper by Falone et al. (2008) reported an interesting finding. The researchers observed that, after magnetic field exposure, the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Thus, aging may make an individual more susceptible to the detrimental effects of ELF EMF. There are other factors that could affect an animal's response to ELF EMF. Janac et al. (2012) reported age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo (in the egg) exposure to ELF EMF, chicks showed memory deficit only when they were under stress. Indeed, Lahijani et al. (2011) reported histological changes in the brain of chicks exposed to ELF EMF in ovo.
 4. The possible medical applications of ELF EMF should be given more attention. Several studies indicate that ELF EMF could enhance recovery of functions after nervous system damage and have protective effects against development of neurodegenerative diseases. Cuccurazzu et al. (2010) reported an ELF EMF-induced neurogenesis and repair of the nervous system after damage. Kumar et al. (2010) and Das et al. (2012) showed an enhanced restoration of functions after spinal injury in the rat. Kumar et al. (2013) further showed that ELF EMF exposure restored spinal cord injury-induced tonic pain and changes in

neurotransmitter concentrations in the brain of the rat. Maestú et al. (2013) reported improvement in pain sensation in fibromyalgia patients after magnetic field stimulation. A possible beneficial effect on cerebral ischemia has been reported by Rauš Balind et al. (2014). Piacentini et al. (2008) reported a promotion of neural differentiation by ELF EMF. Kim et al. (2013) and Bai et al. (2013) reported stimulation by ELF EMF on neural differentiation of stem cells. Effects on stem cells and hippocampal neurogenesis also have been reported by Podda et al. (2013) and Leone et al. (2014). Protective effects of ELF EMF have been reported by Raus et al (2012a, b) after cerebral ischemia, Tassel et al. (2012a, b) on the development of Huntington's Disease, and Manjhi et al. (2013) on spinal cord injury induced osteoporosis. Furthermore, Cvetkovic et al. (2009) reported alteration of EEG by application of certain frequencies of magnetic fields. This may be useful in the treatment of certain neurological disorders such as sleep and psychiatric disorders. Static magnetic field has been shown by Wang et al. (2010) to act like an anti-Parkinson drug. Static magnetic field also has been shown to have antiangiogenesis property (Wang Z, Yang P, Xu H, Qian A, Hu L, Shang P. Inhibitory effects of a gradient static magnetic field on normal angiogenesis. *Bioelectromagnetics*. 30(6):446-453, 2009), which can be translated into an anticancer activity. Use of ELF EMF for cancer treatment has been extensively investigated. There is a study showed that pulsed electromagnetic fields turned on adenosine receptors in brain cancer cells that inhibit cancer growth (Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A₃ adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells. *PLoS One* 7(6):e39317, 2012). Interesting, this effect was not observed when normal brain cells were exposed to magnetic field. The waveform of the fields may play an important role in the effect produced. There are several studies on pulsed (instead of sinusoidal) magnetic fields (Aldinucci et al., 2009; Capone et al., 2009; Cook et al. 2009; Glover et al., 2009) and complex fields (Ross et al., 2008). It has been speculated that intermittent EMF or fields that have a transient nature could be more biologically potent than constant fields. The conditions and parameters of the fields that could produce either detrimental or beneficial effects need further investigation. Furthermore, it is still not clear whether acute (one time) exposure would elicit effects different from chronic/repeated exposure. In the 2007-2012 literature, there are many studies investigated the effects of chronic/repeated exposure. The study by Liu et al. (2008a) indicates that duration of exposure could be an important factor.

5. The majority of the studies used magnetic fields above 0.1 mT (1 gauss; the highest was 8 mT). The intensities are much higher than those in the public environment. Thus, caution should be taken in extrapolating the high-intensity cell and animal studies to environmental human exposure situation. Exposure to magnetic fields of 0.4 μ T (0.0004 mT) has been implication in an increased risk of childhood leukemia. And, the recent report by Li et al. (Li DK, Ferber JR, Odouli R, Quesenberry CP Jr. A Prospective Study of In-utero Exposure to Magnetic Fields and the Risk of Childhood Obesity. *Sci Rep.* 2:540, 2012) on an increased risk of obesity of humans exposed prenatally to magnetic field at 0.25 μ T (0.00025 mT). There is also a report of a blood pressure lowering effect in humans with mild-to-moderate hypertension after exposure to magnetic fields at 1 μ T (0.001mT) (Nishimura T, Tada H, Guo X, Murayama T, Teramukai S, Okano H, Yamada J, Mohri K, Fukushima M. A 1- μ T extremely low-frequency electromagnetic field vs. sham control for mild-to-moderate hypertension: a double-blind, randomized study. *Hypertens Res.* 34(3):372-377, 2011.) Apparently, humans are sensitive to magnetic field at level less than 1 μ T. There are a study by Ross et al (2008) showing 'perception' alternation in human subjects exposed to magnetic field at 10 nT (0.00001 mT), a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and a study by Stevens (2007) indicating changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 μ T (0.005 mT). These data do suggest magnetic fields at very low intensities could cause neurological effects in humans. In the 1990s, there was a series of more than 20 studies published by Reuven Sandyk showing that pulsed magnetic fields at pT (1 pT = 0.000000001 mT) levels could have therapeutic effects on Parkinson's disease and multiple sclerosis (see e.g., Sandyk R. Reversal of cognitive impairment in an elderly Parkinsonian patient by transcranial application of picotesla electromagnetic fields. *Int J Neurosci.* 91(1-2):57-68, 1997, or, search for 'Sandyk R' in the PubMed.) However, Sandyk's findings have never been independently confirmed.
6. In summary, both RF and ELF EMF affect neurological functions and behavior in animals and humans. There is no definite data showing that these effects are detrimental to human health. However, since effects have been observed, it is advisable that one should limit one's exposure to EMF.

Literature on neurological effects of radiofrequency radiation (2007-2014)

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2007 and serve as my comments to help the reader identify the significance of each paper.

(E)-effect observed; **(NE)**- no significant observed; **HU**- human study; **AS**- animal study; **CS**-cell study; **LI**- low intensity/cell tower; **CE**- chronic/repeated exposure; **BE**- behavioral effect; **DE**- developmental effect; **CC**- cellular effects; **CH**-chemical changes; **ME**- morphological effect; **PE**-physiological effect; **EE**- electrophysiological effect; **OX**-oxidative changes; **AD**- age-dependent effect; **SL**- effect on sleep; **MA**- possible medical application; **WS**- waveform specific effect; **IA**- interaction with other factors.

(E) Abdel-Rassoul G, El-Fateh OA, Salem MA, Michael A, Farahat F, El-Batanouny M, Salem E. Neurobehavioral effects among inhabitants around mobile phone base stations. Neurotoxicology. 28(2):434-440, 2007. (HU, CE, BE, LI, SL)

BACKGROUND: There is a general concern on the possible hazardous health effects of exposure to radiofrequency electromagnetic radiations (RFR) emitted from mobile phone base station antennas on the human nervous system. AIM: To identify the possible neurobehavioral deficits among inhabitants living nearby mobile phone base stations. METHODS: A cross-sectional study was conducted on (85) inhabitants living nearby the first mobile phone station antenna in Menoufiya governorate, Egypt, 37 are living in a building under the station antenna while 48 opposite the station. A control group (80) participants were matched with the exposed for age, sex, occupation and educational level. All participants completed a structured questionnaire containing: personal, educational and medical histories; general and neurological examinations; neurobehavioral test battery (NBTB) [involving tests for visuomotor speed, problem solving, attention and memory]; in addition to Eysenck personality questionnaire (EPQ). RESULTS: The prevalence of neuropsychiatric complaints as headache (23.5%), memory changes (28.2%), dizziness (18.8%), tremors (9.4%), depressive symptoms (21.7%), and sleep disturbance (23.5%) were significantly higher among exposed inhabitants than controls: (10%), (5%), (5%), (0%), (8.8%) and (10%), respectively (P<0.05). The NBTB indicated that the exposed inhabitants exhibited a significantly lower performance than controls in one of the tests of attention and short-term auditory memory [Paced Auditory Serial Addition Test (PASAT)]. Also, the inhabitants opposite the station exhibited a lower performance in the problem solving test (block design) than those under the station. All inhabitants exhibited a better performance in the two tests of visuomotor speed (Digit symbol and Trailmaking B) and one test of attention (Trailmaking A) than controls. The last available measures of RFR emitted from the first mobile phone base station antennas in Menoufiya governorate were less than the allowable standard level. CONCLUSIONS AND RECOMMENDATIONS: Inhabitants living nearby mobile phone base stations are at risk for developing neuropsychiatric problems

and some changes in the performance of neurobehavioral functions either by facilitation or inhibition. So, revision of standard guidelines for public exposure to RER from mobile phone base station antennas and using of NBTB for regular assessment and early detection of biological effects among inhabitants around the stations are recommended.

(E) Aboul Ezz HS, Khadrawy YA, Ahmed NA, Radwan NM, El Bakry MM. The effect of pulsed electromagnetic radiation from mobile phone on the levels of monoamine neurotransmitters in four different areas of rat brain. Eur Rev Med Pharmacol Sci. 17(13):1782-1788, 2013. (AS, CE, CH)

BACKGROUND: The use of **mobile** phones is rapidly increasing all over the world. Few studies deal with the effect of electromagnetic radiation (EMR) on monoamine neurotransmitters in the different brain areas of adult rat. AIM: The aim of the present study was to investigate the effect of EMR on the concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the hippocampus, hypothalamus, midbrain and medulla oblongata of adult rats. MATERIALS AND METHODS: Adult rats were exposed daily to EMR (frequency 1800 MHz, specific absorption rate 0.843 W/kg, power density 0.02 mW/cm², modulated at 217 Hz) and sacrificed after 1, 2 and 4 months of daily EMR exposure as well as after stopping EMR for 1 month (after 4 months of daily EMR exposure). Monoamines were determined by high performance liquid chromatography coupled with fluorescence detection (HPLC-FD) using their native properties. RESULTS: The exposure to EMR resulted in significant changes in DA, NE and 5-HT in the four selected areas of adult rat brain. CONCLUSIONS: The exposure of adult rats to EMR may cause disturbances in monoamine neurotransmitters and this may underlie many of the adverse effects reported after EMR including memory, learning, and stress.

***(E) Abramson MJ, Benke GP, Dimitriadis C, Inyang IO, Sim MR, Wolfe RS, Croft RJ. Mobile telephone use is associated with changes in cognitive function in young adolescents. Bioelectromagnetics. 30(8):678-686, 2009. (HU, BE) (*Effects observed probably not caused by exposure to RFR.)**

As part of the Mobile Radiofrequency Phone Exposed Users' Study (MoRPhEUS), a cross-sectional epidemiological study examined cognitive function in secondary school students. We recruited 317, 7th grade students (144 boys, 173 girls, median age 13 years) from 20 schools around Melbourne, Australia. Participants completed an exposure questionnaire based on the Interphone study, a computerised cognitive test battery, and the Stroop colour-word test. The principal exposure metric was the total number of reported mobile phone voice calls per week. Linear regression models were fitted to cognitive test response times and accuracies. Age, gender, ethnicity, socio-economic status and handedness were fitted as covariates and standard errors were adjusted for clustering by school. The accuracy of working memory was poorer, reaction

time for a simple learning task shorter, associative learning response time shorter and accuracy poorer in children reporting more mobile phone voice calls. There were no significant relationships between exposure and signal detection, movement monitoring or estimation. The completion time for Stroop word naming tasks was longer for those reporting more mobile phone voice calls. The findings were similar for total short message service (SMS, also known as text) messages per week, suggesting these cognitive changes were unlikely due to radiofrequency (RF) exposure. Overall, mobile phone use was associated with faster and less accurate responding to higher level cognitive tasks. These behaviours may have been learned through frequent use of a mobile phone.

(NE) Ahlers MT, Ammermüller J. No influence of acute RF exposure (GSM-900, GSM-1800, and UMTS) on mouse retinal ganglion cell responses under constant temperature conditions. Bioelectromagnetics. 2013 Sep 21. doi: 10.1002/bem.21811. [Epub ahead of print] (CS, CC)

Possible non-thermal effects of radio frequency electromagnetic fields (RF-EMF) on retinal ganglion cells were studied in vitro under conditions of constant temperature. Isolated mouse retinæ were exposed to GSM-900, GSM-1800, and universal mobile telecommunication system (UMTS) RF-EMF applying specific absorption rates (SAR) of 0 (sham), 0.02, 0.2, 2, and 20 W/kg. Temperature was kept constant within ± 0.5 to 1 °C for GSM-900 and ± 0.5 °C for GSM-1800 and UMTS. Responses of retinal ganglion cells to light stimuli of three intensities (0.5, 16, and 445 lx) were recorded before, during, and up to 35 min after exposure. Experiments were performed under double-blind conditions. Changes in light responses during and after exposure were determined for each condition (RF-EMF; SAR value; light intensity) with respect to the responses before exposure, respectively. Changes were calculated using the Euclidian distance of the n-dimensional response vectors, respectively. Some changes already occurred during sham (0 W/kg) exposure, reflecting the intrinsic variability in retinal ganglion cell responses. Comparison of the distance values from sham exposure with those from actual exposure yielded no significant differences. In addition, linear regression analysis of the distance values versus SAR values yielded no consistent dependence of light response changes. From these results we conclude that RF-EMF exposure at three mobile phone frequencies (GSM-900, GSM-1800, UMTS) and SARs up to 20 W/kg has no acute effects on retinal ganglion cell responses under constant temperature conditions.

(NE) Aït-Aïssa S, de Gannes FP, Taxile M, Billaudel B, Hurtier A, Haro E, Ruffié G, Athané A, Veyret B, Lagroye I. In Situ Expression of Heat-Shock Proteins and 3-Nitrotyrosine in Brains of Young Rats Exposed to a WiFi Signal In Utero and In Early Life. Radiat Res. 2013 May 10. [Epub ahead of print] (AS, CE, CH, DE, OX)

The bioeffects of exposure to Wireless High-Fidelity (WiFi) signals on the developing nervous systems of young rodents was investigated by assessing the in vivo and in situ expression levels of three stress markers: 3-Nitrotyrosine (3-NT), an oxidative stress marker and two heat-shock proteins (Hsp25 and Hsp70). These biomarkers were measured in the brains of young rats exposed to a 2450 MHz WiFi signal by immunohistochemistry. Pregnant rats were first exposed or sham exposed to WiFi from day 6 to day 21 of gestation. In addition three newborns per litter were further exposed up to 5 weeks old. Daily 2-h exposures were performed blind in a reverberation chamber and whole-body specific absorption rate levels were 0, 0.08, 0.4 and 4 W/kg. 3-NT and stress protein expression was assayed in different areas of the hippocampus and cortex. No significant difference was observed among exposed and sham-exposed groups. These results suggest that repeated exposure to WiFi during gestation and early life has no deleterious effects on the brains of young rats.

(E) Aldad TS, Gan G, Gao XB, Taylor HS. Fetal radiofrequency radiation exposure from 800-1900 MHz-rated cellular telephones affects neurodevelopment and behavior in mice. Sci Rep. 2:312, 2012. (AS, CS, DE, BE, CE, CC)

Neurobehavioral disorders are increasingly prevalent in children, however their etiology is not well understood. An association between prenatal cellular telephone use and hyperactivity in children has been postulated, yet the direct effects of radiofrequency radiation exposure on neurodevelopment remain unknown. Here we used a mouse model to demonstrate that in-utero radiofrequency exposure from cellular telephones does affect adult behavior. Mice exposed in-utero were hyperactive and had impaired memory as determined using the object recognition, light/dark box and step-down assays. Whole cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) revealed that these behavioral changes were due to altered neuronal developmental programming. Exposed mice had dose-responsive impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. We present the first experimental evidence of neuropathology due to in-utero cellular telephone radiation. Further experiments are needed in humans or non-human primates to determine the risk of exposure during pregnancy.

(E) Ammari M, Brillaud E, Gamez C, Lecomte A, Sakly M, Abdelmelek H, de Seze R. Effect of a chronic GSM 900 MHz exposure on glia in the rat brain. Biomed Pharmacother. 62(4):273-281, 2008a. (AS, CE, CC)

Extension of the mobile phone technology raises concern about the health effects of 900 MHz microwaves on the central nervous system (CNS). In this study we measured GFAP expression using immunocytochemistry method, to evaluate glial evolution 10 days after a chronic exposure (5 days a week for 24 weeks) to GSM signal for 45 min/day at a brain-averaged specific absorption rate (SAR)=1.5 W/kg and for 15 min/day at a SAR=6 W/kg in the following rat brain areas: prefrontal cortex (Pfcx), caudate putamen (Cpu), lateral globus pallidus of striatum (LGP), dentate gyrus of hippocampus (DG) and

cerebellum cortex (CCx). In comparison to sham or cage control animals, rats exposed to chronic GSM signal at 6 W/kg have increased GFAP stained surface areas in the brain ($p < 0.05$). But the chronic exposure to GSM at 1.5 W/kg did not increase GFAP expression. Our results indicated that chronic exposure to GSM 900 MHz microwaves (SAR=6 W/kg) may induce persistent astroglia activation in the rat brain (sign of a potential gliosis).

(E) Ammari M, Lecomte A, Sakly M, Abdelmelek H, de-Seze R. Exposure to GSM 900 MHz electromagnetic fields affects cerebral cytochrome c oxidase activity. Toxicology. 250(1):70-74, 2008b. (AS, CE, CH)

The world-wide and rapidly growing use of mobile phones has raised serious concerns about the biological and health-related effects of radio frequency (RF) radiation, particularly concerns about the effects of RFs upon the nervous system. The goal of this study was conducted to measure cytochrome oxidase (CO) levels using histochemical methods in order to evaluate regional brain metabolic activity in rat brain after exposure to a GSM 900 MHz signal for 45 min/day at a brain-averaged specific absorption rate (SAR) of 1.5 W/Kg or for 15 min/day at a SAR of 6 W/Kg over seven days. Compared to the sham and control cage groups, rats exposed to a GSM signal at 6 W/Kg showed decreased CO activity in some areas of the prefrontal and frontal cortex (infralimbic cortex, prelimbic cortex, primary motor cortex, secondary motor cortex, anterior cingulate cortex areas 1 and 2 (Cg1 and Cg2)), the septum (dorsal and ventral parts of the lateral septal nucleus), the hippocampus (dorsal field CA1, CA2 and CA3 of the hippocampus and dental gyrus) and the posterior cortex (retrosplenial agranular cortex, primary and secondary visual cortex, perirhinal cortex and lateral entorhinal cortex). However, the exposure to GSM at 1.5 W/Kg did not affect brain activity. Our results indicate that 6 W/Kg GSM 900 MHz microwaves may affect brain metabolism and neuronal activity in rats.

(NE) Ammari M, Jacquet A, Lecomte A, Sakly M, Abdelmelek H, de Seze R. Effect of head-only sub-chronic and chronic exposure to 900-MHz GSM electromagnetic fields on spatial memory in rats. Brain Inj. 22(13-14):1021-1029, 2008c. (AS, CE, BE)

PRIMARY OBJECTIVE: This study was carried out to investigate the behavioural effects of sub-chronic and chronic head-only exposure to 900 MHz GSM (Global System for Mobile communications) in male rats. **METHODS:** Rats were exposed for 45 minutes per day, at a brain-averaged specific absorption rate (SAR) = 1.5 W Kg⁻¹ or 15 minutes per day at a SAR = 6 W Kg⁻¹, during 8 or 24 weeks. Then, their spatial memory was tested using the radial-arm maze. In the first phase (10 days), rats were trained to visit the eight arms of the maze without returning to an arm already visited. In the second phase (8 days), a 45-minute intra-trial delay was introduced after four visited arms. **RESULTS:** Performance of exposed rats (1.5 or 6 W Kg⁻¹) was compared with that of sham, negative control and positive control rats. Scopolamine treatment in the positive control rats induced deficit in spatial memory task in the second phase of the test.

However, spatial memory task was unaffected in exposed rats. **CONCLUSION:** Sub-chronic and chronic head-only exposure of rats to GSM 900 MHz signal (45-minutes, SAR = 1.5 or 15-minutes, SAR = 6 W Kg(-1)) did not induce spatial memory deficit in the radial-arm maze.

(E) Ammari M, Gamez C, Lecomte A, Sakly M, Abdelmelek H, De Seze R. GFAP expression in the rat brain following sub-chronic exposure to a 900 MHz electromagnetic field signal. Int J Radiat Biol. 86(5):367-375, 2010. (AS, CE, CC)

PURPOSE: The rapid development and expansion of mobile communications contributes to the general debate on the effects of electromagnetic fields emitted by mobile phones on the nervous system. This study aims at measuring the glial fibrillary acidic protein (GFAP) expression in 48 rat brains to evaluate reactive astrogliosis, three and 10 days after long-term head-only sub-chronic exposure to a 900 MHz electromagnetic field (EMF) signal, in male rats. **METHODS:** Sprague-Dawley rats were exposed for 45 min/day at a brain-averaged specific absorption rate (SAR) = 1.5 W/kg or 15 min/day at a SAR = 6 W/kg for five days per week during an eight-week period. GFAP expression was measured by the immunocytochemistry method in the following rat brain areas: Prefrontal cortex, cerebellar cortex, dentate gyrus of the hippocampus, lateral globus pallidus of the striatum, and the caudate putamen. **RESULTS:** Compared to the sham-treated rats, those exposed to the sub-chronic GSM (Global System for mobile communications) signal at 1.5 or 6 W/kg showed an increase in GFAP levels in the different brain areas, three and ten days after treatment. **CONCLUSION:** Our results show that sub-chronic exposures to a 900 MHz EMF signal for two months could adversely affect rat brain (sign of a potential gliosis).

(E) Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010. (AS, CE, CH, BE, MA)

Despite numerous studies, there is no definitive evidence that high-frequency electromagnetic field (EMF) exposure is a risk to human health. To the contrary, this report presents the first evidence that long-term EMF exposure directly associated with cell phone use (918 MHz; 0.25 w/kg) provides cognitive benefits. Both cognitive-protective and cognitive-enhancing effects of EMF exposure were discovered for both normal mice and transgenic mice destined to develop Alzheimer's-like cognitive impairment. The cognitive interference task utilized in this study was designed from, and measure-for-measure analogous to, a human cognitive interference task. In Alzheimer's disease mice, long-term EMF exposure reduced brain amyloid-beta (A β) deposition through A β anti-aggregation actions and increased brain temperature during exposure periods. Several inter-related mechanisms of EMF action are proposed, including increased A β clearance from the brains of Alzheimer's disease mice, increased neuronal activity, and increased cerebral blood flow. Although caution should

be taken in extrapolating these mouse studies to humans, we conclude that EMF exposure may represent a non-invasive, non-pharmacologic therapeutic against Alzheimer's disease and an effective memory-enhancing approach in general.

(E) Arendash GW, Mori T, Dorsey M, Gonzalez R, Tajiri N, Borlongan C. Electromagnetic treatment to old Alzheimer's mice reverses β -amyloid deposition, modifies cerebral blood flow, and provides selected cognitive benefit. PLoS One. 7(4):e35751, 2012. (AS, CE, CH, BE, MA)

Few studies have investigated physiologic and cognitive effects of "long-term" electromagnetic field (EMF) exposure in humans or animals. Our recent studies have provided initial insight into the long-term impact of adulthood EMF exposure (GSM, pulsed/modulated, 918 MHz, 0.25-1.05 W/kg) by showing 6+ months of daily EMF treatment protects against or reverses cognitive impairment in Alzheimer's transgenic (Tg) mice, while even having cognitive benefit to normal mice. Mechanistically, EMF-induced cognitive benefits involve suppression of brain β -amyloid ($A\beta$) aggregation/deposition in Tg mice and brain mitochondrial enhancement in both Tg and normal mice. The present study extends this work by showing that daily EMF treatment given to very old (21-27 month) Tg mice over a 2-month period reverses their very advanced brain $A\beta$ aggregation/deposition. These very old Tg mice and their normal littermates together showed an increase in general memory function in the Y-maze task, although not in more complex tasks. Measurement of both body and brain temperature at intervals during the 2-month EMF treatment, as well as in a separate group of Tg mice during a 12-day treatment period, revealed no appreciable increases in brain temperature (and no/slight increases in body temperature) during EMF "ON" periods. Thus, the neuropathologic/cognitive benefits of EMF treatment occur without brain hyperthermia. Finally, regional cerebral blood flow in cerebral cortex was determined to be reduced in both Tg and normal mice after 2 months of EMF treatment, most probably through cerebrovascular constriction induced by freed/disaggregated $A\beta$ (Tg mice) and slight body hyperthermia during "ON" periods. These results demonstrate that long-term EMF treatment can provide general cognitive benefit to very old Alzheimer's Tg mice and normal mice, as well as reversal of advanced $A\beta$ neuropathology in Tg mice without brain heating. Results further underscore the potential for EMF treatment against AD.

***(E) Arns M, Van Luijelaar G, Sumich A, Hamilton R, Gordon E. Electroencephalographic, personality, and executive function measures associated with frequent mobile phone use. Int J Neurosci. 117(9):1341-1360, 2007. (HU, BE) (*Effects observed probably not caused by exposure to RFR.)**

The present study employs standardized data acquired from the Brain Resource International Database to study the relationship between mobile phone usage, personality, and brain function (n = 300). Based on the frequency and duration of mobile phone usage, three groups were formed. The findings suggest a subtle slowing of

brain activity related to mobile phone use that is not explained by differences in personality. These changes are still within normal physiological ranges. Better executive function in mobile phone users may reflect more focused attention, possibly associated with a cognitive training effect (i.e., frequently making phone calls in distracting places), rather than a direct effect of mobile phone use on cognition.

(E) Bak M, Dudarewicz A, Zmyślony M, Sliwinska-Kowalska M. Effects of GSM signals during exposure to event related potentials (ERPs). Int J Occup Med Environ Health. 23(2):191-199, 2010. (HU, EE)

OBJECTIVES: The primary aim of this work was to assess the effect of electromagnetic field (EMF) from the GSM mobile phone system on human brain function. The assessment was based on the assay of event related potentials (ERPs). **MATERIAL AND METHODS:** The study group consisted of 15 volunteers, including 7 men and 8 women. The test protocol comprised determination of P300 wave in each volunteer during exposure to the EMF. To eliminate possible effects of the applied test procedure on the final result, the test was repeated without EMF exposure. P300 latency, amplitude, and latency of the N1, N2, P2 waves were analysed. **RESULTS:** The statistical analysis revealed an effect of EMF on P300 amplitude. In the experiment with EMF exposure, lower P300 amplitudes were observed only at the time in which the volunteers were exposed to EMF; when the exposure was discontinued, the values of the amplitude were the same as those observed before EMF application. No such change was observed when the experiment was repeated with sham exposure, which may be considered as an indirect proof that lower P300 amplitude values were due to EMF exposure. No statistically significant changes were noted in the latencies of the N1, N2, P2 waves that precede the P300 wave, nor in the latency of the P300 itself. **CONCLUSIONS:** The results suggest that exposure to GSM EMF exerts some effects on CNS, including effects on long latency ERPs.

(E) Barcal J, Vozeh F. Effect of whole-body exposure to high-frequency electromagnetic field on the brain cortical and hippocampal activity in mouse experimental model. NeuroQuantology 5:292-302, 2007. (AS, EE)

Evaluation of the direct registration of brain cortical and hippocampal activity during a high-frequency electromagnetic field (HF-EMF) exposure was performed. Experimental procedures were done under general anesthesia (urethane, 20%, 2g/kg i.p.) in Lurcher mutant mice, wild type (healthy littermates) were used as controls. Animals were exposed to the HF-EMF with frequency corresponding to cellular phones (900 MHz). We used of gel electrodes (silicon tubes or glass microcapillary filled with agar) where the connection with classical electrodes was located out of HF-EMF space. ECoG evaluation showed a distinct shift to lower frequency components but clear effect has been observed only in wild type (healthy) mice whereas in Lurcher mutant mice only gentle differences between frequency spectra were found. Measurement of hippocampal

rhythmicity showed gentle changes with increase of higher frequencies (i.e. opposite effect than in cortex) and changes in theta oscillations registered from a dentate gyrus and CA1 area in both types of animals (healthy and mutant). These findings support an idea about possible influencing the central nervous system by HF-EMF exposure and support also some recent results about possible health risks resulting from cellular phones use.

(E) Bas O, Odaci E, Kaplan S, Acer N, Ucok K, Colakoglu S. 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. Brain Res. 1265:178-185, 2009. (AS, CE, ME)

The effects of electromagnetic fields (EMFs) emitted by mobile phones on humans hold special interest due to their use in close proximity to the brain. The current study investigated the number of pyramidal cells in the cornu ammonis (CA) of the 16-week-old female rat hippocampus following postnatal exposure to a 900 megahertz (MHz) EMF. In this study were three groups of 6 rats: control (Cont), sham exposed (Sham), and EMF exposed (EMF). EMF group rats were exposed to 900 MHz EMF (1 h/day for 28 days) in an exposure tube. Sham group was placed in the exposure tube but not exposed to EMF (1 h/day for 28 days). Cont group was not placed into the exposure tube nor were they exposed to EMF during the study period. In EMF group rats, the specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). All of the rats were sacrificed at the end of the experiment and the number of pyramidal cells in the CA was estimated using the optical fractionator technique. Histopathological evaluations were made on sections of the CA region of the hippocampus. Results showed that postnatal EMF exposure caused a significant decrease of the pyramidal cell number in the CA of the EMF group (P<0.05). Additionally, cell loss can be seen in the CA region of EMF group even at qualitative observation. These results may encourage researchers to evaluate the chronic effects of 900 MHz EMF on teenagers' brains.

(E) Baş O, Sönmez OF, Aslan A, İkinci A, Hancı H, Yıldırım M, Kaya H, Akça M, Odacı E. Pyramidal Cell Loss in the Cornu Ammonis of 32-day-old Female Rats Following Exposure to a 900 Megahertz Electromagnetic Field During Prenatal Days 13–21. NeuroQuantology 11:591-599, 2013. (AS, CE, ME, DE)

The number of studies reporting that the electromagnetic field (EMF) emitted by mobile phones affects human health is increasing by the day. In previous studies we reported that a 900 megahertz (MHz) EMF applied throughout the prenatal period reduced the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. In this study we investigated the effect of a 900 MHz EMF applied on days 13-21 of the prenatal period on the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. For that purpose, pregnant rats were divided into experimental and control groups. Experimental group pregnant rats were exposed to the effect of a

900 MHz EMF on days 13-21 of pregnancy. No procedure was applied to the control group. Newborn female rat pups were added to the study, and no procedure was performed on these after birth. Five newborn female rats were obtained from the experimental group and six from the control group. All female rat pups were decapitated on the postnatal 32nd day, and histological procedures were performed on the brain tissues. Sections were stained with Cresyl fast violet. The optical dissector technique was used to estimate the total number of pyramidal cells in the cornu ammonis. Sections of cornu ammonis were subjected to histopathological evaluations. Our results showed that exposure to 900 MHz EMF during prenatal days 13-21 led to a significant decrease in the number of pyramidal cells in the cornu ammonis of the experimental group female rat pups (P<0.05). Histopathological examination revealed picnotic cells in the cornu ammonis in experimental female rat pups. The pyramidal cell loss in the cornu ammonis may therefore be attributed to exposure to 900 MHz EMF in days 13-21 of the prenatal period.

(E) Boder P, Stankiewicz W, Antkowiak B, Paluch M, Kieliszek J, Sobiech J, Zdanowski R, Wojdas A, Siwicki AK, Skopińska-Różewska E. Suppressive effect of electromagnetic field on analgesic activity of tramadol in rats. Pol J Vet Sci. 15(1):95-100, 2012. (AS, PE, IA)

The electromagnetic fields (EMFs) have been shown to alter animal and human behavior, such as directional orientation, learning, pain perception (nociception or analgesia) and anxiety-related behaviors. The aim of this study was to evaluate the influence of electromagnetic fields of high-frequency microwaves on pain perception and anti-nociceptive activity of tramadol (TRAM) - analgetic effective in the treatment of moderate to severe acute and chronic pain states. Electromagnetic fields exposures of a)1500 MHz frequency and b) modulated, 1800 MHz (which is identical to that generated by mobile phones) were applied. Paw withdrawal latency (PWL) to thermal stimulus was measured in vehicle or tramadol (TRAM) treated animals before and after 30, 60 and 90 minutes from injections. The differences in the level of pain (PWL) between control group and rats exposed to EMF alone in three measurements, were not observed. Tramadol alone significantly increased PWLs to thermal stimulus in comparison to vehicle results at 30 ($p < 0.001$) and 60 minutes ($p < 0.05$) after drug injection. EMF exposure of both frequencies transiently suppressed analgesic effect of tramadol, significantly reducing paw withdrawal latency in animals treated with this drug at 30 minutes from the drug injection.

(E) Bouji M, Lecomte A, Hode Y, de Seze R, Villégier AS. Effects of 900 MHz radiofrequency on corticosterone, emotional memory and neuroinflammation in middle-aged rats. Exp Gerontol. 47(6):444-451, 2012. (AS, CC, BE, AD)

The widespread use of mobile phones raises the question of the effects of electromagnetic fields (EMF, 900 MHz) on the brain. Previous studies reported increased levels of the glial fibrillary acidic protein (GFAP) in the rat's brain after a single exposure to 900 MHz global system for mobile (GSM) signal, suggesting a potential inflammatory process. While this result was obtained in adult rats, no data is currently available in older animals. Since the transition from middle-age to senescence is highly dependent on environment and lifestyle, we studied the reactivity of middle-aged brains to EMF exposure. We assessed the effects of a single 15 min GSM exposure (900 MHz; specific absorption rate (SAR)=6 W/kg) on GFAP expression in young adults (6 week-old) and middle-aged rats (12 month-old). Brain interleukin (IL)-1 β and IL-6, plasmatic levels of corticosterone (CORT), and emotional memory were also assessed. Our data indicated that, in contrast to previously published work, acute GSM exposure did not induce astrocyte activation. Our results showed an IL-1 β increase in the olfactory bulb and enhanced contextual emotional memory in GSM-exposed middle-aged rats, and increased plasmatic levels of CORT in GSM-exposed young adults. Altogether, our data showed an age dependency of reactivity to GSM exposure in neuro-immunity, stress and behavioral parameters. Reproducing these effects and studying their mechanisms may allow a better understanding of mobile phone EMF effects on neurobiological parameters.

(E) Brillaud E, Piotrowski A, de Seze R. Effect of an acute 900 MHz GSM exposure on glia in the rat brain: a time-dependent study. Toxicology. 238(1):23-33, 2007. (AS, CC)

Because of the increasing use of mobile phones, the possible risks of radio frequency electromagnetic fields adverse effects on the human brain has to be evaluated. In this work we measured GFAP expression, to evaluate glial evolution 2, 3, 6 and 10 days after a single GSM exposure (15min, brain averaged SAR=6W/kg, 900 MHz signal) in the rat brain. A statistically significant increase of GFAP stained surface area was observed 2 days after exposure in the frontal cortex and the caudate putamen. A smaller statistically significant increase was noted 3 days after exposure in the same areas and in the cerebellum cortex. Our results confirm the Mausset-Bonnefont et al. study [Mausset-Bonnefont, A.L., Hirbec, H., Bonnefont, X., Privat, A., Vignon, J., de Seze, R., 2004. Acute exposure to GSM 900MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol. Dis. 17, 445-454], showing the existence of glial reactivity after a 15min GSM acute exposure at a brain averaged SAR of 6W/kg. We conclude to a temporary effect, probably due to a hypertrophy of glial cells, with a temporal and a spatial modulation of the effect. Whether this effect could be harmful remains to be studied.

(E) Calabrò E, Condello S, Currò M, Ferlazzo N, Caccamo D, Magazù S, Ientile R. Modulation of heat shock protein response in SH-SY5Y by mobile phone microwaves. World J Biol Chem. 3(2):34-40, 2012. (CS, CH)

AIM: To investigate putative biological damage caused by GSM mobile phone frequencies by assessing electromagnetic fields during mobile phone working. METHODS: Neuron-like cells, obtained by retinoic-acid-induced differentiation of human neuroblastoma SH-SY5Y cells, were exposed for 2 h and 4 h to microwaves at 1800 MHz frequency bands. RESULTS: Cell stress response was evaluated by MTT assay as well as changes in the heat shock protein expression (Hsp20, Hsp27 and Hsp70) and caspase-3 activity levels, as biomarkers of apoptotic pathway. Under our experimental conditions, neither cell viability nor Hsp27 expression nor caspase-3 activity was significantly changed. Interestingly, a significant decrease in Hsp20 expression was observed at both times of exposure, whereas Hsp70 levels were significantly increased only after 4 h exposure. CONCLUSION: The modulation of the expression of Hsps in neuronal cells can be an early response to radiofrequency microwaves.

(E) Cammaerts MC, De Doncker P, Patris X, Bellens F, Rachidi Z, Cammaerts D. GSM 900 MHz radiation inhibits ants' association between food sites and encountered cues. Electromagn Biol Med. 31(2):151-165, 2012. (AS, BE)

The kinetics of the acquisition and loss of the use of olfactory and visual cues were previously obtained in six experimental colonies of the ant *Myrmica sabuleti* meinert 1861, under normal conditions. In the present work, the same experiments were conducted on six other naive identical colonies of *M. sabuleti*, under electromagnetic radiation similar to those surrounding GSM and communication masts. In this situation, no association between food and either olfactory or visual cues occurred. After a recovery period, the ants were able to make such an association but never reached the expected score. Such ants having acquired a weaker olfactory or visual score and still undergoing olfactory or visual training were again submitted to electromagnetic waves. Not only did they lose all that they had memorized, but also they lost it in a few hours instead of in a few days (as under normal conditions when no longer trained). They kept no visual memory at all (instead of keeping 10% of it as they normally do). The impact of GSM 900 MHz radiation was greater on the visual memory than on the olfactory one. These communication waves may have such a disastrous impact on a wide range of insects using olfactory and/or visual memory, i.e., on bees.

(E) Cammaerts MC, Rachidi Z, Bellens F, De Doncker P. Food collection and response to pheromones in an ant species exposed to electromagnetic radiation. Electromagn Biol Med. 2013 Jan 15. [Epub ahead of print] (AS, BE)

We used the ant species *Myrmica sabuleti* as a model to study the impact of electromagnetic waves on social insects' response to their pheromones and their food collection. We quantified *M. sabuleti* workers' response to their trail, area marking and alarm pheromone under normal conditions. Then, we quantified the same responses while under the influence of electromagnetic waves. Under such an influence, ants followed trails for only short distances, no longer arrived at marked areas and no longer orientated themselves to a source of alarm pheromone. Also when exposed to

electromagnetic waves, ants became unable to return to their nest and recruit congeners; therefore, the number of ants collecting food increases only slightly and slowly. After 180 h of exposure, their colonies deteriorated. Electromagnetic radiation obviously affects social insects' behavior and physiology.

(E) Carballo-Quintás M, Martínez-Silva I, Cadarso-Suárez C, Alvarez-Figueiras M, Ares-Pena FJ, López-Martín E. A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains. Neurotoxicology. 32(4):478-494, 2011. (AS, CH)

The acute effects of microwave exposure from the Global System for Mobile Communication (GSM) were studied in rats, using 900 MHz radiation at an intensity similar to mobile phone emissions. Acute subconvulsive doses of picrotoxin were then administered to the rats and an experimental model of seizure-proneness was created from the data. Seventy-two adult male Sprague-Dawley rats underwent immunochemical testing of relevant anatomical areas to measure induction of the c-fos neuronal marker after 90min and 24h, and of the glial fibrillary acidic protein (GFAP) 72h after acute exposure to a 900MHz electromagnetic field (EMF). The experimental set-up facilitated measurement of absorbed power, from which the average specific absorption rate was calculated using the finite-difference time-domain (FDTD) 2h after exposure to EMF radiation at 1.45W/kg in picrotoxin-treated rats and 1.38W/kg in untreated rats. Ninety minutes after radiation high levels of c-fos expression were recorded in the neocortex and paleocortex along with low hippocampus activation in picrotoxin treated animals. Most brain areas, except the limbic cortical region, showed important increases in neuronal activation 24h after picrotoxin and radiation. Three days after picrotoxin treatment, radiation effects were still apparent in the neocortex, dentate gyrus and CA3, but a significant decrease in activity was noted in the piriform and entorhinal cortex. During this time, glial reactivity increased with every seizure in irradiated, picrotoxin-treated brain regions. Our results reveal that c-fos and glial markers were triggered by the combined stress of non-thermal irradiation and the toxic effect of picrotoxin on cerebral tissues.

(E) Cetin H, Nazıroğlu M, Celik O, Yüksel M, Pastacı N, Ozkaya MO. Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring. J Matern Fetal Neonatal Med. 2014 Mar 3. [Epub ahead of print] (AS, CE, CH, OX, DE)

Objectives: The present study determined the effects of mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) exposure on oxidative stress in the brain and liver as well as the element levels in growing rats from pregnancy to 6 weeks of age. Methods: Thirty-two rats and their offspring were equally divided into 3 different groups: the control, 900 MHz, and 1800 MHz groups. The 900 MHz and 1800 MHz groups were exposed to EMR for 60 min/day during pregnancy and neonatal

development. At the 4th, 5th, and 6th weeks of the experiment, brain samples were obtained. Results: Brain and liver glutathione peroxidase (GSH-Px) activities, as well as liver vitamin A and β -carotene concentrations decreased in the EMR groups, although brain iron, vitamin A, and β -carotene concentrations increased in the EMR groups. In the 6th week, selenium concentrations in the brain decreased in the EMR groups. There were no statistically significant differences in glutathione, vitamin E, chromium, copper, magnesium, manganese, and zinc concentrations between the 3 groups. Conclusion: EMR-induced oxidative stress in the brain and liver was reduced during the development of offspring. Mobile phone-induced EMR could be considered as a cause of oxidative brain and liver injury in growing rats.

(NE) Cinel C, Boldini A, Russo R, Fox E. Effects of mobile phone electromagnetic fields on an auditory order threshold task. Bioelectromagnetics. 28(6):493-496, 2007. (HU, BE)

The effect of acute exposure to radio frequency electromagnetic fields (RF EMF) generated by mobile phones on an auditory threshold task was investigated. 168 participants performed the task while exposed to RF EMF in one testing session (either global system for mobile communication (GSM) or unmodulated signals) while in a separate session participants were exposed to sham signals. Lateralization effects were tested by exposing participants either on the left side or on the right side of the head. No significant effect of exposure to RF EMF was detected, suggesting that acute exposure to RF EMFs does not affect performance in the order threshold task.

(NE) Cinel C, Russo R, Boldini A, Fox E. Exposure to mobile phone electromagnetic fields and subjective symptoms: a double-blind study. Psychosom Med. 70(3):345-348, 2008. (HU, BE)

OBJECTIVES: The objective of this study was to examine whether acute exposure to radio frequency electromagnetic fields (REFs) emitted by mobile phone may affect subjective symptoms. METHODS: Three large groups of volunteers (total 496) were exposed to REFs emitted by mobile phones in one session and sham signals in a different session. REF and sham exposure sessions were counterbalanced and double blinded. Participants were exposed to either Global System for Mobile Communication (GSM) or unmodulated signals, and the mobile phone was positioned either on the left or on the right side of the head. Before and after REF and sham exposure participants completed a questionnaire to rate five symptoms. Any changes in the severity of the symptoms after REF exposure were compared with changes after sham exposure. RESULTS: For one group of participants (N = 160), it was found that dizziness was affected by GSM exposure, but this was not consistently found with the other two groups of participants. No other significant effects were found. CONCLUSIONS: We did not find consistent evidence suggesting that exposure to mobile phone REFs affect subjective symptoms. Even though we acknowledge that more research is needed, we

believe that our results give an important contribution to the research on mobile phone use and subjective symptoms.

(E) Croft RJ, Hamblin DL, Spong J, Wood AW, McKenzie RJ, Stough C. The effect of mobile phone electromagnetic fields on the alpha rhythm of human electroencephalogram. *Bioelectromagnetics*. 29(1):1-10, 2008. (HU, EE)

Mobile phones (MP) emit low-level electromagnetic fields that have been reported to affect neural function in humans; however, demonstrations of such effects have not been conclusive. The purpose of the present study was to test one of the strongest findings in the literature; that of increased "alpha" power in response to MP-type radiation. Healthy participants (N = 120) were tested using a double-blind counterbalanced crossover design, with each receiving a 30-min Active and a 30-min Sham Exposure 1 week apart, while electroencephalogram (EEG) data were recorded. Resting alpha power (8-12 Hz) was then derived as a function of time, for periods both during and following exposure. Non-parametric analyses were employed as data could not be normalized. Previous reports of an overall alpha power enhancement during the MP exposure were confirmed (relative to Sham), with this effect larger at ipsilateral than contralateral sites over posterior regions. No overall change to alpha power was observed following exposure cessation; however, there was less alpha power contralateral to the exposure source during this period (relative to ipsilateral). Employing a strong methodology, the current findings support previous research that has reported an effect of MP exposure on EEG alpha power.

(E) Croft RJ, Leung S, McKenzie RJ, Loughran SP, Iskra S, Hamblin DL, Cooper NR. Effects of 2G and 3G mobile phones on human alpha rhythms: Resting EEG in adolescents, young adults, and the elderly. *Bioelectromagnetics*. 31(6):434-444, 2010. (HU, EE, AD, WS)

The present study was conducted to determine whether adolescents and/or the elderly are more sensitive to mobile phone (MP)-related bioeffects than young adults, and to determine this for both 2nd generation (2G) GSM, and 3rd generation (3G) W-CDMA exposures. To test this, resting alpha activity (8-12 Hz band of the electroencephalogram) was assessed because numerous studies have now reported it to be enhanced by MP exposure. Forty-one 13-15 year olds, forty-two 19-40 year olds, and twenty 55-70 year olds were tested using a double-blind crossover design, where each participant received Sham, 2G and 3G exposures, separated by at least 4 days. Alpha activity, during exposure relative to baseline, was recorded and compared between conditions. Consistent with previous research, the young adults' alpha was greater in the 2G compared to Sham condition, however, no effect was seen in the adolescent or the elderly groups, and no effect of 3G exposures was found in any group. The results provide further support for an effect of 2G exposures on resting alpha activity in young adults, but fail to support a similar enhancement in adolescents or the elderly, or in any age group as a function of 3G exposure.

(NE) Curcio G, Valentini E, Moroni F, Ferrara M, De Gennaro L, Bertini M. Psychomotor performance is not influenced by brief repeated exposures to mobile phones. Bioelectromagnetics. 29(3):237-241, 2008. (HU, BE)

The present study investigated the presence of a cumulative effect of brief and repeated exposures to a GSM mobile phone (902.40 MHz, 217 Hz modulated; peak power of 2 W; average power of 0.25 W; SAR = 0.5 W/kg) on psychomotor functions. To this end, after each of 3 15-min exposures, both an acoustic simple reaction time task (SRTT) and a sequential finger tapping task (SFTT) were administered to 24 subjects. The present study was unable to detect the cumulative effects of brief and repeated EMF exposure on human psychomotor performance, although there was a non-statistical trend to shorter reaction times. In summary, these data show an absence of effects with these particular exposure conditions; however, possible cognitive effects induced by different signal characteristics cannot be excluded.

(E) Curcio G, Ferrara M, Limongi T, Tempesta D, Di Sante G, De Gennaro L, Quaresima V, Ferrari M. Acute mobile phones exposure affects frontal cortex hemodynamics as evidenced by functional near-infrared spectroscopy. J Cereb Blood Flow Metab. 29(5):903-910, 2009. (HU, PE)

This study aimed to evaluate by functional near-infrared spectroscopy (fNIRS), the effects induced by an acute exposure (40 mins) to a GSM (Global System for Mobile Communications) signal emitted by a mobile phone (MP) on the oxygenation of the frontal cortex. Eleven healthy volunteers underwent two sessions (Real and Sham exposure) after a crossover, randomized, double-blind paradigm. The whole procedure lasted 60 mins: 10-mins baseline (Bsl), 40-mins (Exposure), and 10-mins recovery (Post-Exp). Together with frontal hemodynamics, heart rate, objective and subjective vigilance, and self-evaluation of subjective symptoms were also assessed. The fNIRS results showed a slight influence of the GSM signal on frontal cortex, with a linear increase in [HHb] as a function of time in the Real exposure condition ($F(4,40)=2.67$; $P=0.04$). No other measure showed any GSM exposure-dependent changes. These results suggest that fNIRS is a convenient tool for safely and noninvasively investigating the cortical activation in MP exposure experimental settings. Given the short-term effects observed in this study, the results should be confirmed on a larger sample size and using a multichannel instrument that allows the investigation of a wider portion of the frontal cortex.

(NE) Curcio G, Nardo D, Perrucci MG, Pasqualetti P, Chen TL, Del Gratta C, Romani GL, Rossini PM. Effects of mobile phone signals over BOLD response while performing a cognitive task. Clin Neurophysiol. 123(1):129-136, 2012. (HU, BE, PE)

OBJECTIVE: The aim of this study was to investigate the effects induced by an exposure to a GSM signal (Global System for Mobile Communication) on brain BOLD (blood-oxygen-level dependent) response, as well as its time course while performing a Go-

NoGo task. **METHODS:** Participants were tested twice, once in presence of a "real" exposure to GSM radiofrequency signal and once under a "sham" exposure (placebo condition). BOLD response of active brain areas and reaction times (RTs) while performing the task were measured both before and after the exposure. **RESULTS:** RTs to the somatosensory task did not change as a function of exposure (real vs sham) to GSM signal. BOLD results revealed significant activations in inferior parietal lobule, insula, precentral and postcentral gyri associated with Go responses after both "real" and "sham" exposure, whereas no significant effects were observed in the ROI analysis. **CONCLUSIONS:** The present fMRI study did not detect any brain activity changes by mobile phones. Also RTs in a somatosensory task resulted unaffected. **SIGNIFICANCE:** No changes in BOLD response have been observed as a consequence of RF-EMFs exposure.

(E) Daniels WM, Pitout IL, Afullo TJ, Mabandla MV. The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat. Metab Brain Dis. 24(4):629-641, 2009. (AS, ME, BE)

Electromagnetic radiation (EMR) is emitted from electromagnetic fields that surround power lines, household appliances and mobile phones. Research has shown that there are connections between EMR exposure and cancer and also that exposure to EMR may result in structural damage to neurons. In a study by Salford et al. (Environ Health Perspect 111:881-883, 2003) the authors demonstrated the presence of strongly stained areas in the brains of rats that were exposed to mobile phone EMR. These darker neurons were particularly prevalent in the hippocampal area of the brain. The aim of our study was to further investigate the effects of EMR. Since the hippocampus is involved in learning and memory and emotional states, we hypothesised that EMR will have a negative impact on the subject's mood and ability to learn. We subsequently performed behavioural, histological and biochemical tests on exposed and unexposed male and female rats to determine the effects of EMR on learning and memory, emotional states and corticosterone levels. We found no significant differences in the spatial memory test, and morphological assessment of the brain also yielded non-significant differences between the groups. However, in some exposed animals there were decreased locomotor activity, increased grooming and a tendency of increased basal corticosterone levels. These findings suggested that EMR exposure may lead to abnormal brain functioning.

***(NE) Danker-Hopfe H, Dorn H, Bornkessel C, Sauter C. Do mobile phone base stations affect sleep of residents? Results from an experimental double-blind sham-controlled field study. Am J Hum Biol. 22(5):613-618, 2010. (HU, BE, LI, SL) (*Effects observed probably not caused by exposure to RFR.)**

OBJECTIVES: The aim of the present double-blind, sham-controlled, balanced randomized cross-over study was to disentangle effects of electromagnetic fields (EMF) and non-EMF effects of mobile phone base stations on objective and subjective sleep

quality. METHODS: In total 397 residents aged 18-81 years (50.9% female) from 10 German sites, where no mobile phone service was available, were exposed to sham and GSM (Global System for Mobile Communications, 900 MHz and 1,800 MHz) base station signals by an experimental base station while their sleep was monitored at their homes during 12 nights. Participants were randomly exposed to real (GSM) or sham exposure for five nights each. Individual measurement of EMF exposure, questionnaires on sleep disorders, overall sleep quality, attitude towards mobile communication, and on subjective sleep quality (morning and evening protocols) as well as objective sleep data (frontal EEG and EOG recordings) were gathered. RESULTS: Analysis of the subjective and objective sleep data did not reveal any significant differences between the real and sham condition. During sham exposure nights, objective and subjective sleep efficiency, wake after sleep onset, and subjective sleep latency were significantly worse in participants with concerns about possible health risks resulting from base stations than in participants who were not concerned. CONCLUSIONS: The study did not provide any evidence for short-term physiological effects of EMF emitted by mobile phone base stations on objective and subjective sleep quality. However, the results indicate that mobile phone base stations as such (not the electromagnetic fields) may have a significant negative impact on sleep quality.

(NE) Danker-Hopfe H, Dorn H, Bahr A, Anderer P, Sauter C. Effects of electromagnetic fields emitted by mobile phones (GSM 900 and WCDMA/UMTS) on the macrostructure of sleep. J Sleep Res. 20(1 Pt 1):73-81, 2011. (HU, BE, SL)

In the present double-blind, randomized, sham-controlled cross-over study, possible effects of electromagnetic fields emitted by Global System for Mobile Communications (GSM) 900 and Wideband Code-Division Multiple Access (WCDMA)/Universal Mobile Telecommunications System (UMTS) cell-phones on the macrostructure of sleep were investigated in a laboratory environment. An adaptation night, which served as screening night for sleep disorders and as an adjustment night to the laboratory environment, was followed by 9 study nights (separated by a 2-week interval) in which subjects were exposed to three exposure conditions (sham, GSM 900 and WCDMA/UMTS). The sample comprised 30 healthy male subjects within the age range 18-30 years (mean \pm standard deviation: 25.3 \pm 2.6 years). A cell-phone usage at maximum radio frequency (RF) output power was simulated and the transmitted power was adjusted in order to approach, but not to exceed, the specific absorption rate (SAR) limits of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines for general public exposure (SAR(10g) = 2.0 W kg⁻¹). In this study, possible effects of long-term (8 h) continuous RF exposure on the central nervous system were analysed during sleep, because sleep is a state in which many confounding intrinsic and extrinsic factors (e.g. motivation, personality, attitude) are eliminated or controlled. Thirteen of 177 variables characterizing the initiation and maintenance of sleep in the GSM 900 and three in the WCDMA exposure condition differed from the sham condition. The few significant results are not indicative of a negative impact on sleep

architecture. From the present results there is no evidence for a sleep-disturbing effect of GSM 900 and WCDMA exposure.

(E) Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Ocak AR. Effect of mobile phone exposure on apoptotic glial cells and status of oxidative stress in rat brain. Electromagn Biol Med. 28(4):342-354, 2009. (AS, CE, CC, OX)

The aim of this study was to investigate the effects of mobile phone exposure on glial cells in brain. The study carried out on 31 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900 MHz microwave. For the study group (n:14), rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group (n:7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. For the cage control (n:10), nothing applied to rats in this group. In this study, rats were euthanized after 10 months of exposure periods and brains were removed. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3, which is a well-known apoptosis marker, and p53. The expression of the proteins was evaluated by a semi-quantitative scoring system. However, total antioxidative capacity (TAC), catalase, total oxidant status (TOS), and oxidative stress index were measured in rat brain. Final score for apoptosis in the exposed group was significantly lower than the sham ($p < 0.001$) and the cage control groups ($p < 0.01$). p53 was not significantly changed by the exposure ($p > 0.05$). The total antioxidant capacity and catalase in the experimental group was found higher than that in the sham group ($p < 0.001$, $p < 0.05$). In terms of the TOS and oxidative stress index, there was no statistically significant difference between exposure and sham groups ($p > 0.05$). In conclusion, the final score for apoptosis, total antioxidant capacity and catalase in rat brain might be altered by 900 MHz radiation produced by a generator to represent exposure of global systems for mobile communication (GSM) cellular phones.

(E) Dasdag S, Akdag MZ, Kizil G, Kizil M, Cakir DU, Yokus B. Effect of 900 MHz radio frequency radiation on beta amyloid protein, protein carbonyl, and malondialdehyde in the brain. Electromagn Biol Med. 31(1):67-74, 2012. (AS, CE, CH, OX)

Recently, many studies have been carried out in relation to 900 MHz radiofrequency radiation (RF) emitted from a mobile phone on the brain. However, there is little data concerning possible mechanisms between long-term exposure of RF radiation and biomolecules in brain. Therefore, we aimed to investigate long-term effects of 900 MHz radiofrequency radiation on beta amyloid protein, protein carbonyl, and malondialdehyde in the rat brain. The study was carried out on 17 Wistar Albino adult male rats. The rat heads in a carousel were exposed to 900 MHz radiofrequency radiation emitted from a generator, simulating mobile phones. For the study group (n: 10), rats were exposed to the radiation 2 h per day (7 days a week) for 10 months. For the sham group (n: 7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. In this study, rats were euthanized after 10 months of exposure and their brains were removed. Beta amyloid protein,

protein carbonyl, and malondialdehyde levels were found to be higher in the brain of rats exposed to 900 MHz radiofrequency radiation. However, only the increase of protein carbonyl in the brain of rats exposed to 900 MHz radiofrequency radiation was found to be statistically significant ($p < 0.001$). In conclusion, 900 MHz radiation emitted from mobile/cellular phones can be an agent to alter some biomolecules such as protein. However, further studies are necessary.

(NE) de Gannes FP, Billaudel B, Taxile M, Haro E, Ruffié G, Lévêque P, Veyret B, Lagroye I. Effects of head-only exposure of rats to GSM-900 on blood-brain barrier permeability and neuronal degeneration. Radiat Res. 172(3):359-367, 2009. (AS, CE, ME, CC)

Salford et al. reported in 2003 that a single 2-h exposure to GSM-900 mobile telephony signals induced brain damage (increased permeability of the blood-brain barrier and presence of dark neurons) 50 days after exposure. In our study, 16 Fischer 344 rats (14 weeks old) were exposed head-only to the GSM-900 signal for 2 h at various brain-averaged SARs (0, 0.14 and 2.0 W/kg) or were used as cage or positive controls. Albumin leakage and neuron degeneration were evaluated 14 and 50 days after exposure. No apoptotic neurons were found 14 days after the last exposure using the TUNEL method. No statistically significant albumin leakage was observed. Neuronal degeneration, assessed using cresyl violet or the more specific marker Fluoro-Jade B, was not significantly different among the tested groups. No apoptotic neurons were detected. The findings of our study did not confirm the previous results of Salford et al.

(E) de Tommaso M, Rossi P, Falsaperla R, Francesco Vde V, Santoro R, Federici A. Mobile phones exposure induces changes of contingent negative variation in humans. Neurosci Lett. 464(2):79-83, 2009. (HU, EE)

Event-related potentials have been largely employed to test effects of GSM emissions on human brain. The aim of the present study was the evaluation of initial contingent negative variation (iCNV) changes, induced by 900 MHz GSM exposure, in a double blind design in healthy volunteers, subjected to a threefold experimental condition, EXPOSED (A), a real GSM phone emitting electromagnetic power, SHAM (B), a real phone where the electromagnetic power was dissipated on an internal load and OFF (C), a phone completely switched-off. Ten healthy right-handed volunteers were evaluated. The CNV was recorded during a 10 min time interval in each of the three experimental conditions A, B, and C, in order to assess the iCNV amplitude and habituation. The iCNV amplitude decreased and habituation increased during both A and B conditions, compared with condition C. This effect was diffuse over the scalp, and there was no significant prevalence of iCNV amplitude reduction on the left side, where the phones were located. Mobile Phones exposures A and B seemed to act on brain electrical activity, reducing the arousal and expectation of warning stimulus. This evidence, limited by the low number of subjects investigated, could be explained in terms of an effect induced by

both the GSM signal and the extremely low frequency magnetic field produced by battery and internal circuits.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Effect of radiofrequency electromagnetic field exposure on in vitro models of neurodegenerative disease. Bioelectromagnetics. 30(7):564-572, 2009. (CS, CE, IA, OX)

In this work we tested viability, proliferation, and vulnerability of neural cells, after continuous radiofrequency (RF) electromagnetic fields exposure (global system for mobile telecommunications (GSM) modulated 900 MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) generated by transverse electromagnetic cells. We used two cellular systems, SN56 cholinergic for example, SN56 cholinergic cell line and rat primary cortical neurons, and well-known neurotoxic challenges, such as glutamate, 25-35AA beta-amyloid, and hydrogen peroxide. Exposure to RF did not change viability/proliferation rate of the SN56 cholinergic cells or viability of cortical neurons. Co-exposure to RF exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons, whereas no cooperative effects of RF with glutamate and 25-35AA beta-amyloid were found. These data suggest that only under particular circumstances exposure to GSM modulated, 900 MHz signal act as a co-stressor for oxidative damage of neural cells.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (CS, ME, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Deshmukh PS, Banerjee BD, Abegaonkar MP, Megha K, Ahmed RS, Tripathi AK, Mediratta PK. Effect of low level microwave radiation exposure on cognitive function and oxidative stress in rats. Indian J Biochem Biophys. 50(2):114-119, 2013a. (AS, LI, CE, BE, OX)

Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. The present study aimed to evaluate the effects of 900 MHz MW radiation exposure on cognitive function and oxidative stress in blood of Fischer rats. Animals were divided into two groups (6 animals/group): Group I (MW-exposed) and Group II (Sham-exposed). Animals were subjected to MW exposure (Frequency 900 MHz; specific absorption rate 8.4738×10^{-5} W/kg) in Gigahertz transverse electromagnetic cell (GTEM) for 30 days (2 h/day, 5 days/week). Subsequently, cognitive function and oxidative stress parameters were examined for each group. Results showed significant impairment in cognitive function and increase in oxidative stress, as evidenced by the increase in levels of MDA (a marker of lipid peroxidation) and protein carbonyl (a marker of protein oxidation) and unaltered GSH content in blood. Thus, the study demonstrated that low level MW radiation had significant effect on cognitive function and was also capable of leading to oxidative stress.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats. Toxicol Int. 20(1):19-24, 2013b. (AS, LI, CE, CH)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and **2450 MHz**. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 **MHz** at specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: Animals exposed to 1800 **MHz** at SAR 5.835×10^{-4} W/kg and Group IV: Animals exposed to **2450 MHz** at SAR 6.672×10^{-4} W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Divan HA, Kheifets L, Obel C, Olsen J. Prenatal and postnatal exposure to cell phone use and behavioral problems in children. Epidemiology. 19(4):523-529, 2008. (HU, DE, BE)

BACKGROUND: The World Health Organization has emphasized the need for research into the possible effects of radiofrequency fields in children. We examined the association between prenatal and postnatal exposure to cell phones and behavioral problems in young children. **METHODS:** Mothers were recruited to the Danish National Birth Cohort early in pregnancy. When the children of those pregnancies reached 7 years of age in 2005 and 2006, mothers were asked to complete a questionnaire regarding the current health and behavioral status of children, as well as past exposure to cell phone use. Mothers evaluated the child's behavior problems using the Strength and Difficulties Questionnaire. **RESULTS:** Mothers of 13,159 children completed the follow-up questionnaire reporting their use of cell phones during pregnancy as well as current cell phone use by the child. Greater odds ratios for behavioral problems were observed for children who had possible prenatal or postnatal exposure to cell phone use. After adjustment for potential confounders, the odds ratio for a higher overall behavioral problems score was 1.80 (95% confidence interval = 1.45-2.23) in children with both prenatal and postnatal exposure to cell phones. **CONCLUSIONS:** Exposure to cell phones prenatally-and, to a lesser degree, postnatally-was associated with behavioral difficulties such as emotional and hyperactivity problems around the age of school entry. These associations may be noncausal and may be due to unmeasured confounding. If real, they would be of public health concern given the widespread use of this technology.

(NE) Divan HA, Kheifets L, Olsen J. Prenatal cell phone use and developmental milestone delays among infants. Scand J Work Environ Health. 37(4):341-348, 2011. (HU, DE, BE)

OBJECTIVE: The aim of this study was to examine if prenatal use of cell phones by pregnant mothers is associated with developmental milestones delays among offspring up to 18 months of age. **METHODS:** Our work is based upon the Danish National Birth Cohort (DNBC), which recruited pregnant mothers from 1996-2002, and was initiated to collect a variety of detailed information regarding in utero exposures and various health outcomes. At the end of 2008, over 41,000 singleton, live births had been followed with the Age-7 questionnaire, which collected cell phone use exposure for mothers during pregnancy. Outcomes for developmental milestones were obtained from telephone interviews completed by mothers at age 6 and 18 months postpartum. **RESULTS:** A logistic regression model estimated the odds ratios (OR) for developmental milestone delays, adjusted for potential confounders. Less than 5% of children at age 6 and 18 months had cognitive/language or motor developmental delays. At 6 months, the adjusted OR was 0.8 [95% confidence interval (95% CI) 0.7-1.0] for cognitive/language delay and 0.9 (95% CI 0.8-1.1) for motor development delay. At 18 months, the adjusted OR were 1.1 (95% CI 0.9-1.3) and 0.9 (95% CI 0.8-1.0) for cognitive/language and motor development delay, respectively. **CONCLUSIONS:** No evidence of an association between prenatal cell phone use and motor or cognitive/language developmental delays among infants at 6 and 18 months of age was observed. Even when considering dose-response associations for cell phone, associations were null.

(E) Divan HA, Kheifets L, Obel C, Olsen J. Cell phone use and behavioural problems in young children. J Epidemiol Community Health. 66(6):524-529, 2012. (HU, DE, BE)

BACKGROUND: Potential health effects of cell phone use in children have not been adequately examined. As children are using cell phones at earlier ages, research among this group has been identified as the highest priority by both national and international organisations. The authors previously reported results from the Danish National Birth Cohort (DNBC), which looked at prenatal and postnatal exposure to cell phone use and behavioural problems at age 7 years. Exposure to cell phones prenatally, and to a lesser degree postnatally, was associated with more behavioural difficulties. The original analysis included nearly 13 000 children who reached age 7 years by November 2006. **METHODS:** To see if a larger, separate group of DNBC children would produce similar results after considering additional confounders, children of mothers who might better represent current users of cell phones were analysed. This 'new' dataset consisted of 28 745 children with completed Age-7 Questionnaires to December 2008. **RESULTS:** The highest OR for behavioural problems were for children who had both prenatal and postnatal exposure to cell phones compared with children not exposed during either time period. The adjusted effect estimate was 1.5 (95% CI 1.4 to 1.7). **CONCLUSIONS:** The findings of the previous publication were replicated in this separate group of participants demonstrating that cell phone use was associated with behavioural problems at age 7 years in children, and this association was not limited to early users of the technology. Although weaker in the new dataset, even with further control for an extended set of potential confounders, the associations remained.

(NE) Dogan M, Turtay MG, Oguzturk H, Samdanci E, Turkoz Y, Tasdemir S, Alkan A, Bakir S. Effects of electromagnetic radiation produced by 3G mobile phones on rat brains: magnetic resonance spectroscopy, biochemical, and histopathological evaluation. Hum Exp Toxicol. 31(6):557-564, 2012. (AS, CE, OX, CC, CH)

Objective: The effects of electromagnetic radiation (EMR) produced by a third-generation (3G) mobile phone (MP) on rat brain tissues were investigated in terms of magnetic resonance spectroscopy (MRS), biochemistry, and histopathological evaluations. Methods: The rats were randomly assigned to two groups: Group 1 is composed of 3G-EMR-exposed rats (n = 9) and Group 2 is the control group (n = 9). The first group was subjected to EMR for 20 days. The control group was not exposed to EMR. Choline (Cho), creatinin (Cr), and N-acetylaspartate (NAA) levels were evaluated by MRS. Catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were measured by spectrophotometric method. Histopathological analyses were carried out to evaluate apoptosis in the brain tissues of both groups. Results: In MRS, NAA/Cr, Cho/Cr, and NAA/Cho ratios were not significantly different between Groups 1 and 2. Neither the oxidative stress parameters, CAT and GSH-Px, nor the number of apoptotic cells were significantly different between Groups 1 and 2. Conclusions: Usage of short-term 3G MP does not seem to have a harmful effect on rat brain tissue.

(E) Dragicevic N, Bradshaw PC, Mamcarz M, Lin X, Wang L, Cao C, Arendash GW. Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: a mechanism for electromagnetic field-induced cognitive benefit? Neuroscience 185:135-149, 2011. (AS, CE, CC, OX, MA)

We have recently reported that long-term exposure to high frequency electromagnetic field (EMF) treatment not only prevents or reverses cognitive impairment in Alzheimer's transgenic (Tg) mice, but also improves memory in normal mice. To elucidate the possible mechanism(s) for these EMF-induced cognitive benefits, brain mitochondrial function was evaluated in aged Tg mice and non-transgenic (NT) littermates following 1 month of daily EMF exposure. In Tg mice, EMF treatment enhanced brain mitochondrial function by 50-150% across six established measures, being greatest in cognitively-important brain areas (e.g. cerebral cortex and hippocampus). EMF treatment also increased brain mitochondrial function in normal aged mice, although the enhancement was not as robust and less widespread compared to that of Tg mice. The EMF-induced enhancement of brain mitochondrial function in Tg mice was accompanied by 5-10 fold increases in soluble A β 1-40 within the same mitochondrial preparations. These increases in mitochondrial soluble amyloid- β peptide (A β) were apparently due to the ability of EMF treatment to disaggregate A β oligomers, which are believed to be the form of A β causative to mitochondrial dysfunction in Alzheimer's disease (AD). Finally, the EMF-induced mitochondrial enhancement in both Tg and normal mice occurred through non-thermal effects because brain temperatures were either stable or decreased during/after EMF treatment. These results collectively suggest that brain mitochondrial enhancement may be a primary mechanism through which EMF treatment provides cognitive benefit to both Tg and NT mice. Especially in the context that mitochondrial dysfunction is an early and prominent characteristic of Alzheimer's pathogenesis, EMF treatment could have profound value in the disease's prevention and treatment through intervention at the mitochondrial level.

(E) Eberhardt JL, Persson BR, Brun AE, Salford LG, Malmgren LO. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. Electromagn Biol Med. 27(3):215-229, 2008. (AS, ME, CC, LI)

We investigated the effects of global system for mobile communication (GSM) microwave exposure on the permeability of the blood-brain barrier and signs of neuronal damage in rats using a real GSM programmable mobile phone in the 900 MHz band. Ninety-six non-anaesthetized rats were either exposed to microwaves or sham exposed in TEM-cells for 2 h at specific absorption rates of average whole-body Specific Absorption Rates (SAR) of 0.12, 1.2, 12, or 120 mW/kg. The rats were sacrificed after a recovery time of either 14 or 28 d, following exposure and the extravasation of albumin, its uptake into neurons, and occurrence of damaged neurons was assessed. Albumin extravasation and also its uptake into neurons was seen to be enhanced after 14 d (Kruskal Wallis test: p = 0.02 and 0.002, respectively), but not after a 28 d recovery

period. The occurrence of dark neurons in the rat brains, on the other hand, was enhanced later, after 28 d ($p = 0.02$). Furthermore, in the 28-d brain samples, neuronal albumin uptake was significantly correlated to occurrence of damaged neurons (Spearman $r = 0.41$; $p < 0.01$).

(NE) Eltiti S, Wallace D, Ridgewell A, Zougkou K, Russo R, Sepulveda F, Fox E. Short-term exposure to mobile phone base station signals does not affect cognitive functioning or physiological measures in individuals who report sensitivity to electromagnetic fields and controls. Bioelectromagnetics. 30(7):556-563, 2009. (HU, BE, LI)

Individuals who report sensitivity to electromagnetic fields often report cognitive impairments that they believe are due to exposure to mobile phone technology. Previous research in this area has revealed mixed results, however, with the majority of research only testing control individuals. Two studies using control and self-reported sensitive participants found inconsistent effects of mobile phone base stations on cognitive functioning. The aim of the present study was to clarify whether short-term (50 min) exposure at 10 mW/m² to typical Global System for Mobile Communication (GSM) and Universal Mobile Telecommunications System (UMTS) base station signals affects attention, memory, and physiological endpoints in sensitive and control participants. Data from 44 sensitive and 44 matched-control participants who performed the digit symbol substitution task (DSST), digit span task (DS), and a mental arithmetic task (MA), while being exposed to GSM, UMTS, and sham signals under double-blind conditions were analyzed. Overall, cognitive functioning was not affected by short-term exposure to either GSM or UMTS signals in the current study. Nor did exposure affect the physiological measurements of blood volume pulse (BVP), heart rate (HR), and skin conductance (SC) that were taken while participants performed the cognitive tasks.

(E) Eser O, Songur A, Aktas C, Karavelioglu E, Caglar V, Aylak F, Ozguner F, Kanter M. The effect of electromagnetic radiation on the rat brain: an experimental study. Turk Neurosurg. 23(6):707-715, 2013. (AS, CE, OX, ME)

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum. MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and 2450 MHz. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months. RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups

and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1 β level was significantly increased in the EMR groups in the brain stem. **CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.**

(E) Favre D. Mobile phone-induced honeybee worker piping *Apidologie* 42:270–279, 2011. (AS, BE)

The worldwide maintenance of the honeybee has major ecological, economic, and political implications. In the present study, electromagnetic waves originating from mobile phones were tested for potential effects on honeybee behavior. Mobile phone handsets were placed in the close vicinity of honeybees. The sound made by the bees was recorded and analyzed. The audiograms and spectrograms revealed that active mobile phone handsets have a dramatic impact on the behavior of the bees, namely by inducing the worker piping signal. In natural conditions, worker piping either announces the swarming process of the bee colony or is a signal of a disturbed bee colony.

(NE) Finnie JW, Blumbergs PC, Cai Z, Manavis J. Expression of the water channel protein, aquaporin-4, in mouse brains exposed to mobile telephone radiofrequency fields. *Pathology*. 41(5):473-475, 2009. (AS, CE, CC)

AIM: To determine whether exposure to mobile telephone radiofrequency (RF) fields, either acutely or long-term, produces up-regulation of the water channel protein, aquaporin-4 (AQP-4). **METHODS:** Using a purpose-designed exposure system at 900 MHz, mice were given a single, far-field whole body exposure at a specific absorption rate of 4 W/kg for 60 minutes or a similar exposure on 5 successive days/week for 104 weeks. Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by restraint in the exposure module. A positive control group was given a clostridial toxin known to cause microvascular endothelial injury, severe vasogenic oedema and upregulation of AQP-4. Brains were perfusion fixed with 4% paraformaldehyde, coronal sections cut from six levels, and immunostained for the principal water channel protein in brain, AQP-4. **RESULTS:** There was no increase in AQP-4 expression in brains exposed to mobile phone microwaves compared to control (sham exposed and freely moving caged mice) brains after short or protracted exposure, while AQP-4 was substantially upregulated in the brains of mice given the clostridial toxin. **CONCLUSION: Brains exposed to mobile telephone RF fields for a short (60 minutes) or long (2 years) duration did not show any immunohistochemically detectable up-regulation of the water channel protein, AQP-4, suggesting that there was no significant increase in blood-brain barrier permeability.**

(NE) Finnie JW, Chidlow G, Blumbergs PC, Manavis J, Cai Z. Heat shock protein induction in fetal mouse brain as a measure of stress after whole of gestation

exposure to mobile telephony radiofrequency fields. Pathology. 41(3):276-279, 2009. (AS, LE, CC, DE)

AIM: To determine whether whole of gestation exposure of fetal mouse brain to mobile telephone radiofrequency fields produces a stress response detectable by induction of heat shock proteins (HSPs). **METHODS:** Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by restraint in the exposure module. Immediately prior to parturition on day 19, fetal brains were collected, fixed in 4% paraformaldehyde and paraffin-embedded. Three coronal sections encompassing a wide range of anatomical regions were cut from each brain and any stress response detected by immunostaining for HSP25, 32 and 70.

RESULTS: There was no induction of HSP32 or 70 in any brains, while HSP25 expression was limited to two brainstem nuclei and occurred consistently in exposed and non-exposed brains. **CONCLUSION:** Whole of gestation exposure of fetal mouse brains to mobile phone radiofrequency fields did not produce any stress response using HSPs as an immunohistochemical marker.

(NE) Finnie JW, Cai Z, Manavis J, Helps S, Blumbergs PC. Microglial activation as a measure of stress in mouse brains exposed acutely (60 minutes) and long-term (2 years) to mobile telephone radiofrequency fields. Pathology. 42(2):151-154, 2010. (AS, CE, CC)

AIM: To determine whether acute or long-term exposure of the brain to mobile telephone radiofrequency (RF) fields produces activation of microglia, which normally respond rapidly to any change in their microenvironment. **METHODS:** Using a purpose designed exposure system at 900 MHz, mice were given a single, far-field whole body exposure at a specific absorption rate (SAR) of 4 W/kg for 60 min (acute) or on five successive days per week for 104 weeks (long-term). Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by immobilisation in the exposure module. Positive control brains subjected to a stab wound were also included to confirm the ability of microglia to react to any neural stress. Brains were perfusion-fixed with 4% paraformaldehyde and representative regions of the cerebral cortex and hippocampus immunostained for ionised calcium binding adaptor molecule (Iba1), a specific microglial marker. **RESULTS:** There was no increase in microglial Iba1 expression in brains short or long-term exposed to mobile telephony microwaves compared to control (sham-exposed or freely moving caged mice) brains, while substantial microglial activation occurred in damaged positive control neural tissue. **CONCLUSION:** Acute (60 minutes) or longer duration (2 years) exposure of murine brains to mobile telephone RF fields did not produce any microglial activation detectable by Iba1 immunostaining.

(E) Fragopoulou AF, Miltiadous P, Stamatakis A, Stylianopoulou F, Koussoulakos SL, Margaritis LH. Whole body exposure with GSM 900MHz affects spatial memory in mice. Pathophysiology. 17(3):179-187, 2010. (AS, BE)

Extended work has been performed worldwide on the effects of mobile phone radiation upon rats' cognitive functions, however there is great controversy to the existence or not of deficits. The present work has been designed in order to test the effects of mobile phone radiation on spatial learning and memory in mice *Mus musculus* Balb/c using the Morris water maze (a hippocampal-dependent spatial memory task), since there is just one other study on mice with very low SAR level (0.05W/kg) showing no effects. We have applied a 2h daily dose of pulsed GSM 900MHz radiation from commercially available mobile phone for 4 days at SAR values ranging from 0.41 to 0.98W/kg. Statistical analysis revealed that during learning, exposed animals showed a deficit in transferring the acquired spatial information across training days (increased escape latency and distance swam, compared to the sham-exposed animals, on the first trial of training days 2-4). Moreover, during the memory probe-trial sham-exposed animals showed the expected preference for the target quadrant, while the exposed animals showed no preference, indicating that the exposed mice had deficits in consolidation and/or retrieval of the learned spatial information. Our results provide a basis for more thorough investigations considering reports on non-thermal effects of electromagnetic fields (EMFs).

(E) Fragopoulou AF, Samara A, Antonelou MH, Xanthopoulou A, Papadopoulou A, Vougas K, Koutsogiannopoulou E, Anastasiadou E, Stravopodis DJ, Tsangaris GT, Margaritis LH. Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation. Electromagn Biol Med. 31(4):250-274, 2012. (AS, CE, CH, LI)

The objective of this study was to investigate the effects of two sources of electromagnetic fields (EMFs) on the proteome of cerebellum, hippocampus, and frontal lobe in Balb/c mice following long-term whole body irradiation. Three equally divided groups of animals (6 animals/group) were used; the first group was exposed to a typical mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 h daily for 8 months, the second group was exposed to a wireless DECT base (Digital Enhanced Cordless Telecommunications/Telephone) at a SAR level range of 0.012-0.028 W/kg for 8 h/day also for 8 months and the third group comprised the sham-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from both EMF sources altered significantly ($p < 0.05$) the expression of 143 proteins in total (as low as 0.003 fold downregulation up to 114 fold overexpression). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected

proteins confirmed the proteomics data. The observed protein expression changes may be related to brain plasticity alterations, indicative of oxidative stress in the nervous system or involved in apoptosis and might potentially explain human health hazards reported so far, such as headaches, sleep disturbance, fatigue, memory deficits, and brain tumor long-term induction under similar exposure conditions.

(NE) Fritzer G, Göder R, Friege L, Wachter J, Hansen V, Hinze-Selch D, Aldenhoff JB. Effects of short- and long-term pulsed radiofrequency electromagnetic fields on night sleep and cognitive functions in healthy subjects. Bioelectromagnetics. 28(4):316-325, 2007. (HU, BE, EE, SL)

There has been wide public discussion on whether the electromagnetic fields of mobile telephones and their base stations affect human sleep or cognitive functioning. As there is evidence for learning and memory-consolidating effects of sleep and particularly of REM sleep, disturbance of sleep by radiofrequency electromagnetic fields might also impair cognitive functions. Previously realized sleep studies yielded inconsistent results regarding short-term exposure. Moreover, data are lacking on the effect that short- and long-term exposure might have on sleep as well as on cognitive functions. Therefore, 10 healthy young male subjects were included and nocturnal sleep was recorded during eight consecutive nights. In the second, third, and last night, we investigated polysomnographic night sleep and cognitive functions. After the adaptation and baseline nights, the participants were exposed to a defined radiofrequency electromagnetic field during the following six nights. We analyzed polysomnographic night sleep according to Rechtschaffen and Kales [1968, Manual of Standardized Terminology, Techniques and Scoring System for Sleep of Human Subjects] as well as by power spectra and correlation dimension. Cognitive functions were investigated by an array of neuropsychological tests. Data analysis was done by comparing the baseline night with the first and last exposure night and the first two sleep cycles of the respective nights. We did not find significant effects, either on conventional sleep parameters or on power spectra and correlation dimension, nor were there any significant effects on cognitive functions. With our results, we are unable to reveal either short-term or cumulative long-term effects of radiofrequency electromagnetic fields on night sleep and cognitive functions in healthy young male subjects.

(E) Gao X, Luo R, Ma B, Wang H, Liu T, Zhang J, Lian Z, Cui X. [Interference of vitamin E on the brain tissue damage by electromagnetic radiation of cell phone in pregnant and fetal rats]. Wei Sheng Yan Jiu. 42(4):642-646, 2013.[Article in Chinese] (AS, CE, ME, OX, DE)

OBJECTIVE: To investigate the interference of vitamin E on brain tissue damage by electromagnetic radiation of cell phone in pregnant and fetal rats. METHODS: 40 pregnant rats were randomly divided into five groups (positive control, negative control, low, middle and high dosage of vitamin E groups). The low, middle and high dosage of

vitamin E groups were supplemented with 5, 15 and 30 mg/ml vitamin E respectively since the first day of pregnancy. And the negative control group and the positive control group were given peanut oil without vitamin E. All groups except for the negative control group were exposed to 900MHz intensity of cell phone radiation for one hour each time, three times per day for 21 days. After accouchement, the right hippocampus tissue of fetal rats in each group was taken and observed under electron microscope. The vitality of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA) in pregnant and fetal rats' brain tissue were tested. RESULTS: Compared with the negative control group, the chondriosomes in neuron and neuroglia of brain tissues was swelling, mild edema was found around the capillary, chromatin was concentrated and collected, and bubbles were formed in vascular endothelial cells (VEC) in the positive fetal rat control group, whereas the above phenomenon was un-conspicuous in the middle and high dosage of vitamin E groups. We can see uniform chromatin, abundant mitochondrion, rough endoplasmic reticulum and free ribosomes in the high dosage group. The apoptosis has not found in all groups' sections. In the antioxidase activity analysis, compared with the negative control group, the vitality of SOD and GSH-Px significantly decreased and the content of MDA significantly increased both in the pregnant and fetal rats positive control group ($P < 0.05$). In fetal rats, the vitality of SOD and GSH-Px significantly increased in the brain tissues of all three different vitamin E dosages groups when compared with the positive control group, and the content of MDA was found significantly decreased in both middle and high dosage of vitamin E groups ($P < 0.05$). The same results have also been found in high dosage pregnant rat group, but in middle dosage group only SOD activity was found increased with significance ($P < 0.05$). With the dosage increase of vitamin E, the vitality of SOD and GSH-Px was increasing and the content of MDA was decreasing. CONCLUSION: Under the experimental dosage, vitamin E has certain interference on damage of antioxidant capacity and energy metabolism induced by electromagnetic radiation of cell phone in pregnant rats and fetal rats.

(NE) Grafström G, Nittby H, Brun A, Malmgren L, Persson BR, Salford LG, Eberhardt J. Histopathological examinations of rat brains after long-term exposure to GSM-900 mobile phone radiation. Brain Res Bull. 77(5):257-263, 2008. (AS, CE, ME, CH, LI)

In order to mimic the real life situation, with often life-long exposure to the electromagnetic fields emitted by mobile phones, we have investigated in a rat model the effects of repeated exposures under a long period to Global System for Mobile Communication-900 MHz (GSM-900) radiation. Out of a total of 56 rats, 32 were exposed once weekly in a 2-h period, for totally 55 weeks, at different average whole-body specific absorption rates (SAR) (of in average 0.6 and 60 mW/kg at the initiation of the experimental period). The animals were exposed in a transverse electromagnetic transmission line chamber (TEM-cell) to radiation emitted by a GSM-900 test phone. Sixteen animals were sham exposed and eight animals were cage controls, which never left the animal house. After behavioural tests, 5-7 weeks after the last exposure, the brains were evaluated for histopathological alterations such as albumin extravasation,

dark neurons, lipofuscin aggregation and signs of cytoskeletal and neuritic neuronal changes of the type seen in human ageing. In this study, no significant alteration of any these histopathological parameters was found, when comparing the GSM exposed animals to the sham exposed controls.

(NE) Guxens M, van Eijsden M, Vermeulen R, Loomans E, Vrijkotte TG, Komhout H, van Strien RT, Huss A. Maternal cell phone and cordless phone use during pregnancy and behaviour problems in 5-year-old children. J Epidemiol Community Health. 2013 Feb 5. [Epub ahead of print] (HU, DE, BE)

BACKGROUND: A previous study found an association between maternal cell phone use during pregnancy and maternal-reported child behaviour problems at age 7. Together with cell phones, cordless phones represent the main exposure source of radiofrequency-electromagnetic fields to the head. Therefore, we assessed the association between maternal cell phone and cordless phone use during pregnancy and teacher-reported and maternal-reported child behaviour problems at age 5. **METHODS:** The study was embedded in the Amsterdam Born Children and their Development study, a population-based birth cohort study in Amsterdam, the Netherlands (2003-2004). Teachers and mothers reported child behaviour problems using the Strength and Difficulties Questionnaire at age 5. Maternal cell phone and cordless phone use during pregnancy was asked when children were 7 years old. **RESULTS:** A total of 2618 children were included. As compared to non-users, those exposed to prenatal cell phone use showed an increased but non-significant association of having teacher-reported overall behaviour problems, although without dose-response relationship with the number of calls (OR=2.12 (95% CI 0.95 to 4.74) for <1 call/day, OR=1.58 (95% CI 0.69 to 3.60) for 1-4 calls/day and OR=2.04 (95% CI 0.86 to 4.80) for ≥ 5 calls/day). ORs for having teacher-reported overall behaviour problems across categories of cordless phone use were below 1 or close to unity. Associations of maternal cell phone and cordless phone use with maternal-reported overall behaviour problems remained non-significant. Non-significant associations were found for the specific behaviour problem subscales. **CONCLUSION:** Our results do not suggest that maternal cell phone or cordless phone use during pregnancy increases the odds of behaviour problems in their children.

(NE) Haarala C, Takio F, Rintee T, Laine M, Koivisto M, Revonsuo A, Hämäläinen H. Pulsed and continuous wave mobile phone exposure over left versus right hemisphere: effects on human cognitive function. Bioelectromagnetics. 28(4):289-295, 2007. (HU, BE)

The possible effects of continuous wave (CW) and pulse modulated (PM) electromagnetic field (EMF) on human cognition was studied in 36 healthy male subjects. They performed cognitive tasks while exposed to CW, PM, and sham EMF. The subjects performed the same tasks twice during each session; once with left-sided and once with right-sided exposure. The EMF conditions were spread across three testing sessions, each session separated by 1 week. The exposed hemisphere, EMF condition,

and test order were counterbalanced over all subjects. We employed a double-blind design: both the subject and the experimenter were unaware of the EMF condition. The EMF was created with a signal generator connected via amplifier to a dummy phone antenna, creating a power output distribution similar to the original commercial mobile phone. The EMF had either a continuous power output of 0.25 W (CW) or pulsed power output with a mean of 0.25 W. An additional control group of 16 healthy male volunteers performed the same tasks without any exposure equipment to see if mere presence of the equipment could have affected the subjects' performance. No effects were found between the different EMF conditions, separate hemisphere exposures, or between the control and experimental group. In conclusion, the current results indicate that normal mobile phones have no discernible effect on human cognitive function as measured by behavioral tests.

(E) Haghani M, Shabani M, Moazzami K. Maternal mobile phone exposure adversely affects the electrophysiological properties of Purkinje neurons in rat offspring. Neuroscience. 2013 Jul 29. pii: S0306-4522(13)00643-X. doi: 10.1016/j.neuroscience.2013.07.049. [Epub ahead of print] (AS, CE, EE, CC, DE) no behavioral effect.

Electromagnetic field (EMF) radiations emitted from mobile phones may cause structural damage to neurons. With the increased usage of mobile phones worldwide, concerns about their possible effects on the nervous system are rising. In the present study, we aimed to elucidate the possible effects of prenatal EMF exposure on the cerebellum of offspring Wistar rats. Rats in EMF group were exposed to 900 MHz Pulse-EMF irradiation for six hours per day during all gestation period. Ten offspring's per each group were evaluated for behavioral and electrophysiological evaluations. Cerebellum - related behavioral dysfunctions were analyzed using motor learning and cerebellum-dependent functional tasks (Accelerated Rotarod, Hanging and Open field tests). Whole cell- patch clamp recordings were used for electrophysiological evaluations. The results of the present study failed to show any behavioral abnormalities in rats exposed to chronic EMF radiation. However, whole cell patch clamp recordings revealed decreased neuronal excitability of Purkinje cells in rats exposed to EMF. The most prominent changes included afterhyperpolarization amplitude, spike frequency, half width and first spike latency. In conclusion, the results of the present study show that prenatal EMF exposure results in altered electrophysiological properties of Purkinje neurons. However, these changes may not be severe enough to alter the cerebellum-dependent functional tasks.

(E) Hao D, Yang L, Chen S, Tong J, Tian Y, Su B, Wu S, Zeng Y. Effects of long-term electromagnetic field exposure on spatial learning and memory in rats. Neurol Sci. 2012 Feb 24. [Epub ahead of print] (AS, CE, BE, CC, EE)

With the development of communications industry, mobile phone plays an important role in daily life. Whether or not the electromagnetic radiation emitted by mobile phone causes any adverse effects on brain function has become of a great concern. This paper investigated the effect of electromagnetic field on spatial learning and memory in rats. 32 trained Wistar rats were divided into two groups: exposure group and control group. The exposure group was exposed to 916 MHz, 10w/m² mobile phone electromagnetic field (EMF) 6 h a day, 5 days a week, 10 weeks. The completion time, number of total errors and the neuron discharge signals were recorded while the rats were searching for food in an eight-arm radial maze at every weekend. The neuron signals of one exposed rat and one control rat in the maze were obtained by the implanted microelectrode arrays in their hippocampal regions. It can be seen that during the weeks 4-5 of the experiment, the average completion time and error rate of the exposure group were longer and larger than that of control group ($p < 0.05$). During the weeks 1-3 and 6-9, they were close to each other. The hippocampal neurons showed irregular firing patterns and more spikes with shorter interspike interval during the whole experiment period. It indicates that the 916 MHz EMF influence learning and memory in rats to some extent in a period during exposure, and the rats can adapt to long-term EMF exposure.

(E) Hardell L, Söderqvist F, Carlberg M, Zetterberg H, Mild KH. Exposure to wireless phone emissions and serum beta-trace protein. Int J Mol Med. 26(2):301-306, 2010. (HU, CH, SL)

The lipocalin type of prostaglandin D synthase or beta-trace protein is synthesized in the choroid plexus, lepto-meninges and oligodendrocytes of the central nervous system and is secreted into the cerebrospinal fluid. beta-trace protein is the key enzyme in the synthesis of prostaglandin D₂, an endogenous sleep-promoting neurohormone in the brain. Electromagnetic fields (EMF) in the radio frequency (RF) range have in some studies been associated with disturbed sleep. We studied the concentration of beta-trace protein in blood in relation to emissions from wireless phones. This study included 62 persons aged 18-30 years. The concentration of beta-trace protein decreased with increasing number of years of use of a wireless phone yielding a negative beta coefficient = -0.32, 95% confidence interval -0.60 to -0.04. Also cumulative use in hours gave a negative beta coefficient, although not statistically significant. Of the 62 persons, 40 participated in an experimental study with 30 min exposure to an 890-MHz GSM signal. No statistically significant change of beta-trace protein was found. In a similar study of the remaining 22 participants with no exposure, beta-trace protein increased significantly over time, probably due to a relaxed situation. EMF emissions may down-regulate the synthesis of beta-trace protein. This mechanism might be involved in sleep disturbances reported in persons exposed to RF fields. The results must be interpreted with caution since use of mobile and cordless phones were self-reported. Awareness of exposure condition in the experimental study may have influenced beta-trace protein concentrations.

(NE) Hareuveny R, Eliyahu I, Luria R, Meiran N, Margaliot M. Cognitive effects of cellular phones: a possible role of non-radiofrequency radiation factors. *Bioelectromagnetics*. 32(7):585-588, 2011. (See also: Luria et al., 2009) (HU, BE)

Some studies found that cognitive functions of human beings may be altered while exposed to radiofrequency radiation (RFR) emitted by cellular phones. In two recent studies, we have found that experiment duration and exposure side (i.e., phone's location--right or left) may have a major influence on the detection of such effects. In this brief follow-up experiment, 29 right-handed male subjects were divided into two groups. Each subject had two standard cellular phones attached to both sides of his head. The subjects performed a spatial working memory task that required either a left-hand or a right-hand response under one of the two exposure conditions: left side of the head or right side. Contrary to our previous studies, in this work external antennas located far away from the subjects were connected to the cellular phones. This setup prevents any emission of RFR from the internal antenna, thus drastically reducing RFR exposure. Despite that, the results remain similar to those obtained in our previous work. These results indicate that some of the effects previously attributed to RFR can be the result of some confounders.

(NE) Heinrich S, Thomas S, Heumann C, von Kries R, Radon K. Association between exposure to radiofrequency electromagnetic fields assessed by dosimetry and acute symptoms in children and adolescents: a population based cross-sectional study. *Environ Health*. 9:75, 2010. (HU, BE)

BACKGROUND: The increase in numbers of mobile phone users was accompanied by some concern that exposure to radiofrequency electromagnetic fields (RF EMF) might adversely affect acute health especially in children and adolescents. The authors investigated this potential association using personal dosimeters. METHODS: A 24-hour exposure profile of 1484 children and 1508 adolescents was generated in a population-based cross-sectional study in Germany between 2006 and 2008 (participation 52%). Personal interview data on socio-demographic characteristics, self-reported exposure and potential confounders were collected. Acute symptoms were assessed twice during the study day using a symptom diary. RESULTS: Only few of the large number of investigated associations were found to be statistically significant. At noon, adolescents with a measured exposure in the highest quartile during morning hours reported a statistically significant higher intensity of headache (Odd Ratio: 1.50; 95% confidence interval: 1.03, 2.19). At bedtime, adolescents with a measured exposure in the highest quartile during afternoon hours reported a statistically significant higher intensity of irritation in the evening (4th quartile 1.79; 1.23, 2.61), while children reported a statistically significant higher intensity of concentration problems (4th quartile 1.55; 1.02, 2.33). CONCLUSIONS: *We observed few statistically significant results which are not consistent over the two time points.* Furthermore, when the 10% of the participants with the highest exposure are taken into consideration the significant results of the main analysis could not be confirmed. Based on the pattern of these results, we assume

that the few observed significant associations are not causal but rather occurred by chance.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J.

Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. *Bioelectromagnetics*. 28(2):99-108, 2007. (CS, CH, LI)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Hirose H, Sasaki A, Ishii N, Sekijima M, Iyama T, Nojima T, Ugawa Y. 1950 MHz IMT-2000 field does not activate microglial cells in vitro. *Bioelectromagnetics*. 31(2):104-112, 2010. (CS, CC)

Given the widespread use of the cellular phone today, investigation of potential biological effects of radiofrequency (RF) fields has become increasingly important. In particular, much research has been conducted on RF effects on brain function. To examine any biological effects on the central nervous system (CNS) induced by 1950 MHz modulation signals, which are controlled by the International Mobile Telecommunication-2000 (IMT-2000) cellular system, we investigated the effect of RF fields on microglial cells in the brain. We assessed functional changes in microglial cells by examining changes in immune reaction-related molecule expression and cytokine production after exposure to a 1950 MHz Wideband Code Division Multiple Access (W-

CDMA) RF field, at specific absorption rates (SARs) of 0.2, 0.8, and 2.0 W/kg. Primary microglial cell cultures prepared from neonatal rats were subjected to an RF or sham field for 2 h. Assay samples obtained 24 and 72 h after exposure were processed in a blind manner. Results showed that the percentage of cells positive for major histocompatibility complex (MHC) class II, which is the most common marker for activated microglial cells, was similar between cells exposed to W-CDMA radiation and sham-exposed controls. No statistically significant differences were observed between any of the RF field exposure groups and the sham-exposed controls in percentage of MHC class II positive cells. Further, no remarkable differences in the production of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and interleukin-6 (IL-6) were observed between the test groups exposed to W-CDMA signal and the sham-exposed negative controls. These findings suggest that exposure to RF fields up to 2 W/kg does not activate microglial cells in vitro.

(E) Hountala CD, Maganioti AE, Papageorgiou CC, Nanou ED, Kyprianou MA, Tsiafakis VG, Rabavilas AD, Capsalis CN. The spectral power coherence of the EEG under different EMF conditions. Neurosci Lett. 441(2):188-192, 2008. (HU, EE)

The present study introduces the concept of spectral power coherence (SPC), which reflects the pattern of coordination of the four basic EEG bands (delta, theta, alpha, and beta) at a specific location of the brain. The SPC was calculated for the pre-stimulus EEG signal during an auditory memory task under different electromagnetic field (EMF) conditions (900 MHz and 1800 MHz). The results showed that delta rhythm is less consequential in the overall cooperation between the bands than the higher frequency theta, alpha and beta rhythms. Additionally, it has been shown that the radiation effect on SPC is different for the two genders. In the absence of radiation males exhibit higher overall SPC than females. These differences disappear in the presence of 900 MHz and are reversed in the presence of 1800 MHz.

(E) Hung CS, Anderson C, Horne JA, McEvoy P. Mobile phone 'talk-mode' signal delays EEG-determined sleep onset. Neurosci Lett. 421(1):82-86, 2007. (HU, EE, BE, WS, SL)

Mobile phones signals are pulse-modulated microwaves, and EEG studies suggest that the extremely low-frequency (ELF) pulse modulation has sleep effects. However, 'talk', 'listen' and 'standby' modes differ in the ELF (2, 8, and 217Hz) spectral components and specific absorption rates, but no sleep study has differentiated these modes. We used a GSM900 mobile phone controlled by a base-station simulator and a test SIM card to simulate these three specific modes, transmitted at 12.5% (23dBm) of maximum power. At weekly intervals, 10 healthy young adults, sleep restricted to 6h, were randomly and single-blind exposed to one of: talk, listen, standby and sham (nil signal) modes, for 30 min, at 13:30 h, whilst lying in a sound-proof, lit bedroom, with a thermally insulated silent phone beside the right ear. Bipolar EEGs were recorded continuously, and subjective ratings of sleepiness obtained every 3 min (before, during and after exposure). After exposure the phone and base-station were switched off, the bedroom

darkened, and a 90 min sleep opportunity followed. We report on sleep onset using: (i) visually scored latency to onset of stage 2 sleep, (ii) EEG power spectral analysis. There was no condition effect for subjective sleepiness. Post-exposure, sleep latency after talk mode was markedly and significantly delayed beyond listen and sham modes. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset. It is possible that 2, 8, 217Hz modulation may differentially affect sleep onset.

(E) İkinci A, Odacı E, Yıldırım M, Kaya H, Akça M, Hancı H, Aslan A, Sönmez OF, Baş O. The Effects of Prenatal Exposure to a 900 Megahertz Electromagnetic Field on Hippocampus Morphology and Learning Behavior in Rat Pups. NeuroQuantology. 11(4):582-590, 2013. (AS, BE, ME, CE, DE)

The purpose of this study was to examine the effect on hippocampus morphology and learning behavior in rat pups following prenatal exposure to a 900 megahertz (MHz) electromagnetic field (EMF). Female Sprague Dawley rats weighing 180-250 g were left to mate with males. The following day, pregnant rats identified as such by the vaginal smear test were divided into two groups, control (n=3) and EMF (n=3). No procedures were performed on the control group. The rats in the EMF group were exposed to 900 MHz EMF on days 13 to 21 of pregnancy, for 1 h a day. Female rat pups were removed from their mothers at 22 days old. We then established two newborn rat groups, a 13 member control group and a 10 member EMF group. Radial arm maze and passive avoidance tests were used to measure rat pups' learning and memory performance. All rats were decapitated on the postnatal 32nd day. Routine histological procedures were performed on the brain tissues, and sections were stained with Cresyl fast violet. The radial arm maze (p=0.007) and passive avoidance (p=0.032) tests were administered to both groups under identical conditions, and compromised learning behavior was determined in the EMF group rats. Morphological compromise was also determined in the EMF group sections. Our results show that the application of a 900 MHz EMF in the prenatal period adversely affected female pups' learning behavior and also resulted in histopathological changes appearing in the hippocampus.

(E) Imge EB, Kiliçoğlu B, Devrim E, Cetin R, Durak I. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C - a preliminary study. Int J Radiat Biol. 86(12):1044-1049, 2010. (AS, CE, CH, OX)

PURPOSE: To evaluate effects of mobile phone use on brain tissue and a possible protective role of vitamin C. **MATERIALS AND METHODS:** Forty female rats were divided into four groups randomly (Control, mobile phone, mobile phone plus vitamin C and, vitamin C alone). The mobile phone group was exposed to a mobile phone signal

(900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated with vitamin C administered orally (per os). The vitamin C group was also treated with vitamin C per os for four weeks. Then, the animals were sacrificed and brain tissues were dissected to be used in the analyses of malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'-nucleotidase (5'-NT). **RESULTS:** Mobile phone use caused an inhibition in 5'-NT and CAT activities as compared to the control group. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. **CONCLUSION:** Our results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

(NE) Inomata-Terada S, Okabe S, Arai N, Hanajima R, Terao Y, Frubayashi T, Ugawa Y. Effects of high frequency electromagnetic field (EMF) emitted by mobile phones on the human motor cortex. Bioelectromagnetics. 28(7):553-561, 2007. (HU, EE)

We investigated whether the pulsed high frequency electromagnetic field (EMF) emitted by a mobile phone has short term effects on the human motor cortex. We measured motor evoked potentials (MEPs) elicited by single pulse transcranial magnetic stimulation (TMS), before and after mobile phone exposure (active and sham) in 10 normal volunteers. Three sites were stimulated (motor cortex (CTX), brainstem (BST) and spinal nerve (Sp)). The short interval intracortical inhibition (SICI) of the motor cortex reflecting GABAergic interneuronal function was also studied by paired pulse TMS method. MEPs to single pulse TMS were also recorded in two patients with multiple sclerosis showing temperature dependent neurological symptoms (hot bath effect). Neither MEPs to single pulse TMS nor the SICI was affected by 30 min of EMF exposure from mobile phones or sham exposure. In two MS patients, mobile phone exposure had no effect on any parameters of MEPs even though conduction block occurred at the corticospinal tracts after taking a bath. As far as available methods are concerned, we did not detect any short-term effects of 30 min mobile phone exposure on the human motor cortical output neurons or interneurons even though we can not exclude the possibility that we failed to detect some mild effects due to a small sample size in the present study. This is the first study of MEPs after electromagnetic exposure from a mobile phone in neurological patients.

(NE) Irlenbusch L, Bartsch B, Cooper J, Herget I, Marx B, Raczek J, Thoss F. Influence of a 902.4 MHz GSM signal on the human visual system: investigation of the discrimination threshold. Bioelectromagnetics. 28(8):648-654, 2007. (HU, EE, LI)

The proximity of a mobile phone to the human eye raises the question as to whether radiofrequency (RF) electromagnetic fields (EMF) affect the visual system. A basic characteristic of the human eye is its light sensitivity, making the visual discrimination

threshold (VDThr) a suitable parameter for the investigation of potential effects of RF exposure on the eye. The VDThr was measured for 33 subjects under standardized conditions. Each subject took part in two experiments (RF-exposure and sham-exposure experiment) on different days. In each experiment, the VDThr was measured continuously in time intervals of about 10 s for two periods of 30 min, having a break of 5 min in between. The sequence of the two experiments was randomized, and the study was single blinded. During the RF exposure, a GSM signal of 902.4 MHz (pulsed with 217 Hz) was applied to the subjects. The power flux density of the electromagnetic field at the subject location (in the absence of the subject) was 1 W/m², and numerical dosimetry calculations determined corresponding maximum local averaged specific absorption rate (SAR) values in the retina of $SAR(1\text{ g}) = 0.007\text{ W/kg}$ and $SAR(10\text{ g}) = 0.003\text{ W/kg}$. No statistically significant differences in the VDThr were found in comparing the data obtained for RF exposure with those for sham exposure.

(E) Jing J, Yuhua Z, Xiao-qian Y, Rongping J, Dong-mei G, Xi C. The influence of microwave radiation from cellular phone on fetal rat brain. Electromagn Biol Med. 31(1):57-66, 2012. (AS, CE, CH, OX, DE)

The increasing use of cellular phones in our society has brought focus on the potential detrimental effects to human health by microwave radiation. The aim of our study was to evaluate the intensity of oxidative stress and the level of neurotransmitters in the brains of fetal rats chronically exposed to cellular phones. The experiment was performed on pregnant rats exposed to different intensities of microwave radiation from cellular phones. Thirty-two pregnant rats were randomly divided into four groups: CG, GL, GM, and GH. CG accepted no microwave radiation, GL group radiated 10 min each time, GM group radiated 30 min, and GH group radiated 60 min. The 3 experimental groups were radiated 3 times a day from the first pregnant day for consecutively 20 days, and on the 21st day, the fetal rats were taken and then the contents of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), noradrenaline (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in the brain were assayed. Compared with CG, there were significant differences ($P < 0.05$) found in the contents of SOD, GSH-Px, and MDA in GM and GH; the contents of SOD and GSH-Px decreased and the content of MDA increased. The significant content differences of NE and DA were found in fetal rat brains in GL and GH groups, with the GL group increased and the GH group decreased. Through this study, we concluded that receiving a certain period of microwave radiation from cellular phones during pregnancy has certain harm on fetal rat brains.

(E) Jorge-Mora T, Folgueiras MA, Leiro-Vidal JM, Jorge-Barreiro FJ, Ares-Pena FJ, López-Martin E. Exposure to 2.45 GHz Microwave Radiation Provokes Cerebral Changes in Induction of Hsp-90 α/β Heat Shock Protein in Rat. Prog Electromagn Res, 100:351-379, 2010. (AS, CC, CH)

Physical agents such as non-ionizing continuous-wave 2.45 GHz radiation may cause damage that alters cellular homeostasis and may trigger activation of the genes that encode heat shock proteins (HSP). We used Enzyme-Linked ImmunoSorbent Assay (ELISA) and immunohistochemistry to analyze the changes in levels of HSP-90 and its distribution in the brain of Sprague-Dawley rats, ninety minutes and twenty-four hours after acute (30min) continuous exposure to 2.45 GHz radiation in a the Gigahertz Transverse Electromagnetic (GTEM cell). In addition, we studied further indicators of neuronal insult: dark neurons, chromatin condensation and nucleus fragmentation, which were observed under optical conventional or fluorescence microscopy after DAPI staining. The cellular distribution of protein HSP-90 in the brain increased with each corresponding SAR (0.034 ± 3.10^{-3} , 0.069 ± 5.10^{-3} , 0.27 ± 21.10^{-3} W/kg), in hypothalamic nuclei, limbic cortex and somatosensorial cortex after exposure to the radiation. At twenty-four hours post-irradiation, levels of HSP-90 protein remained high in all hypothalamic nuclei for all SARs, and in the parietal cortex, except the limbic system, HSP-90 levels were lower than in non-irradiated rats, almost half the levels in rats exposed to the highest power radiation. Non-apoptotic cellular nuclei and some dark neurons were found ninety minutes and twenty-four hours after maximal SAR exposure. The results suggest that acute exposure to electromagnetic fields triggered an imbalance in anatomical HSP- 90 levels but the anti-apoptotic mechanism is probably sufficient to compensate the non-ionizing stimulus. Further studies are required to determine the regional effects of chronic electromagnetic pollution on heat shock proteins and their involvement in neurological processes and neuronal damage.

(NE) Joubert V, Leveque P, Cueille M, Bourthoumieu S, Yardin C. No apoptosis is induced in rat cortical neurons exposed to GSM phone fields. Bioelectromagnetics. 28(2):115-121, 2007. (CS, CC)

The aim of this study was to investigate the radiofrequency (RF) electromagnetic fields (EMF) effects on neuronal apoptosis in vitro. Primary cultured neurons from cortices of embryonic Wistar rats were exposed to a 900-MHz global system for mobile communication (GSM) RF field for 24 h in a wire-patch cell. The average-specific absorption rate (SAR) used was 0.25 W/kg. Apoptosis rate was assessed immediately or 24 h after exposure using three methods: (i) DAPI staining; (ii) flow cytometry using double staining with TdT-mediated dUTP nick-end labeling (TUNEL) and propidium iodide (PI); and (iii) measurement of caspase-3 activity by fluorimetry. No statistically significant difference in the apoptosis rate was observed between controls and 24 h GSM-exposed neurons, either 0 h or 24 h post-exposure. All three methods used to assess apoptosis were concordant. These results showed that, under the conditions of experiment used, GSM-exposure does not significantly increase the apoptosis rate in rat primary neuronal cultures. This work is in accordance with other studies performed on cell lines and, to our knowledge, is the first one performed on cultured cortical neurons.

****(E) Joubert, V., Bourthoumieu, S., Leveque, P. and Yardin, C. Apoptosis is Induced by Radiofrequency Fields through the Caspase-Independent Mitochondrial Pathway in Cortical Neurons. Radiat. Res. 169, 38-45, 2008. (CS, CC)**

In the present study, we investigated whether continuous-wave (CW) radiofrequency (RF) fields induce neuron apoptosis in vitro. Rat primary neuronal cultures were exposed to a CW 900 MHz RF field with a specific absorption rate (SAR) of 2 W/kg for 24 h. During exposure, an increase of 2 degrees C was measured in the medium; control experiments with neurons exposed to 39 degrees C were then performed. Apoptosis was assessed by condensation of nuclei with 4',6-diamino-2-phenylindole (DAPI) staining observed with an epifluorescence microscope and fragmentation of DNA with TdT-mediated dUTP nick-end labeling (TUNEL) analyzed by flow cytometry. A statistically significant difference in the rate of apoptosis was found in the RF-field-exposed neurons compared to the sham-, 37 degrees C- and 39 degrees C-exposed neurons either 0 or 24 h after exposure using both methods. To assess whether the observed apoptosis was caspase-dependent or -independent, assays measuring caspase 3 activity and apoptosis-inducing factor (AIF) labeling were performed. No increase in the caspase 3 activity was found, whereas the percentage of AIF-positive nuclei in RF-field-exposed neurons was increased by three- to sevenfold compared to other conditions. Our results show that, under the experimental conditions used, exposure of primary rat neurons to CW RF fields may induce a caspase-independent pathway to apoptosis that involves AIF.

(E) Júnior LC, Guimarães ED, Musso CM, Stabler CT, Garcia RM, Mourão-Júnior CA, Andreazzi AE. Behavior and memory evaluation of Wistar rats exposed to 1.8 GHz radiofrequency electromagnetic radiation. Neurol Res. 2014 Jan 27;1743132813Y0000000276. [Epub ahead of print] (AS, CE, BE)

Background: The development of communication systems has brought great social and economic benefits to society. As mobile phone use has become widespread, concerns have emerged regarding the potential adverse effects of radiofrequency electromagnetic radiation (RF-EMR) used by these devices. Objective: To verify potential effects of mobile phone radiation on the central nervous system (CNS) in an animal model. Methods: Male Wistar rats (60 days old) were exposed to RF-EMR from a Global System for Mobile (GSM) cell phone (1.8 GHz) for 3 days. At the end of the exposure, the following behavioral tests were performed: open field and object recognition. Results: Our results showed that exposed animals did not present anxiety patterns or working memory impairment, but stress behavior actions were observe. Conclusion: Given the results of the present study, we speculate that RF-EMR does not promote CNS impairment, but suggest that it may lead to stressful behavioral patterns.

(NE) Kang KA, Lee HC, Lee JJ, Hong MN, Park MJ, Lee YS, Choi HD, Kim N, Ko YK, Lee JS. Effects of combined radiofrequency radiation exposure on levels of reactive oxygen species in neuronal cells. J Radiat Res. 2013 Oct 8. [Epub ahead of print] (CS, OX, IA)

The objective of this study was to investigate the effects of the combined RF radiation (837 MHz CDMA plus 1950 MHz WCDMA) signal on levels of intracellular reactive oxygen species (ROS) in neuronal cells. Exposure of the combined RF signal was conducted at specific absorption rate values of 2 W/kg of CDMA plus 2 W/kg of WCDMA for 2 h. Co-exposure to combined RF radiation with either H₂O₂ or menadione was also performed. The experimental exposure groups were incubator control, sham-exposed, combined RF radiation-exposed with or without either H₂O₂ or menadione groups. The intracellular ROS level was measured by flow cytometry using the fluorescent probe dichlorofluorescein diacetate. Intracellular ROS levels were not consistently affected by combined RF radiation exposure alone in a time-dependent manner in U87, PC12 or SH-SY5Y cells. In neuronal cells exposed to combined RF radiation with either H₂O₂ or menadione, intracellular ROS levels showed no statically significant alteration compared with exposure to menadione or H₂O₂ alone. These findings indicate that neither combined RF radiation alone nor combined RF radiation with menadione or H₂O₂ influences the intracellular ROS level in neuronal cells such as U87, PC12 or SH-SY5Y.

(E) Kaprana AE, Chimona TS, Papadakis CE, Velegrakis SG, Vardiambasis IO, Adamidis G, Velegrakis GA. Auditory brainstem response changes during exposure to GSM-900 radiation: an experimental study. Audiol Neurootol. 16(4):270-276, 2011. (HU, EE)

The objective of the present study was to investigate the possible electrophysiological time-related changes in auditory pathway during mobile phone electromagnetic field exposure. Thirty healthy rabbits were enrolled in an experimental study of exposure to GSM-900 radiation for 60 min and auditory brainstem responses (ABRs) were recorded at regular time-intervals during exposure. The study subjects were radiated via an adjustable power and frequency radio transmitter for GSM-900 mobile phone emission simulation, designed and manufactured according to the needs of the experiment. The mean absolute latency of waves III-V showed a statistically significant delay ($p < 0.05$) after 60, 45 and 15 min of exposure to electromagnetic radiation of 900 MHz, respectively. Interwave latency I-III was found to be prolonged after 60 min of radiation exposure in correspondence to wave III absolute latency delay. Interwave latencies I-V and III-V were found with a statistically significant delay ($p < 0.05$) after 30 min of radiation. No statistically significant delay was found for the same ABR parameters in recordings from the ear contralateral to the radiation source at 60 min radiation exposure compared with baseline ABR. The ABR measurements returned to baseline recordings 24 h after the exposure to electromagnetic radiation of 900 MHz. The

prolongation of interval latencies I-V and III-V indicates that exposure to electromagnetic fields emitted by mobile phone can affect the normal electrophysiological activity of the auditory system, and these findings fit the pattern of general responses to a stressor.

(E) Karaca E, Durmaz B, Aktug H, Yildiz T, Guducu C, Irgi M, Koksai MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol. 106(1):53-58, 2012. (CS, CH)

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorption rate (SAR) 0.725 W/kg signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNI) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNI rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Kumar S, Behari J. 900-MHz microwave radiation promotes oxidation in rat brain. Electromagn Biol Med. 30(4):219-234, 2011. (AS, CE, CH, OX)

Recently, there have been several reports referring to detrimental effects due to radio frequency electromagnetic fields (RF-EMF) exposure. Special attention was given to investigate the effect of mobile phone exposure on the rat brain. Since the integrative mechanism of the entire body lies in the brain, it is suggestive to analyze its biochemical aspects. For this, 35-day old Wistar rats were exposed to a mobile phone for 2 h per day for a duration of 45 days where specific absorption rate (SAR) was 0.9 W/Kg. Animals were divided in two groups: sham exposed (n = 6) and exposed group (n = 6). Our observations indicate a significant decrease (P < 0.05) in the level of glutathione peroxidase, superoxide dismutase, and an increase in catalase activity. Moreover, protein kinase shows a significant decrease in exposed group (P < 0.05) of hippocampus and whole brain. Also, a significant decrease (P < 0.05) in the level of pineal melatonin and a significant increase (P < 0.05) in creatine kinase and caspase 3 was observed in exposed group of whole brain as compared with sham exposed. Finally, a significant increase in the level of ROS (reactive oxygen species) (P < 0.05) was also recorded. The study concludes that a reduction or an increase in antioxidative enzyme activities, protein kinase C, melatonin, caspase 3, and creatine kinase are related to overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications.

(E) Khullar S1, Sood A2, Sood S3. Auditory Brainstem Responses and EMFs Generated by Mobile Phones. Indian J Otolaryngol Head Neck Surg. 65(Suppl 3):645-649, 2013. (HU, EE)

There has been a manifold increase in the number of mobile phone users throughout the world with the current number of users exceeding 2 billion. However this advancement in technology like many others is accompanied by a progressive increase in the frequency and intensity of electromagnetic waves without consideration of the health consequences. The aim of our study was to advance our understanding of the potential adverse effects of GSM mobile phones on auditory brainstem responses (ABRs). 60 subjects were selected for the study and divided into three groups of 20 each based on their usage of mobile phones. Their ABRs were recorded and analysed for latency of waves I-V as well as interpeak latencies I-III, I-V and III-V (in ms). Results revealed no significant difference in the ABR parameters between group A (control group) and group B (subjects using mobile phones for maximum 30 min/day for 5 years). However the latency of waves was significantly prolonged in group C (subjects using mobile phones for 10 years for a maximum of 30 min/day) as compared to the control group. Based on our findings we concluded that long term exposure to mobile phones may affect conduction in the peripheral portion of the auditory pathway. However more research needs to be done to study the long term effects of mobile phones particularly of newer technologies like smart phones and 3G.

(NE) Kim HS, An YS, Paik MJ, Lee YS, Choi HD, Kim BC, Paek JK, Kim N, Ahn YH. The effects of exposure to 915 MHz radiofrequency identification on cerebral glucose metabolism in rat: A [F-18] FDG micro-PET study. Int J Radiat Biol. 2013 May 7. [Epub ahead of print] (AS, CE, CC, CH)

Purpose: We investigated the effect of whole-body exposure to 915-MHz radiofrequency identification (RFID) on rat cortical glucose metabolism by using ¹⁸F-deoxyglucose positron emission tomography (FDG-PET). Materials and methods: Male Sprague-Dawley rats were divided into three groups: Cage-control, sham-exposed and RFID-exposed groups. Rats were exposed to the 915-MHz RFID for 8 h daily, 5 days per week, for 2 or 16 weeks. The whole-body average specific absorption rate (SAR) was 4 W/kg for the field of the 915 MHz RFID signal. FDG-PET images were obtained the day after RFID exposure, using micro-PET with a FDG tracer. With a Xeleris functional imaging workstation, absolute values in regions of interest (ROI) in the frontal, temporal and parietal cortexes and cerebellum were measured. Cortical ROI values were normalized to the cerebellar value and compared. Results: The data showed that the relative cerebral glucose metabolic rate was unchanged in the frontal, temporal and parietal cortexes of the 915 MHz RFID-exposed rats, compared with rats in cage-control and sham-exposed groups. Conclusion: Our results suggest that 915 MHz RFID radiation exposure did not cause a significant long lasting effect on glucose metabolism in the rat brain.

(NE) Kim TH, Huang TQ, Jang JJ, Kim MH, Kim HJ, Lee JS, Pack JK, Seo JS, Park WY. Local exposure of 849 MHz and 1763 MHz radiofrequency radiation to mouse heads does not induce cell death or cell proliferation in brain. Exp Mol Med. 40(3):294-303, 2008. (AS, CE, CC) Erratum in: Exp Mol Med. 2008 Aug 31;40(4):477. Kim, Tae-Hyung [corrected to Kim, Tae-Hyung].

Even though there is no direct evidence to prove the cellular and molecular changes induced by radiofrequency (RF) radiation itself, we cannot completely exclude the possibility of any biological effect of mobile phone frequency radiation. We established a carousel-type exposure chamber for 849 MHz or 1763 MHz of mobile phone RF radiation to expose RF to the heads of C57BL mice. In this chamber, animals were irradiated intermittently at 7.8 W/kg for a maximum of 12 months. During this period, the body weights of 3 groups-sham, 849 MHz RF, and 1763 MHz RF-did not show any differences between groups. The brain tissues were obtained from 3 groups at 6 months and 12 months to examine the differences in histology and cell proliferation between control and RF exposure groups, but we could not find any change upon RF radiation. Likewise, we could not find changes in the expression and distribution of NeuN and GFAP in hippocampus and cerebellum, or in cell death by TUNEL assay in RF exposure groups. From these data, we conclude that the chronic exposure to 849 MHz and 1763 MHz RF radiation at a 7.8 W/kg specific absorption rate (SAR) could not induce cellular alterations such as proliferation, death, and reactive gliosis.

(NE) Kleinlogel H, Dierks T, Koenig T, Lehmann H, Minder A, Berz R. Effects of weak mobile phone - electromagnetic fields (GSM, UMTS) on well-being and resting EEG. Bioelectromagnetics. 29(6):479-487, 2008a. (HU, BE, EE)

Modern mobile phones emit electromagnetic fields (EMFs) ranging from 900 to 2000 MHz which are suggested to have an influence on well-being, attention and neurological parameters in mobile phone users. To date most studies have investigated Global System for Mobile Communications (GSM)-EMF and only very few studies were concerned with Universal Mobile Telecommunications System (UMTS)-EMF. Consequently, we tested the effects of both types of EMF, 1950 MHz UMTS (SAR 0.1 and 1 W/kg) and pulsed 900 MHz GSM (1 W/kg), on well-being and vigilance-controlled resting electroencephalogram (eyes closed) in 15 healthy, right-handed subjects. A double-blind, randomised, crossover application of the test procedure was used. Neither the UMTS- nor the GSM-EMF produced any significant changes in the measured parameters compared to sham exposure. The results do not give any evidence for a deleterious effect of the EMF on normal healthy mobile phone users.

(NE) Kleinlogel H, Dierks T, Koenig T, Lehmann H, Minder A, Berz R. Effects of weak mobile phone - electromagnetic fields (GSM, UMTS) on event related potentials and cognitive functions. Bioelectromagnetics. 29(6):488-497, 2008b. (HU, EE, BE)

Modern mobile phones emit electromagnetic fields (EMF) ranging from 900 to 2000 MHz which are suggested to have an influence on well-being, attention and neurological parameters in mobile phone users. Until now most studies have investigated Global System for Mobile Communications (GSM)-EMF and only very few studies have focused on Universal Mobile Telecommunications System (UMTS)-EMF. Therefore, we tested the effects of both types of unilaterally presented EMF, 1950 UMTS (0.1 and 1 W/kg) and pulsed 900 MHz GSM (1 W/kg), on visually evoked occipital P100, the P300 of a continuous performance test, auditory evoked central N100 and the P300 during an oddball task as well as on the respective behavioral parameters, reaction time and false reactions, in 15 healthy, right handed subjects. A double-blind, randomized, crossover application of the test procedure was used. Neither the UMTS- nor the GSM-EMF produced any significant changes in the measured parameters compared to sham exposure. The results do not give any evidence for a deleterious effect of the EMF on normal healthy mobile phone users.

(E) Köktürk S, Yardimoglu M, Celikozlu SD, Dolanbay EG, Cimbiz A. Effect of *Lycopersicon esculentum* extract on apoptosis in the rat cerebellum, following prenatal and postnatal exposure to an electromagnetic field. *Exp Ther Med.* 6(1):52-56, 2013. (AS, CE, DE, CC)

The expansion of mobile phone technology has raised concerns regarding the effect of 900-MHz electromagnetic field (EMF) exposure on the central nervous system. At present, the developing human brain is regularly exposed to mobile telephones, pre- and postnatally. Several studies have demonstrated the acute effects of EMF exposure during pre- or postnatal periods; however, the chronic effects of EMF exposure are less understood. Thus, the aim of the present study was to determine the chronic effects of EMF on the pre- and postnatal rat cerebellum. The control group was maintained in the same conditions as the experimental groups, without the exposure to EMF. In the EMF1 group, the rats were exposed to EMF during pre- and postnatal periods (until postnatal day 80). In the EMF2 group, the rats were also exposed to EMF pre- and postnatally; in addition, however, they were provided with a daily oral supplementation of *Lycopersicon esculentum* extract (2 g/kg). The number of caspase-3-labeled Purkinje neurons and granule cells present in the rats in the control and experimental groups were then counted. The neurodegenerative changes were studied using cresyl violet staining, and these changes were evaluated. In comparison with the control animals, the EMF1 group demonstrated a significant increase in the number of caspase-3-labeled Purkinje neurons and granule cells present in the cerebellum ($P < 0.001$). However, in comparison with the EMF1 group, the EMF2 group exhibited significantly fewer caspase-3-labeled Purkinje neurons and granule cells in the cerebellum. In the EMF1 group, the Purkinje neurons were revealed to have undergone dark neuron degenerative changes. However, the presence of dark Purkinje neurons was reduced in the EMF2 group, compared with the EMF1 group. The results indicated that apoptosis and

neurodegeneration in rats exposed to EMF during pre- and postnatal periods may be reduced with *Lycopersicon esculentum* extract therapy.

(NE) Krause CM, Pesonen M, Haarala Björnberg C, Hämäläinen H. Effects of pulsed and continuous wave 902 MHz mobile phone exposure on brain oscillatory activity during cognitive processing. *Bioelectromagnetics*. 28(4):296-308, 2007. (HU, EE)

The aim of the current double-blind studies was to partially replicate the studies by Krause et al. [2000ab, 2004] and to further investigate the possible effects of electromagnetic fields (EMF) emitted by mobile phones (MP) on the event-related desynchronisation/synchronisation (ERD/ERS) EEG (electroencephalogram) responses during cognitive processing. Two groups, both consisting of 36 male participants, were recruited. One group performed an auditory memory task and the other performed a visual working memory task in six exposure conditions: SHAM (no EMF), CW (continuous wave EMF) and PM (pulse modulated EMF) during both left- and right-side exposure, while the EEG was recorded. In line with our previous studies, we observed that the exposure to EMF had modest effects on brain oscillatory responses in the alpha frequency range (approximately 8-12 Hz) and had no effects on the behavioural measures. The effects on the EEG were, however, varying, unsystematic and inconsistent with previous reports. We conclude that the effects of EMF on brain oscillatory responses may be subtle, variable and difficult to replicate for unknown reasons.

(E) Kumar RS, Sareesh NN, Nayak S, Mailankot M. Hypoactivity of Wistar rats exposed to mobile phone on elevated plus maze. *Indian J Physiol Pharmacol*. 53(3):283-286, 2009. (AS, BE)

No abstract available. From discussion section: "In conclusion, our preliminary results indicate mobile phone exposure induced behavioral changes in rats, expressed as deficit in open arm exploration on elevated plus-maze."

(E) Kumlin T, Iivonen H, Miettinen P, Juvonen A, van Groen T, Puranen L, Pitkääho R, Juutilainen J, Tanila H. Mobile phone radiation and the developing brain: behavioral and morphological effects in juvenile rats. *Radiat Res*. 168(4):471-479, 2007. (AS, CE, ME, BE)

The increasing use of mobile phones by children and teenagers has raised concerns about their safety. Addressing such concerns is difficult, because no data are available on possible effects from long-term exposure to radiofrequency (RF) fields during the development of the nervous system. Possible morphological and functional changes were evaluated in the central nervous system of young male Wistar rats exposed to 900 MHz mobile phone signal for 2 h/day on 5 days/week. After 5 weeks of exposure at whole-body average specific energy absorption rates of 0.3 or 3.0 W/kg or sham exposure, six rats per group were examined histologically, and the remaining 18 rats per

group were subjected to behavioral tests. No degenerative changes, dying neurons, or effects on the leakage of the blood-brain barrier were detected. No group differences were observed in the open-field test, plus maze test or acoustic startle response tests. In the water maze test, however, significantly improved learning (P = 0.012) and memory (P = 0.01) were detected in rats exposed to RF fields. The results do not indicate a serious threat to the developing brain from mobile phone radiation at intensities relevant to human exposure. However, the interesting finding of improved learning and memory warrants further studies.

(NE) Kwon MS, Jääskeläinen SK, Toivo T, Hämäläinen H. No effects of mobile phone electromagnetic field on auditory brainstem response. Bioelectromagnetics. 31(1):48-55, 2010a. (HU, EE)

The present study investigated the possible effects of the electromagnetic field (EMF) emitted by an ordinary GSM mobile phone (902.4 MHz pulsed at 217 Hz) on brainstem auditory processing. Auditory brainstem responses (ABR) were recorded in 17 healthy young adults, without a mobile phone at baseline, and then with a mobile phone on the ear under EMF-off and EMF-on conditions. The amplitudes, latencies, and interwave intervals of the main ABR components (waves I, III, V) were compared among the three conditions. ABR waveforms showed no significant differences due to exposure, suggesting that short-term exposure to mobile phone EMF did not affect the transmission of sensory stimuli from the cochlea up to the midbrain along the auditory nerve and brainstem auditory pathways.

(NE) Kwon MS, Huotilainen M, Shestakova A, Kujala T, Näätänen R, Hämäläinen H. No effects of mobile phone use on cortical auditory change-detection in children: an ERP study. Bioelectromagnetics. 31(3):191-199, 2010b. (HU, EE)

We investigated the effect of mobile phone use on the auditory sensory memory in children. Auditory event-related potentials (ERPs), P1, N2, mismatch negativity (MMN), and P3a, were recorded from 17 children, aged 11-12 years, in the recently developed multi-feature paradigm. This paradigm allows one to determine the neural change-detection profile consisting of several different types of acoustic changes. During the recording, an ordinary GSM (Global System for Mobile Communications) mobile phone emitting 902 MHz (pulsed at 217 Hz) electromagnetic field (EMF) was placed on the ear, over the left or right temporal area (SAR(1g) = 1.14 W/kg, SAR(10g) = 0.82 W/kg, peak value = 1.21 W/kg). The EMF was either on or off in a single-blind manner. We found that a short exposure (two 6 min blocks for each side) to mobile phone EMF has no statistically significant effects on the neural change-detection profile measured with the MMN. Furthermore, the multi-feature paradigm was shown to be well suited for studies of perception accuracy and sensory memory in children. However, it should be noted that the present study only had sufficient statistical power to detect a large effect size.

(NE) Kwon MS, Vorobyev V, Kännälä S, Laine M, Rinne JO, Toivonen T, Johansson J, Teräs M, Joutsa J, Tuominen L, Lindholm H, Alanko T, Hämäläinen H. No effects of short-term GSM mobile phone radiation on cerebral blood flow measured using positron emission tomography. *Bioelectromagnetics*. 33(3):247-256, 2012. (HU, PE)

The present study investigated the effects of 902.4 MHz global system for mobile communications (GSM) mobile phone radiation on cerebral blood flow using positron emission tomography (PET) with the (15) O-water tracer. Fifteen young, healthy, right-handed male subjects were exposed to phone radiation from three different locations (left ear, right ear, forehead) and to sham exposure to test for possible exposure effects on brain regions close to the exposure source. Whole-brain [¹⁵O]H₂O-PET images were acquired 12 times, 3 for each condition, in a counterbalanced order. Subjects were exposed for 5 min in each scan while performing a simple visual vigilance task. Temperature was also measured in the head region (forehead, eyes, cheeks, ear canals) during exposure. The exposure induced a slight temperature rise in the ear canals but did not affect brain hemodynamics and task performance. The results provided no evidence for acute effects of short-term mobile phone radiation on cerebral blood flow.

(E) Kwon MS, Vorobyev V, Kännälä S, Laine M, Rinne JO, Toivonen T, Johansson J, Teräs M, Lindholm H, Alanko T, Hämäläinen H. GSM mobile phone radiation suppresses brain glucose metabolism. *J Cereb Blood Flow Metab*. 31(12):2293-2301, 2011. (HU, PE)

We investigated the effects of mobile phone radiation on cerebral glucose metabolism using high-resolution positron emission tomography (PET) with the (18)F-deoxyglucose (FDG) tracer. A long half-life (109 minutes) of the (18)F isotope allowed a long, natural exposure condition outside the PET scanner. Thirteen young right-handed male subjects were exposed to a pulse-modulated 902.4 MHz Global System for Mobile Communications signal for 33 minutes, while performing a simple visual vigilance task. Temperature was also measured in the head region (forehead, eyes, cheeks, ear canals) during exposure. (18)F-deoxyglucose PET images acquired after the exposure showed that relative cerebral metabolic rate of glucose was significantly reduced in the temporoparietal junction and anterior temporal lobe of the right hemisphere ipsilateral to the exposure. Temperature rise was also observed on the exposed side of the head, but the magnitude was very small. The exposure did not affect task performance (reaction time, error rate). Our results show that short-term mobile phone exposure can locally suppress brain energy metabolism in humans.

(NE) Kwon MS, Kujala T, Huutilainen M, Shestakova A, Näätänen R, Hämäläinen H. Preattentive auditory information processing under exposure to the 902 MHz GSM mobile phone electromagnetic field: a mismatch negativity (MMN) study. *Bioelectromagnetics*. 30(3):241-248, 2009. (HU, EE)

Previous studies on the effects of the mobile phone electromagnetic field (EMF) on various event-related potential (ERP) components have yielded inconsistent and even contradictory results, and often failed in replication. The mismatch negativity (MMN) is an auditory ERP component elicited by infrequent (deviant) stimuli differing in some physical features from the repetitive frequent (standard) stimuli in a sound sequence. The MMN provides a sensitive measure for cortical auditory stimulus feature discrimination, regardless of attention and other contaminating factors. In this study, MMN responses to duration, intensity, frequency, and gap changes were recorded in healthy young adults ($n = 17$), using a multifeature paradigm including several types of auditory change in the same stimulus sequence, while a GSM mobile phone was placed on either ear with the EMF (902 MHz pulsed at 217 Hz; SAR(1g) = 1.14 W/kg, SAR(10g) = 0.82 W/kg, peak value = 1.21 W/kg, measured with an SAM phantom) on or off. An MMN was elicited by all deviant types, while its amplitude and latency showed no significant differences due to EMF exposure for any deviant types. In the present study, we found no conclusive evidence that acute exposure to GSM mobile phone EMF affects cortical auditory change detection processing reflected by the MMN.

(E) Lee KS, Choi JS, Hong SY, Son TH, Yu K. Mobile phone electromagnetic radiation activates MAPK signaling and regulates viability in *Drosophila*. *Bioelectromagnetics*. 29(5):371-379, 2008. (AS, CC)

Mobile phones are widely used in the modern world. However, biological effects of electromagnetic radiation produced by mobile phones are largely unknown. In this report, we show biological effects of the mobile phone 835 MHz electromagnetic field (EMF) in the *Drosophila* model system. When flies were exposed to the specific absorption rate (SAR) 1.6 W/kg, which is the proposed exposure limit by the American National Standards Institute (ANSI), more than 90% of the flies were viable even after the 30 h exposure. However, in the SAR 4.0 W/kg strong EMF exposure, viability dropped from the 12 h exposure. These EMF exposures triggered stress response and increased the production of reactive oxygen species. The EMF exposures also activated extracellular signal regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling, but not p38 kinase signaling. Interestingly, SAR 1.6 W/kg activated mainly ERK signaling and expression of an anti-apoptotic gene, whereas SAR 4.0 W/kg strongly activated JNK signaling and expression of apoptotic genes. In addition, SAR 4.0 W/kg amplified the number of apoptotic cells in the fly brain. These findings demonstrate that the exposure limit on electromagnetic radiation proposed by ANSI triggered ERK-survival signaling but the strong electromagnetic radiation activated JNK-apoptotic signaling in *Drosophila*.

(E) Leung S, Croft RJ, McKenzie RJ, Iskra S, Silber B, Cooper NR, O'Neill B, Cropley V, Diaz-Trujillo A, Hamblin D, Simpson D. Effects of 2G and 3G mobile phones on performance and electrophysiology in adolescents, young adults and older adults. *Clin Neurophysiol*. 122(11):2203-2216, 2011. (HU, AD, BE, EE)

OBJECTIVE: This study examined sensory and cognitive processing in adolescents, young adults and older adults, when exposed to 2nd (2G) and 3rd (3G) generation mobile phone signals. **METHODS:** Tests employed were the auditory 3-stimulus oddball and the N-back. Forty-one 13-15 year olds, forty-two 19-40 year olds and twenty 55-70 year olds were tested using a double-blind cross-over design, where each participant received Sham, 2G and 3G exposures, separated by at least 4 days. **RESULTS:** 3-Stimulus oddball task: Behavioural: accuracy and reaction time of responses to targets were not affected by exposure. Electrophysiological: augmented N1 was found in the 2G condition (independent of age group). N-back task: Behavioural: the combined groups performed less accurately during the 3G exposure (compared to Sham), with post hoc tests finding this effect separately in the adolescents only. Electrophysiological: delayed ERD/ERS responses of the alpha power were found in both 3G and 2G conditions (compared to Sham; independent of age group). **CONCLUSION:** Employing tasks tailored to each individual's ability level, this study provides support for an effect of acute 2G and 3G exposure on human cognitive function. **SIGNIFICANCE:** The subtlety of mobile phone effect on cognition in our study suggests that it is important to account for individual differences in future mobile phone research.

(NE) Lipping T, Rorarius M, Jäntti V, Annala K, Mennander A, Ferenets R, Toivonen T, Toivo T, Värri A, Korpinen L. Using the nonlinear control of anaesthesia-induced hypersensitivity of EEG at burst suppression level to test the effects of radiofrequency radiation on brain function. Nonlinear Biomed Phys. 3(1):5, 2009. (AS, IA, EE)

BACKGROUND: In this study, investigating the effects of mobile phone radiation on test animals, eleven pigs were anaesthetised to the level where burst-suppression pattern appears in the electroencephalogram (EEG). At this level of anaesthesia both human subjects and animals show high sensitivity to external stimuli which produce EEG bursts during suppression. The burst-suppression phenomenon represents a nonlinear control system, where low-amplitude EEG abruptly switches to very high amplitude bursts. This switching can be triggered by very minor stimuli and the phenomenon has been described as hypersensitivity. To test if also radio frequency (RF) stimulation can trigger this nonlinear control, the animals were exposed to pulse modulated signal of a GSM mobile phone at 890 MHz. In the first phase of the experiment electromagnetic field (EMF) stimulation was randomly switched on and off and the relation between EEG bursts and EMF stimulation onsets and endpoints were studied. In the second phase a continuous RF stimulation at 31 W/kg was applied for 10 minutes. The ECG, the EEG, and the subcutaneous temperature were recorded. **RESULTS:** No correlation between the exposure and the EEG burst occurrences was observed in phase I measurements. No significant changes were observed in the EEG activity of the pigs during phase II measurements although several EEG signal analysis methods were applied. The temperature measured subcutaneously from the pigs' head increased by 1.6 degrees C and the heart rate by 14.2 bpm on the average during the 10 min exposure periods. **CONCLUSION:** The hypothesis that RF radiation would produce sensory stimulation of

somatosensory, auditory or visual system or directly affect the brain so as to produce EEG bursts during suppression was not confirmed.

(E) Liu ML, Wen JQ, Fan YB. Potential protection of green tea polyphenols against 1800 MHz electromagnetic radiation-induced injury on rat cortical neurons. Neurotox Res. 20(3):270-276, 2011. (CS, IA, CC, OX)

Radiofrequency electromagnetic fields (EMF) are harmful to public health, but the certain anti-irradiation mechanism is not clear yet. The present study was performed to investigate the possible protective effects of green tea polyphenols against electromagnetic radiation-induced injury in the cultured rat cortical neurons. In this study, green tea polyphenols were used in the cultured cortical neurons exposed to 1800 MHz EMFs by the mobile phone. We found that the mobile phone irradiation for 24 h induced marked neuronal cell death in the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) and TUNEL (TdT mediated biotin-dUTP nicked-end labeling) assay, and protective effects of green tea polyphenols on the injured cortical neurons were demonstrated by testing the content of Bcl-2 Associated X protein (Bax) in the immunoprecipitation assay and Western blot assay. In our study results, the mobile phone irradiation-induced increases in the content of active Bax were inhibited significantly by green tea polyphenols, while the contents of total Bax had no marked changes after the treatment of green tea polyphenols. Our results suggested a neuroprotective effect of green tea polyphenols against the mobile phone irradiation-induced injury on the cultured rat cortical neurons.

(E) Liu YX, Tai JL, Li GQ, Zhang ZW, Xue JH, Liu HS, Zhu H, Cheng JD, Liu YL, Li AM, Zhang Y. Exposure to 1950-MHz TD-SCDMA Electromagnetic Fields Affects the Apoptosis of Astrocytes via Caspase-3-Dependent Pathway. PLoS One. 7(8):e42332, 2012. (CS, CC)

The usage of mobile phone increases globally. However, there is still a paucity of data about the impact of electromagnetic fields (EMF) on human health. This study investigated whether EMF radiation would alter the biology of glial cells and act as a tumor-promoting agent. We exposed rat astrocytes and C6 glioma cells to 1950-MHz TD-SCDMA for 12, 24 and 48 h respectively, and found that EMF exposure had differential effects on rat astrocytes and C6 glioma cells. A 48 h of exposure damaged the mitochondria and induced significant apoptosis of astrocytes. Moreover, caspase-3, a hallmark of apoptosis, was highlighted in astrocytes after 48 h of EMF exposure, accompanied by a significantly increased expression of bax and reduced level of bcl-2. The tumorigenicity assays demonstrated that astrocytes did not form tumors in both control and exposure groups. In contrast, the unexposed and exposed C6 glioma cells show no significant differences in both biological feature and tumor formation ability. Therefore, our results implied that exposure to the EMF of 1950-MHz TD-SCDMA may not promote the tumor formation, but continuous exposure damaged the mitochondria of astrocytes and induce apoptosis through a caspase-3-dependent pathway with the involvement of bax and bcl-2.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, CC, WS)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Loughran SP, McKenzie RJ, Jackson ML, Howard ME, Croft RJ. Individual differences in the effects of mobile phone exposure on human sleep: rethinking the problem. Bioelectromagnetics. 33(1):86-93, 2012. (HU, EE, SL)

Mobile phone exposure-related effects on the human electroencephalogram (EEG) have been shown during both waking and sleep states, albeit with slight differences in the frequency affected. This discrepancy, combined with studies that failed to find effects, has led many to conclude that no consistent effects exist. We hypothesised that these differences might partly be due to individual variability in response, and that mobile phone emissions may in fact have large but differential effects on human brain activity. Twenty volunteers from our previous study underwent an adaptation night followed by two experimental nights in which they were randomly exposed to two conditions (Active and Sham), followed by a full-night sleep episode. The EEG spectral power was increased in the sleep spindle frequency range in the first 30 min of non-rapid eye movement (non-REM) sleep following Active exposure. This increase was more prominent in the participants that showed an increase in the original study. These results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that previous negative results are not strong evidence for a lack of an effect and, given the far-reaching implications of mobile phone research, we may need to rethink the interpretation of results and the manner in which research is conducted in this field.

(NE) Loughran SP, Benz DC, Schmid MR, Murbach M, Kuster N, Achermann P. No increased sensitivity in brain activity of adolescents exposed to mobile phone-like emissions. Clin Neurophysiol. 124(7):1303-1308, 2013. (HU, BE, EE, AD)

OBJECTIVE: To examine the potential sensitivity of adolescents to radiofrequency electromagnetic field (RF EMF) exposures, such as those emitted by mobile phones.

METHODS: In a double-blind, randomized, crossover design, 22 adolescents aged 11-13 years (12 males) underwent three experimental sessions in which they were exposed to mobile phone-like RF EMF signals at two different intensities, and a sham session. During exposure cognitive tasks were performed and waking EEG was recorded at three time-points subsequent to exposure (0, 30 and 60 min). **RESULTS:** No clear significant effects of RF EMF exposure were found on the waking EEG or cognitive performance.

CONCLUSIONS: Overall, the current study was unable to demonstrate exposure-related effects previously observed on the waking EEG in adults, and also provides further support for a lack of an influence of mobile phone-like exposure on cognitive performance. **SIGNIFICANCE:** Adolescents do not appear to be more sensitive than adults to mobile phone RF EMF emissions.

(E) Lowden A, Akerstedt T, Ingre M, Wiholm C, Hillert L, Kuster N, Nilsson JP, Arnetz B. Sleep after mobile phone exposure in subjects with mobile phone-related symptoms. Bioelectromagnetics. 32(1):4-14, 2011. (HU, EE, SL)

Several studies show increases in activity for certain frequency bands (10-14 Hz) and visually scored parameters during sleep after exposure to radiofrequency electromagnetic fields. A shortened REM latency has also been reported. We investigated the effects of a double-blind radiofrequency exposure (884 MHz, GSM signaling standard including non-DTX and DTX mode, time-averaged 10 g psSAR of 1.4 W/kg) on self-evaluated sleepiness and objective EEG measures during sleep. Forty-eight subjects (mean age 28 years) underwent 3 h of controlled exposure (7:30-10:30 PM; active or sham) prior to sleep, followed by a full-night polysomnographic recording in a sleep laboratory. The results demonstrated that following exposure, time in Stages 3 and 4 sleep (SWS, slow-wave sleep) decreased by 9.5 min (12%) out of a total of 78.6 min, and time in Stage 2 sleep increased by 8.3 min (4%) out of a total of 196.3 min compared to sham. The latency to Stage 3 sleep was also prolonged by 4.8 min after exposure. Power density analysis indicated an enhanced activation in the frequency ranges 0.5-1.5 and 5.75-10.5 Hz during the first 30 min of Stage 2 sleep, with 7.5-11.75 Hz being elevated within the first hour of Stage 2 sleep, and bands 4.75-8.25 Hz elevated during the second hour of Stage 2 sleep. No pronounced power changes were observed in SWS or for the third hour of scored Stage 2 sleep. No differences were found between controls and subjects with prior complaints of mobile phone-related symptoms. The results confirm previous findings that RF exposure increased the EEG alpha range in the sleep EEG, and indicated moderate impairment of SWS. Furthermore, reported differences in sensitivity to mobile phone use were not reflected in sleep parameters.

(E) Lu Y, Xu S, He M, Chen C, Zhang L, Liu C, Chu F, Yu Z, Zhou Z, Zhong M. Glucose administration attenuates spatial memory deficits induced by chronic low-power-density microwave exposure. *Physiol Behav.* 106(5):631-637, 2012. (AS, CE, BE)

Extensive evidence indicates that glucose administration attenuates memory deficits in rodents and humans, and cognitive impairment has been associated with reduced glucose metabolism and uptake in certain brain regions including the hippocampus. In the present study, we investigated whether glucose treatment attenuated memory deficits caused by chronic low-power-density microwave (MW) exposure, and the effect of MW exposure on hippocampal glucose uptake. We exposed Wistar rats to 2.45 GHz pulsed MW irradiation at a power density of 1 mW/cm² for 3 h/day, for up to 30 days. MW exposure induced spatial learning and memory impairments in rats. Hippocampal glucose uptake was also reduced by MW exposure in the absence or presence of insulin, but the levels of blood glucose and insulin were not affected. However, these spatial memory deficits were reversed by systemic glucose treatment. Our results indicate that glucose administration attenuates the spatial memory deficits induced by chronic low-power-density MW exposure, and reduced hippocampal glucose uptake may be associated with cognitive impairment caused by MW exposure.

(E) Luria R, Eliyahu I, Hareuveny R, Margaliot M, Meiran N. Cognitive effects of radiation emitted by cellular phones: the influence of exposure side and time. *Bioelectromagnetics.* 30(3):198-204, 2009. (See also Hareuveny et al., 2011) (HU, BE)

This study examined the time dependence effects of exposure to radiofrequency radiation (RFR) emitted by standard GSM cellular phones on the cognitive functions of humans. A total of 48 healthy right-handed male subjects performed a spatial working memory task (that required either a left-hand or a right-hand response) while being exposed to one of two GSM phones placed at both sides of the head. The subjects were randomly divided into three groups. Each group was exposed to one of three exposure conditions: left-side of the head, right-side, or sham-exposure. The experiment consisted of 12 blocks of trials. Response times (RTs) and accuracy of the responses were recorded. It was found that the average RT of the right-hand responses under left-side exposure condition was significantly longer than those of the right-side and sham-exposure groups averaged together during the first two time blocks. These results confirmed the existence of an effect of exposure on RT, as well as the fact that exposure duration (together with the responding hand and the side of exposure) may play an important role in producing detectable RFR effects on performance. Differences in these parameters might be the reason for the failure of certain studies to detect or replicate RFR effects.

(E) Lustenberger C, Murbach M, Durr R, Schmid MR, Kuster N, Achermann P, Huber R. Stimulation of the brain with radiofrequency electromagnetic field pulses affects

sleep-dependent performance improvement. Brain Stimul 6(5):805-811, 2013. (HU, BE, EE, SL)

Background: Sleep-dependent performance improvements seem to be closely related to sleep spindles (12–15 Hz) and sleep slow-wave activity (SWA, 0.75–4.5 Hz). Pulse-modulated radiofrequency electromagnetic fields (RF EMF, carrier frequency 900 MHz) are capable to modulate these electroencephalographic (EEG) characteristics of sleep. Objective: The aim of our study was to explore possible mechanisms how RF EMF affects cortical activity during sleep and to test whether such effects on cortical activity during sleep interact with sleep-dependent performance changes. Methods: Sixteen male subjects underwent 2 experimental nights, one of them with all-night 0.25–0.8 Hz pulsed RF EMF exposure. All-night EEG was recorded. To investigate RF EMF induced changes in overnight performance improvement, subjects were trained for both nights on a motor task in the evening and the morning. Results: We obtained good sleep quality in all subjects under both conditions (mean sleep efficiency > 90%). After pulsed RF EMF we found increased SWA during exposure to pulse-modulated RF EMF compared to sham exposure ($P < 0.05$) toward the end of the sleep period. Spindle activity was not affected. Moreover, subjects showed an increased RF EMF burst-related response in the SWA range, indicated by an increase in event-related EEG spectral power and phase changes in the SWA range. Notably, during exposure, sleep-dependent performance improvement in the motor sequence task was reduced compared to the sham condition (-20.1% , $P = 0.03$). Conclusion: The changes in the time course of SWA during the exposure night may reflect an interaction of RF EMF with the renormalization of cortical excitability during sleep, with a negative impact on sleep-dependent performance improvement.

(E) Lv B, Chen Z, Wu T, Shao Q, Yan D, Ma L, Lu K, Xie Y. The alteration of spontaneous low frequency oscillations caused by acute electromagnetic fields exposure. Clin Neurophysiol. 2013 Sep 4. pii: S1388-2457(13)00976-0. doi: 10.1016/j.clinph.2013.07.018. [Epub ahead of print] (HU, EE, PE)

OBJECTIVE: The motivation of this study is to evaluate the possible alteration of regional resting state brain activity induced by the acute radiofrequency **electromagnetic** field (RF-EMF) exposure (30min) of Long Term Evolution (LTE) signal. METHODS: We designed a controllable near-field LTE RF-EMF exposure environment. Eighteen subjects participated in a double-blind, crossover, randomized and counterbalanced experiment including two sessions (real and sham exposure). The

radiation source was close to the right ear. Then the resting state fMRI signals of human brain were collected before and after the exposure in both sessions. We measured the amplitude of low frequency fluctuation (ALFF) and fractional ALFF (fALFF) to characterize the spontaneous brain activity. RESULTS: We found the decreased ALFF value around in left superior temporal gyrus, left middle temporal gyrus, right superior temporal gyrus, right medial frontal gyrus and right paracentral lobule after the real exposure. And the decreased fALFF value was also detected in right medial frontal gyrus and right paracentral lobule. CONCLUSIONS: The study provided the evidences that 30min LTE RF-EMF exposure modulated the spontaneous low frequency fluctuations in some brain regions. SIGNIFICANCE: With resting state fMRI, we found the alteration of spontaneous low frequency fluctuations induced by the acute LTE RF-EMF exposure.

(E) Maaroufi K, Had-Aissouni L, Melon C, Sakly M, Abdelmelek H, Poucet B, Save E. Spatial learning, monoamines and oxidative stress in rats exposed to 900MHz electromagnetic field in combination with iron overload. Behav Brain Res. 2013 Oct 18. pii: S0166-4328(13)00624-4. doi: 10.1016/j.bbr.2013.10.016. [Epub ahead of print] (AS, CE, BE, CH)

The increasing use of mobile phone technology over the last decade raises concerns about the impact of high frequency electromagnetic fields (EMF) on health. More recently, a link between EMF, iron overload in the brain and neurodegenerative disorders including Parkinson's and Alzheimer's diseases has been suggested. Co-exposure to EMF and brain iron overload may have a greater impact on brain tissues and cognitive processes than each treatment by itself. To examine this hypothesis, Long-Evans rats submitted to 900MHz exposure or combined 900MHz EMF and iron overload treatments were tested in various spatial learning tasks (navigation task in the Morris water maze, working memory task in the radial-arm maze, and object exploration task involving spatial and non spatial processing). Biogenic monoamines and metabolites (dopamine, serotonin) and oxidative stress were measured. Rats exposed to EMF were impaired in the object exploration task but not in the navigation and working memory tasks. They also showed alterations of monoamine content in several brain areas but mainly in the hippocampus. Rats that received combined treatment did not show greater behavioral and neurochemical deficits than EMF-exposed rats. None of the two treatments produced global oxidative stress. These results show that there is an impact of EMF on the brain and cognitive processes but this impact is revealed only in a task exploiting spontaneous exploratory activity. In contrast, there are no synergistic effects between EMF and a high content of iron in the brain.

(E) Maganioti AE, Hountala CD, Papageorgiou CC, Kyprianou MA, Rabavilas AD, Capsalis CN. Principal component analysis of the P600 waveform: RF and gender effects. Neurosci Lett. 478(1):19-23, 2010. (HU, EE)

The aim of the present study was to examine the patterns of activation of the P600 waveform of the event-related potentials (ERP), applying principal component analysis (PCA) and repeated measures ANOVA, and whether these patterns are RF and gender dependent. The ERPs of thirty-nine healthy subjects (20 male and 19 female) were recorded during an auditory memory task in the presence and absence of RF, similar to that emitted by mobile phones. Both PCA and ANOVA produced congruent results, showing that activation of the P600 component occurs early and more intensely in the region of the posterior electrodes and in a less intense manner in the central electrodes. Conversely, the activation at the anterior electrodes arises later with a considerably reduced intensity. In the absence of RF female subjects exhibited significantly lower amplitudes at anterior electrodes and earlier latencies at central electrodes than male subjects. These differences disappear in the presence of RF. Consequently, the P600 component follows distinct patterns of activation in the anterior, central and posterior brain areas and gender differences are observed simultaneously at several electrodes within these areas. Finally, the gender-related functional architecture with regard the P600 component appears to be RF sensitive. In conclusion, the application of the PCA procedure provides an adequate model of the spatially distributed event-related dynamics that correspond to the P600 waveform.

(E) Mandalà M, Colletti V, Sacchetto L, Manganotti P, Ramat S, Marcocci A, Colletti L. Effect of Bluetooth headset and mobile phone electromagnetic fields on the human auditory nerve. *Laryngoscope*. 2013 Apr 25. doi: 10.1002/lary.24103. [Epub ahead of print] (HU, EE)

OBJECTIVES/HYPOTHESIS: The possibility that long-term mobile phone use increases the incidence of astrocytoma, glioma and acoustic neuroma has been investigated in several studies. Recently, our group showed that direct exposure (in a surgical setting) to cell phone electromagnetic fields (EMFs) induces deterioration of auditory evoked cochlear nerve compound action potential (CNAP) in humans. To verify whether the use of Bluetooth devices reduces these effects, we conducted the present study with the same experimental protocol. STUDY DESIGN: Randomized trial. METHODS: Twelve patients underwent retrosigmoid vestibular neurectomy to treat definite unilateral Ménière's disease while being monitored with acoustically evoked CNAPs to assess direct mobile phone exposure or alternatively the EMF effects of Bluetooth headsets. RESULTS: We found no short-term effects of Bluetooth EMFs on the auditory nervous structures, whereas direct mobile phone EMF exposure confirmed a significant decrease in CNAPs amplitude and an increase in latency in all subjects. CONCLUSIONS: The outcomes of the present study show that, contrary to the finding that the latency and amplitude of CNAPs are very sensitive to EMFs produced by the tested mobile phone, the EMFs produced by a common Bluetooth device do not induce any significant change in cochlear nerve activity. The conditions of exposure, therefore, differ from those of everyday life, in which various biological tissues may reduce the EMF affecting the cochlear nerve. Nevertheless, these novel findings may have important safety implications.

(E) Masuda H, Hirata A, Kawai H, Wake K, Watanabe S, Arima T, Poullietier de Gannes F, Lagroye I, Veyret B. Local exposure of the rat cortex to radiofrequency electromagnetic fields increases local cerebral blood flow along with temperature. J Appl Physiol. 110(1):142-148, 2011. (AS, PE)

Few studies have shown that local exposure to radiofrequency electromagnetic fields (RF) induces intensity-dependent physiological changes, especially in the brain. The aim of the present study was to detect reproducible responses to local RF exposure in the parietal cortex of anesthetized rats and to determine their dependence on RF intensity. The target cortex tissue was locally exposed to 2-GHz RF using a figure-eight loop antenna within a range of averaged specific absorption rates (10.5, 40.3, 130, and 263 W/kg averaged over 4.04 mg) in the target area. Local cerebral blood flow (CBF) and temperatures in three regions (target area, rectum, and calf hypodermis) were measured using optical fiber blood flow meters and thermometers during RF exposure. All parameters except for the calf hypodermis temperature increased significantly in exposed animals compared with sham-exposed ones during 18-min exposures. Dependence of parameter values on exposure intensity was analyzed using linear regression models. The elevation of local CBF was correlated with temperature rise in both target and rectum at the end of RF exposure. However, the local CBF elevation seemed to be elevated by the rise in target temperature, but not by that of the rectal temperature, in the early part of RF exposure or at low-intensity RF exposure. These findings suggest that local RF exposure of the rat cortex drives a regulation of CBF accompanied by a local temperature rise, and our findings may be helpful for discussing physiological changes in the local cortex region, which is locally exposed to RF.

(E) Maskey D, Kim M, Aryal B, Pradhan J, Choi IY, Park KS, Son T, Hong SY, Kim SB, Kim HG, Kim MJ. Effect of 835 MHz radiofrequency radiation exposure on calcium binding proteins in the hippocampus of the mouse brain. Brain Res. 1313:232-241, 2010a. (AS, CE, ME, CH)

Worldwide expansion of mobile phones and electromagnetic field (EMF) exposure has raised question of their possible biological effects on the brain and nervous system. Radiofrequency (RF) radiation might alter intracellular signaling pathways through changes in calcium (Ca²⁺) permeability across cell membranes. Changes in the expression of calcium binding proteins (CaBP) like calbindin D28-k (CB) and calretinin (CR) could indicate impaired Ca²⁺ homeostasis due to EMF exposure. CB and CR expression were measured with immunohistochemistry in the hippocampus of mice after EMF exposure at 835 MHz for different exposure times and absorption rates, 1 h/day for 5 days at a specific absorption rate (SAR)=1.6 W/kg, 1 h/day for 5 days at SAR=4.0 W/kg, 5 h/day for 1 day at SAR=1.6 W/kg, 5 h/day for 1 day at SAR=4.0 W/kg, daily exposure for 1 month at SAR=1.6 W/kg. Body weights did not change significantly. CB immunoreactivity (IR) displayed moderate staining of cells in the cornu ammonis (CA) areas and prominently stained granule cells. CR IR revealed prominently stained

pyramidal cells with dendrites running perpendicularly in the CA area. Exposure for 1 month produced almost complete loss of pyramidal cells in the CA1 area. CaBP differences could cause changes in cellular Ca(2+) levels, which could have deleterious effect on normal hippocampal functions concerned with neuronal connectivity and integration.

(E) Maskey D, Pradhan J, Aryal B, Lee CM, Choi IY, Park KS, Kim SB, Kim HG, Kim MJ. Chronic 835-MHz radiofrequency exposure to mice hippocampus alters the distribution of calbindin and GFAP immunoreactivity. Brain Res. 1346:237-246, 2010b. (AS, CE, ME, CH)

Exponential interindividual handling in wireless communication system has raised possible doubts in the biological aspects of radiofrequency (RF) exposure on human brain owing to its close proximity to the mobile phone. In the nervous system, calcium (Ca(2+)) plays a critical role in releasing neurotransmitters, generating action potential and membrane integrity. Alterations in intracellular Ca(2+) concentration trigger aberrant synaptic action or cause neuronal apoptosis, which may exert an influence on the cellular pathology for learning and memory in the hippocampus. Calcium binding proteins like calbindin D28-K (CB) is responsible for the maintaining and controlling Ca(2+) homeostasis. Therefore, in the present study, we investigated the effect of RF exposure on rat hippocampus at 835 MHz with low energy (specific absorption rate: SAR=1.6 W/kg) for 3 months by using both CB and glial fibrillary acidic protein (GFAP) specific antibodies by immunohistochemical method. Decrease in CB immunoreactivity (IR) was noted in exposed (E1.6) group with loss of interneurons and pyramidal cells in CA1 area and loss of granule cells. Also, an overall increase in GFAP IR was observed in the hippocampus of E1.6. By TUNEL assay, apoptotic cells were detected in the CA1, CA3 areas and dentate gyrus of hippocampus, which reflects that chronic RF exposure may affect the cell viability. In addition, the increase of GFAP IR due to RF exposure could be well suited with the feature of reactive astrogliosis, which is an abnormal increase in the number of astrocytes due to the loss of nearby neurons. Chronic RF exposure to the rat brain suggested that the decrease of CB IR accompanying apoptosis and increase of GFAP IR might be morphological parameters in the hippocampus damages.

(E) Maskey D, Kim HJ, Kim HG, Kim MJ. Calcium-binding proteins and GFAP immunoreactivity alterations in murine hippocampus after 1 month of exposure to 835 MHz radiofrequency at SAR values of 1.6 and 4.0 W/kg. Neurosci Lett. 506(2):292-296, 2012. (AS, CE, ME, CH)

Widespread use of wireless mobile communication has raised concerns of adverse effect to the brain owing to the proximity during use due to the electromagnetic field emitted by mobile phones. Changes in calcium ion concentrations via binding proteins can disturb calcium homeostasis; however, the correlation between calcium-binding protein (CaBP) immunoreactivity (IR) and glial cells has not been determined with different SAR values. Different SAR values [1.6 (E1.6 group) and 4.0 (E4 group) W/kg]

were applied to determine the distribution of calbindin D28-k (CB), calretinin (CR), and glial fibrillary acidic protein (GFAP) IR in murine hippocampus. Compared with sham control group, decreased CB and CR IRs, loss of CB and CR immunoreactive cells and increased GFAP IR exhibiting hypertrophic cytoplasmic processes were noted in both experimental groups. E4 group showed a prominent decrement in CB and CR IR than the E1.6 group due to down-regulation of CaBP proteins and neuronal loss. GFAP IR was more prominent in the E4 group than the E1.6 group. Decrement in the CaBPs can affect the calcium-buffering capacity leading to cell death, while increased GFAP IR and changes in astrocyte morphology, may mediate brain injury due to radiofrequency exposure.

(E) Maskey D, Kim MJ. Immunohistochemical Localization of Brain-derived Neurotrophic Factor and Glial Cell Line-derived Neurotrophic Factor in the Superior Olivary Complex of Mice after Radiofrequency Exposure. Neuroscience Letters. Available online February 16, 2014. (AS, CE, CH)

Raising health concerns about the biological effects from radiofrequency exposure, even with conflicting results, has prompted calls for formulation of a guideline of the biological safety level. Given the close proximity between a mobile phone and the ear, it has been suggested that the central auditory system may be detrimentally influenced by radiofrequency exposure. In the auditory system, neurotrophins are important in the regulation of neuron survival, especially mammalian cochlear neurons. Neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) present in the auditory system are responsible for the maintenance of auditory neurons. BDNF and GDNF may protect against acoustic trauma and prevent from hearing defect. The present study applied radiofrequency at a specific absorption rate (SAR) of 1.6 W/kg (E1.6) or 0 W/kg group to determine the distribution of BDNF and GDNF in the nuclei of superior olivary complex (SOC). In the E1.6 group, significant decrements of BDNF immunoreactivity (IR) were noted in the lateral superior olive, medial superior olive, superior paraolivary nucleus and medial nucleus of the trapezoid body. GDNF IR was also significantly decreased ($p < 0.001$) in all SOC nuclei of the E1.6 group. The decrease in the IR of these neurotrophic factors in the SOC of the E1.6 group suggests a detrimental effect of RF exposure in the auditory nuclei.

(E) Mathur R. Effect of chronic intermittent exposure to AM radiofrequency field on responses to various types of noxious stimuli in growing rats. Electromagn Biol Med. 27(3):266-276, 2008. (AS, CE, BE)

There are several reports of altered pain sensation after exposure (from a few minutes to hours in single or repeated doses for 2-3 weeks) to electromagnetic fields (EMF) in adults. The commonly utilized noxious stimulus is radiant heat. The nociceptive responses are known to be influenced by characteristics of stimulus, organism, and environment. We studied the pattern of nociceptive responses to various noxious stimuli in growing rats exposed to radiofrequency field (73.5 MHz amplitude modulated,

16 Hz power density 1.33 mw/cm², SAR = 0.4 w/kg) for 45 d (2 h/d). Threshold current for stimulation of nociceptive afferents to mediate motor response of tail (TF), vocalization during stimulus (VD), and vocalization after discharge (VA); the withdrawal latency of tail (TFL) and hind paw (HPL) to thermal noxious stimulus and tonic pain responses were recorded in every rat. The TFL was not affected, HPL was decreased ($p < 0.01$), and the thresholds of TF and VD were not affected, while, that of VA was significantly decreased. The tonic pain rating was decreased ($p < 0.01$). A decrease in the threshold of VA ($p < 0.01$) is indicative of an increase in the emotional component of the response to the phasic pain, whereas a decrease in the pain rating indicates analgesia in response to the tonic pain. The results of our study suggest that chronic (45 d), intermittent (2 h/d) amplitude modulated RF field exposure to the peripubertal rat increases the emotional component of phasic pain over a basal euanalgesic state, while late response to tonic pain is decreased. The data suggest that amplitude modulated RF field differentially affects the mechanisms involved in the processing of various noxious stimuli.

(E) Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Abegaonkar MP. Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. Indian J Exp Biol. 50(12):889-896, 2012. (AS, LI, CE, BE, OX, CH)

Public concerns over possible adverse effects of microwave radiation emitted by mobile phones on health are increasing. To evaluate the intensity of oxidative stress, cognitive impairment and inflammation in brain of Fischer rats exposed to microwave radiation, male Fischer-344 rats were exposed to 900 MHz microwave radiation (SAR = 5.953×10^{-4} W/kg) and 1800 MHz microwave radiation (SAR = 5.835×10^{-4} W/kg) for 30 days (2 h/day). Significant impairment in cognitive function and induction of oxidative stress in brain tissues of microwave exposed rats were observed in comparison with sham exposed groups. Further, significant increase in level of cytokines (IL-6 and TNF- α) was also observed following microwave exposure. Results of the present study indicated that increased oxidative stress due to microwave exposure may contribute to cognitive impairment and inflammation in brain.

(E) Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res. 1169:120-124, 2007. (AS, CE, OX)

This study was designed to demonstrate the effects of 900-MHz electromagnetic field (EMF) emitted from cellular phone on brain tissue and also blood malondialdehyde (MDA), glutathione (GSH), retinol (vitamin A), vitamin D(3) and tocopherol (vitamin E) levels, and catalase (CAT) enzyme activity of guinea pigs. Fourteen male guinea pigs, weighing 500-800 g were randomly divided into one of two experimental groups: control and treatment (EMF-exposed), each containing seven animals. Animals in treatment group were exposed to 890- to 915-MHz EMF (217-Hz pulse rate, 2-W maximum peak power, SAR 0.95 w/kg) of a cellular phone for 12 h/day (11-h 45-min

stand-by and 15-min spiking mode) for 30 days. Control guinea pigs were housed in a separate room without exposing EMF of a cellular phone. Blood samples were collected through a cardiac puncture and brains were removed after decapitation for the biochemical analysis at the end of the 30 days of experimental period. It was found that the MDA level increased ($P < 0.05$), GSH level and CAT enzyme activity decreased ($P < 0.05$), and vitamins A, E and D(3) levels did not change ($P > 0.05$) in the brain tissues of EMF-exposed guinea pigs. In addition, MDA, vitamins A, D(3) and E levels, and CAT enzyme activity increased ($P < 0.05$), and GSH level decreased ($P < 0.05$) in the blood of EMF-exposed guinea pigs. It was concluded that electromagnetic field emitted from cellular phone might produce oxidative stress in brain tissue of guinea pigs. However, more studies are needed to demonstrate whether these effects are harmful or/and affect the neural functions.

(NE) Mohler E, Frei P, Braun-Fahrländer C, Fröhlich J, Neubauer G, Rösli M; Qualifex Team. Effects of everyday radiofrequency electromagnetic-field exposure on sleep quality: a cross-sectional study. Radiat Res. 174(3):347-356, 2010. (HU, SL)

The aim of this cross-sectional study was to investigate the association between exposure to various sources of radiofrequency electromagnetic fields (RF EMFs) in the everyday environment and sleep quality, which is a common public health concern. We assessed self-reported sleep disturbances and daytime sleepiness in a random population sample of 1,375 inhabitants from the area of Basel, Switzerland. Exposure to environmental far-field RF EMFs was predicted for each individual using a prediction model that had been developed and validated previously. Self-reported cordless and mobile phone use as well as objective mobile phone operator data for the previous 6 months were also considered in the analyses. In multivariable regression models, adjusted for relevant confounders, no associations between environmental far-field RF EMF exposure and sleep disturbances or excessive daytime sleepiness were observed. The 10% most exposed participants had an estimated risk for sleep disturbances of 1.11 (95% CI: 0.50 to 2.44) and for excessive daytime sleepiness of 0.58 (95% CI: 0.31 to 1.05). Neither mobile phone use nor cordless phone use was associated with decreased sleep quality. The results of this large cross-sectional study did not indicate an impairment of subjective sleep quality due to exposure from various sources of RF EMFs in everyday life.

(NE) Mohler E, Frei P, Fröhlich J, Braun-Fahrländer C, Rösli M; QUALIFEX-team. Exposure to radiofrequency electromagnetic fields and sleep quality: a prospective cohort study. PLoS One. 7(5):e37455, 2012. (HU, SL)

BACKGROUND: There is persistent public concern about sleep disturbances due to radiofrequency electromagnetic field (RF-EMF) exposure. The aim of this prospective cohort study was to investigate whether sleep quality is affected by mobile phone use or by other RF-EMF sources in the everyday environment. METHODS: We conducted a prospective cohort study with 955 study participants aged between 30 and 60 years.

Sleep quality and daytime sleepiness was assessed by means of standardized questionnaires in May 2008 (baseline) and May 2009 (follow-up). We also asked about mobile and cordless phone use and asked study participants for consent to obtain their mobile phone connection data from the mobile phone operators. Exposure to environmental RF-EMF was computed for each study participant using a previously developed and validated prediction model. In a nested sample of 119 study participants, RF-EMF exposure was measured in the bedroom and data on sleep behavior was collected by means of actigraphy during two weeks. Data were analyzed using multivariable regression models adjusted for relevant confounders. RESULTS: In the longitudinal analyses neither operator-recorded nor self-reported mobile phone use was associated with sleep disturbances or daytime sleepiness. Also, exposure to environmental RF-EMF did not affect self-reported sleep quality. The results from the longitudinal analyses were confirmed in the nested sleep study with objectively recorded exposure and measured sleep behavior data. CONCLUSIONS: We did not find evidence for adverse effects on sleep quality from RF-EMF exposure in our everyday environment.

(E) Mohammed HS, Fahmy HM, Radwah NM, Elsayed AA. Non-thermal continuous and modulated electromagnetic radiation fields effects on sleep EEG of rats. J Adv Res 4(2) 181-187, 2013. (AS, EE, SL, WS)

In the present study, the alteration in the sleep EEG in rats due to chronic exposure to low-level non-thermal electromagnetic radiation was investigated. Two types of radiation fields were used; 900 MHz *unmodulated* wave and 900 MHz *modulated* at 8 and 16 Hz waves. Animals has exposed to radiation fields for 1 month (1 h/day). EEG power spectral analyses of exposed and control animals during slow wave sleep (SWS) and rapid eye movement sleep (REM sleep) revealed that the REM sleep is more susceptible to modulated radiofrequency radiation fields (RFR) than the SWS. The latency of REM sleep increased due to radiation exposure indicating a change in the ultradian rhythm of normal sleep cycles. The cumulative and irreversible effect of radiation exposure was proposed and the interaction of the extremely low frequency radiation with the similar EEG frequencies was suggested.

(E) Moretti D, Garenne A, Haro E, Poullietier de Gannes F, Lagroye I, Lévêque P, Veyret B, Lewis N. In-vitro exposure of neuronal networks to the GSM-1800 signal. Bioelectromagnetics. 2013 Aug 1. doi: 10.1002/bem.21805. [Epub ahead of print] (CS, EE)

The central nervous system is the most likely target of mobile telephony radiofrequency (RF) field exposure in terms of biological effects. Several electroencephalography (EEG)

studies have reported variations in the alpha-band power spectrum during and/or after RF exposure, in resting EEG and during sleep. In this context, the observation of the spontaneous electrical activity of neuronal networks under RF exposure can be an efficient tool to detect the occurrence of low-level RF effects on the nervous system. Our research group has developed a dedicated experimental setup in the GHz range for the simultaneous exposure of neuronal networks and monitoring of electrical activity. A transverse electromagnetic (TEM) cell was used to expose the neuronal networks to GSM-1800 signals at a SAR level of 3.2 W/kg. Recording of the neuronal electrical activity and detection of the extracellular spikes and bursts under exposure were performed using microelectrode arrays (MEAs). This work provides the proof of feasibility and preliminary results of the integrated investigation regarding exposure setup, culture of the neuronal network, recording of the electrical activity, and analysis of the signals obtained under RF exposure. In this pilot study on 16 cultures, there was a 30% reversible decrease in firing rate (FR) and bursting rate (BR) during a 3 min exposure to RF. Additional experiments are needed to further characterize this effect.

(NE) Nakatani-Enomoto S, Furubayashi T, Ushiyama A, Groiss SJ, Ueshima K, Sokejima S, Simba AY, Wake K, Watanabe SI, Nishikawa M, Miyawaki K, Taki M, Ugawa Y. Effects of electromagnetic fields emitted from W-CDMA-like mobile phones on sleep in humans. *Bioelectromagnetics*. 2013 Aug 22. doi: 10.1002/bem.21809. [Epub ahead of print] (HU, EE, SL)

In this study, we investigated subjective and objective effects of mobile phones using a Wideband Code Division Multiple Access (W-CDMA)-like system on human sleep. Subjects were 19 volunteers. Real or sham electromagnetic field (EMF) exposures for 3 h were performed before their usual sleep time on 3 consecutive days. They were exposed to real EMF on the second or third experimental day in a double-blind design. Sleepiness and sleep insufficiency were evaluated the next morning. Polysomnograms were recorded for analyses of the sleep variables and power spectra of electroencephalograms (EEG). No significant differences were observed between the two conditions in subjective feelings. Sleep parameters including sleep stage percentages and EEG power spectra did not differ significantly between real and sham exposures. We conclude that continuous wave EMF exposure for 3 h from a W-CDMA-like system has no detectable effects on human sleep.

(E) Narayanan SN, Kumar RS, Potu BK, Nayak S, Mailankot M. Spatial memory performance of Wistar rats exposed to mobile phone. *Clinics (Sao Paulo)*. 64(3):231-234, 2009. (AS, CE, BE)

INTRODUCTION: With the tremendous increase in number of mobile phone users world wide, the possible risks of this technology have become a serious concern. OBJECTIVE: We tested the effects of mobile phone exposure on spatial memory performance. MATERIALS AND METHODS: Male Wistar rats (10-12 weeks old) were exposed to 50

missed calls/day for 4 weeks from a GSM (900/1800 MHz) mobile phone in vibratory mode (no ring tone). After the experimental period, the animals were tested for spatial memory performance using the Morris water maze test. **RESULTS:** Both phone exposed and control animals showed a significant decrease in escape time with training. Phone exposed animals had significantly (approximately 3 times) higher mean latency to reach the target quadrant and spent significantly (approximately 2 times) less time in the target quadrant than age- and sex-matched controls. **CONCLUSION:** Mobile phone exposure affected the acquisition of learned responses in Wistar rats. This in turn points to the poor spatial navigation and the object place configurations of the phone-exposed animals.

(E) Narayanan SN, Kumar RS, Potu BK, Nayak S, Bhat PG, Mailankot M. Effect of radio-frequency electromagnetic radiations (RF-EMR) on passive avoidance behaviour and hippocampal morphology in Wistar rats. Ups J Med Sci. 115(2):91-96, 2010. (AS, CE, ME, BE)

INTRODUCTION: The interaction of mobile phone radio-frequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. **OBJECTIVE:** We evaluated the effect of RF-EMR from mobile phones on passive avoidance behaviour and hippocampal morphology in rats. **MATERIALS AND METHODS:** Healthy male albino Wistar rats were exposed to RF-EMR by giving 50 missed calls (within 1 hour) per day for 4 weeks, keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in vibratory mode (no ring tone) in the cage. After the experimental period, passive avoidance behaviour and hippocampal morphology were studied. **RESULTS:** Passive avoidance behaviour was significantly affected in mobile phone RF-EMR-exposed rats demonstrated as shorter entrance latency to the dark compartment when compared to the control rats. Marked morphological changes were also observed in the CA(3) region of the hippocampus of the mobile phone-exposed rats in comparison to the control rats. **CONCLUSION:** Mobile phone RF-EMR exposure significantly altered the passive avoidance behaviour and hippocampal morphology in rats.

(E) Narayanan SN, Kumar RS, Paval J, Kedage V, Bhat MS, Nayak S, Bhat PG. Analysis of emotionality and locomotion in radio-frequency electromagnetic radiation exposed rats. Neurol Sci. 34(7):1117-1124, 2013. (AS, CE, BE)

In the current study the modulatory role of mobile phone radio-frequency electromagnetic radiation (RF-EMR) on emotionality and locomotion was evaluated in adolescent rats. Male albino Wistar rats (6-8 weeks old) were randomly assigned into the following groups having 12 animals in each group. Group I (Control): they remained in the home cage throughout the experimental period. Group II (Sham exposed): they were exposed to mobile phone in switch-off mode for 28 days, and Group III (RF-EMR exposed): they were exposed to RF-EMR (900 MHz) from an active GSM (Global system for mobile communications) mobile phone with a peak power density of 146.60 $\mu\text{W}/\text{cm}^2$ for 28 days. On 29th day, the animals were tested for emotionality and

locomotion. Elevated plus maze (EPM) test revealed that, percentage of entries into the open arm, percentage of time spent on the open arm and distance travelled on the open arm were significantly reduced in the RF-EMR exposed rats. Rearing frequency and grooming frequency were also decreased in the RF-EMR exposed rats. Defecation boli count during the EPM test was more with the RF-EMR group. No statistically significant difference was found in total distance travelled, total arm entries, percentage of closed arm entries and parallelism index in the RF-EMR exposed rats compared to controls. Results indicate that mobile phone radiation could affect the emotionality of rats without affecting the general locomotion.

(E) Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariante JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca(2+) channels in brain and dorsal root ganglion in rat. *Physiol Behav.* 105(3):683-692, 2012. (AS, CE, CH, EE, OX)

We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca(2+) influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR+melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca(2+) values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyl-diphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca(2+) influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca(2+) channels.

(E) Ning W, Xu SJ, Chiang H, Xu ZP, Zhou SY, Yang W, Luo JH. Effects of GSM 1800 MHz on dendritic development of cultured hippocampal neurons. *Acta Pharmacol Sin.* 28(12):1873-1880, 2007. (CS, CE, DE, ME)

AIM: To evaluate the effects of global system for mobile communications (GSM) 1800 MHz microwaves on dendritic filopodia, dendritic arborization, and spine maturation during development in cultured hippocampal neurons in rats. **METHODS:** The cultured hippocampal neurons were exposed to GSM 1800 MHz microwaves with 2.4 and 0.8 W/kg, respectively, for 15 min each day from 6 days in vitro (DIV6) to DIV14. The subtle structures of dendrites were displayed by transfection with farnesylated enhanced green fluorescent protein (F-GFP) and GFP-actin on DIV5 into the hippocampal neurons. **RESULTS:** There was a significant decrease in the density and mobility of dendritic filopodia at DIV8 and in the density of mature spines at DIV14 in the neurons exposed to

GSM 1800 MHz microwaves with 2.4 W/kg. In addition, the average length of dendrites per neuron at DIV10 and DIV14 was decreased, while the dendritic arborization was unaltered in these neurons. However, there were no significant changes found in the neurons exposed to the GSM 1800 MHz microwaves with 0.8 W/kg. **CONCLUSION:** These data indicate that the chronic exposure to 2.4 W/kg GSM 1800 MHz microwaves during the early developmental stage may affect dendritic development and the formation of excitatory synapses of hippocampal neurons in culture.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (AS, CH, LI)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nittby H, Grafström G, Tian DP, Malmgren L, Brun A, Persson BR, Salford LG, Eberhardt J. Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics. 29(3):219-232, 2008. (AS, CE, BE, LI)

Considering the frequent use of mobile phones, we have directed attention to possible implications on cognitive functions. In this study we investigated in a rat model the long-term effects of protracted exposure to Global System for Mobile Communication-900 MHz (GSM-900) radiation. Out of a total of 56 rats, 32 were exposed for 2 h each week

for 55 weeks to radio-frequency electromagnetic radiation at different SAR levels (0.6 and 60 mW/kg at the initiation of the experimental period) emitted by a (GSM-900) test phone. Sixteen animals were sham exposed and eight animals were cage controls, which never left the animal house. After this protracted exposure, GSM-900 exposed rats were compared to sham exposed controls. Effects on exploratory behaviour were evaluated in the open-field test, in which no difference was seen. Effects on cognitive functions were evaluated in the episodic-like memory test. In our study, GSM exposed rats had impaired memory for objects and their temporal order of presentation, compared to sham exposed controls ($P = 0.02$). Detecting the place in which an object was presented was not affected by GSM exposure. Our results suggest significantly reduced memory functions in rats after GSM microwave exposure ($P = 0.02$).

(E) Nittby H, Brun A, Eberhardt J, Malmgren L, Persson BR, Salford LG. Increased blood-brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology. 16(2-3):103-112, 2009. (AS, ME, LI)

Microwaves were for the first time produced by humans in 1886 when radio waves were broadcasted and received. Until then microwaves had only existed as a part of the cosmic background radiation since the birth of universe. By the following utilization of microwaves in telegraph communication, radars, television and above all, in the modern mobile phone technology, mankind is today exposed to microwaves at a level up to 10(20) times the original background radiation since the birth of universe. Our group has earlier shown that the electromagnetic radiation emitted by mobile phones alters the permeability of the blood-brain barrier (BBB), resulting in albumin extravasation immediately and 14 days after 2h of exposure. In the background section of this report, we present a thorough review of the literature on the demonstrated effects (or lack of effects) of microwave exposure upon the BBB. Furthermore, we have continued our own studies by investigating the effects of GSM mobile phone radiation upon the blood-brain barrier permeability of rats 7 days after one occasion of 2h of exposure. Forty-eight rats were exposed in TEM-cells for 2h at non-thermal specific absorption rates (SARs) of 0mW/kg, 0.12mW/kg, 1.2mW/kg, 12mW/kg and 120mW/kg. Albumin extravasation over the BBB, neuronal albumin uptake and neuronal damage were assessed. Albumin extravasation was enhanced in the mobile phone exposed rats as compared to sham controls after this 7-day recovery period (Fisher's exact probability test, $p=0.04$ and Kruskal-Wallis, $p=0.012$), at the SAR-value of 12mW/kg (Mann-Whitney, $p=0.007$) and with a trend of increased albumin extravasation also at the SAR-values of 0.12mW/kg and 120mW/kg. There was a low, but significant correlation between the exposure level (SAR-value) and occurrence of focal albumin extravasation ($r(s)=0.33$; $p=0.04$). The present findings are in agreement with our earlier studies where we have seen increased BBB permeability immediately and 14 days after exposure. We here discuss the present findings as well as the previous results of altered BBB permeability from our and other laboratories.

(E) Nittby H, Moghadam MK, Sun W, Malmgren L, Eberhardt J, Persson BR, Salford LG. Analgetic effects of non-thermal GSM-1900 radiofrequency electromagnetic fields in the land snail *Helix pomatia*. *Int J Radiat Biol.* 88(3):245-252, 2012. (AS, BE, MA, LI)

PURPOSE: To investigate whether mobile phone radiation might affect snail nociception, employing radiofrequency (RF) electromagnetic fields (EMF) which, to our knowledge, have hitherto not been studied in a snail model. Exposure to extremely low frequency (ELF) magnetic fields has however been shown to significantly affect nociceptive responses. **MATERIALS AND METHODS:** In the present study, we exposed 29 land snails of the strain *Helix pomatia* to global system for mobile communications (GSM) EMF at 1900 MHz at the non-thermal level 48 mW/kg for 1 hour each and 29 snails were sham controls. The experiments took place during the onset of summer, with all snails being well out of hibernation. Before and after GSM or sham exposure, the snails were subjected to thermal pain by being placed on a hot plate. The reaction time for retraction from the hot plate was measured by two blinded observers. **RESULTS:** Comparing the reaction pattern of each snail before and after exposure, the GSM-exposed snails were less sensitive to thermal pain as compared to the sham controls, indicating that RF exposure induces a significant analgesia (Mann-Whitney $p < 0.001$). **CONCLUSION:** This study might support earlier findings, describing beneficial effects of EMF exposure upon nociception.

(E) Noor NA, Mohammed HS, Ahmed NA, Radwan NM. Variations in amino acid neurotransmitters in some brain areas of adult and young male albino rats due to exposure to mobile phone radiation. *Eur Rev Med Pharmacol Sci.* 15(7):729-742, 2011. (AS, CE, CH, AD)

BACKGROUND AND OBJECTIVES: Mobile phone radiation and health concerns have been raised, especially following the enormous increase in the use of wireless mobile telephony throughout the world. The present study aims to investigate the effect of one hour daily exposure to electromagnetic radiation (EMR) with frequency of 900 Mz (SAR 1.165 w/kg, power density 0.02 mW/cm²) on the levels of amino acid neurotransmitters in the midbrain, cerebellum and medulla of adult and young male albino rats.

MATERIALS AND METHODS: Adult and young rats were divided into two main groups (treated and control). The treated group of both adult and young rats was exposed to EMR for 1 hour daily. The other group of both adult and young animals was served as control. The determination of amino acid levels was carried out after 1 hour, 1 month, 2 months and 4 months of EMR exposure as well as after stopping radiation. **RESULTS:** Data of the present study showed a significant increase in both excitatory and inhibitory amino acids in the cerebellum of adult and young rats and midbrain of adult animals after 1 hour of EMR exposure. In the midbrain of adult animals, there was a significant increase in glycine level after 1 month followed by significant increase in GABA after 4 months. Young rats showed significant decreases in the midbrain excitatory amino acids. In the medulla, the equilibrium ratio percent (ER%) calculations showed a state of neurochemical inhibition after 4 months in case of adult animals, whereas in young

animals, the neurochemical inhibitory state was observed after 1 month of exposure due to significant decrease in glutamate and aspartate levels. This state was converted to excitation after 4 months due to the increase in glutamate level. **CONCLUSION:** The present changes in amino acid concentrations may underlie the reported adverse effects of using mobile phones.

(E) Ntzouni MP, Stamatakis A, Stylianopoulou F, Margaritis LH. Short-term memory in mice is affected by mobile phone radiation. Pathophysiology. 18(3):193-199, 2011. (AS, CE, BE)

The effects of mobile phone electromagnetic fields (EMFs) were studied on a non-spatial memory task (Object Recognition Task - ORT) that requires entorhinal cortex function. The task was applied to three groups of mice *Mus musculus* C57BL/6 (exposed, sham-exposed and control) combined with 3 different radiation exposure protocols. In the first protocol designated "acute exposure", mice 45 days old (PND45 - postnatal day 45) were exposed to mobile phone (MP) radiation (SAR value 0.22W/kg) during the habituation, the training and the test sessions of the ORT, but not during the 10min inter-trial interval (ITI) where consolidation of stored object information takes place. On the second protocol designated "chronic exposure-I", the same mice were exposed for 17 days for 90min/per day starting at PND55 to the same MP radiation. ORT recognition memory was performed at PND72 with radiation present only during the ITI phase. In the third protocol designated "chronic exposure-II", mice continued to be exposed daily under the same conditions up to PND86 having received radiation for 31 days. One day later the ORT test was performed without irradiation present in any of the sessions. The ORT-derived discrimination indices in all three exposure protocols revealed a major effect on the "chronic exposure-I" suggesting a possible severe interaction of EMF with the consolidation phase of recognition memory processes. This may imply that the primary EMF target may be the information transfer pathway connecting the entorhinal-parahippocampal regions which participate in the ORT memory task.

(E) Ntzouni MP, Skouroliakou A, Kostomitsopoulos N, Margaritis LH. Transient and cumulative memory impairments induced by GSM 1.8 GHz cell phone signal in a mouse model. Electromagn Biol Med. 2013 Jan 15. [Epub ahead of print] (AS, CE, BE)

This study was designed to investigate the transient and cumulative impairments in spatial and non-spatial memory of C57Bl/6J mice exposed to GSM 1.8 GHz signal for 90 min daily by a typical cellular (mobile) phone at a specific absorption rate value of 0.11 W/kg. Free-moving male mice 2 months old were irradiated in two experimental protocols, lasting for 66 and for 148 days respectively. Each protocol used three groups of animals (n = 8 each for exposed, sham exposed and controls) in combination with two behavioural paradigms, the object recognition task and the object location task sequentially applied at different time points. One-way analysis of variance revealed statistically significant impairments of both types of memory gradually accumulating, with more pronounced effects on the spatial memory. The impairments persisted even

2 weeks after interruption of the 8 weeks daily exposure, whereas the memory of mice as detected by both tasks showed a full recovery approximately 1 month later. Intermittent every other day exposure for 1 month had no effect on both types of memory. The data suggest that visual information processing mechanisms in hippocampus, perirhinal and entorhinal cortex are gradually malfunctioning upon long-term daily exposure, a phenotype that persists for at least 2 weeks after interruption of radiation, returning to normal memory performance levels 4 weeks later. It is postulated that cellular repair mechanisms are operating to eliminate the memory affecting molecules. The overall contribution of several possible mechanisms to the observed cumulative and transient impairments in spatial and non-spatial memory is discussed.

(NE) Nylund R, Kuster N, Leszczynski D. Analysis of proteome response to the mobile phone radiation in two types of human primary endothelial cells. Proteome Sci. 8:52, 2010. (CS, CH, WS)

BACKGROUND: Use of mobile phones has widely increased over the past decade. However, in spite of the extensive research, the question of potential health effects of the mobile phone radiation remains unanswered. We have earlier proposed, and applied, proteomics as a tool to study biological effects of the mobile phone radiation, using as a model human endothelial cell line EA.hy926. Exposure of EA.hy926 cells to 900 MHz GSM radiation has caused statistically significant changes in expression of numerous proteins. However, exposure of EA.hy926 cells to 1800 MHz GSM signal had only very small effect on cell proteome, as compared with 900 MHz GSM exposure. In the present study, using as model human primary endothelial cells, we have examined whether exposure to 1800 MHz GSM mobile phone radiation can affect cell proteome.

RESULTS: Primary human umbilical vein endothelial cells and primary human brain microvascular endothelial cells were exposed for 1 hour to 1800 MHz GSM mobile phone radiation at an average specific absorption rate of 2.0 W/kg. The cells were harvested immediately after the exposure and the protein expression patterns of the sham-exposed and radiation-exposed cells were examined using two dimensional difference gel electrophoresis-based proteomics (2DE-DIGE). There were observed numerous differences between the proteomes of human umbilical vein endothelial cells and human brain microvascular endothelial cells (both sham-exposed). These differences are most likely representing physiological differences between endothelia in different vascular beds. However, the exposure of both types of primary endothelial cells to mobile phone radiation did not cause any statistically significant changes in protein expression.

CONCLUSIONS: Exposure of primary human endothelial cells to the mobile phone radiation, 1800 MHz GSM signal for 1 hour at an average specific absorption rate of 2.0 W/kg, does not affect protein expression, when the proteomes were examined immediately after the end of the exposure and when the false discovery rate correction was applied to analysis. This observation agrees with our earlier study showing that the 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation.

(NE) O'Connor RP, Madison SD, Leveque P, Roderick HL, Bootman MD. Exposure to GSM RF fields does not affect calcium homeostasis in human endothelial cells, rat pheocromocytoma cells or rat hippocampal neurons. PLoS One. 5(7):e11828, 2010. (CS, CC, CH)

In the course of modern daily life, individuals are exposed to numerous sources of electromagnetic radiation that are not present in the natural environment. The strength of the electromagnetic fields from sources such as hairdryers, computer display units and other electrical devices is modest. However, in many home and office environments, individuals can experience perpetual exposure to an "electromagnetic smog", with occasional peaks of relatively high electromagnetic field intensity. This has led to concerns that such radiation can affect health. In particular, emissions from mobile phones or mobile phone masts have been invoked as a potential source of pathological electromagnetic radiation. Previous reports have suggested that cellular calcium (Ca²⁺) homeostasis is affected by the types of radiofrequency fields emitted by mobile phones. In the present study, we used a high-throughput imaging platform to monitor putative changes in cellular Ca²⁺ during exposure of cells to 900 MHz GSM fields of differing power (specific absorption rate 0.012-2 W/Kg), thus mimicking the type of radiation emitted by current mobile phone handsets. Data from cells experiencing the 900 Mhz GSM fields were compared with data obtained from paired experiments using continuous wave fields or no field. We employed three cell types (human endothelial cells, PC-12 neuroblastoma and primary hippocampal neurons) that have previously been suggested to be sensitive to radiofrequency fields. Experiments were designed to examine putative effects of radiofrequency fields on resting Ca²⁺, in addition to Ca²⁺ signals evoked by an InsP(3)-generating agonist. Furthermore, we examined putative effects of radiofrequency field exposure on Ca²⁺ store emptying and store-operated Ca²⁺ entry following application of the Ca²⁺ATPase inhibitor thapsigargin. Multiple parameters (e.g., peak amplitude, integrated Ca²⁺ signal, recovery rates) were analysed to explore potential impact of radiofrequency field exposure on Ca²⁺ signals. Our data indicate that 900 MHz GSM fields do not affect either basal Ca²⁺ homeostasis or provoked Ca²⁺ signals. Even at the highest field strengths applied, which exceed typical phone exposure levels, we did not observe any changes in cellular Ca²⁺ signals. We conclude that under the conditions employed in our experiments, and using a highly-sensitive assay, we could not detect any consequence of RF exposure.

(E) Odaci E, Bas O, Kaplan S. Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Res. 1238:224-229, 2008. (AS, CE, DE, ME)

Electromagnetic fields (EMFs) inhibit the formation and differentiation of neural stem cells during embryonic development. In this study, the effects of prenatal exposure to EMF on the number of granule cells in the dentate gyrus of 4-week-old rats were investigated. This experiment used a control (Cont) group and an EMF exposed (EMF)

group (three pregnant rats each group). The EMF group consisted of six offspring (n=6) of pregnant rats that were exposed to an EMF of up to 900 megahertz (MHz) for 60 min/day between the first and last days of gestation. The control group consisted of five offspring (n=5) of pregnant rats that were not treated at all. The offspring were sacrificed when they were 4 weeks old. The numbers of granule cells in the dentate gyrus were analyzed using the optical fractionator technique. The results showed that prenatal EMF exposure caused a decrease in the number of granule cells in the dentate gyrus of the rats ($P < 0.01$). This suggests that prenatal exposure to a 900 MHz EMF affects the development of the dentate gyrus granule cells in the rat hippocampus. Cell loss might be caused by an inhibition of granule cell neurogenesis in the dentate gyrus.

(E) Odacı E, İkinci A, Yıldırım M, Kaya H, Akça M, Hancı H, Sönmez OF, Aslan A, Okuyan M, Baş O. The Effects of 900 Megahertz Electromagnetic Field Applied in the Prenatal Period on Spinal Cord Morphology and Motor Behavior in Female Rat Pups. NeuroQuantology 11:573-581, 2013. (AS, CE, DE, BE, ME)

This study investigated the effect of a 900 megahertz (MHz) electromagnetic field (EMF) applied in the prenatal period on the spinal cord and motor behavior of female rat pups. Beginning of the study, female Sprague Dawley rats (180–250 g) were left to mate with male rats. Rats identified as pregnant were then divided into control (n=3) and EMF groups (n=3). The EMF group was exposed to 1-h 900 MHz EMF daily between days 13 and 21 of pregnancy. At 21 days old, rat pups were removed from their mothers and divided into two newborn rat groups, control (n=13) and EMF (n=10). The rotarod test was applied to the rat pups to assess motor functions and the open field test to evaluate locomotor activity. On day 32 of the study, the rat pups were decapitated, and the spinal cord in the upper thoracic region was removed. Following routine histological tests, they were stained with Cresyl fast violet. Rotarod test results revealed a significant increase in EMF group rat pups' motor functions ($p=0.037$). However, no difference was observed in the open field test results ($p > 0.05$). In the EMF group' rat pups, we observed pathological changes in the spinal cord. On the basis of our results, 900 MHz EMF applied in the prenatal period affected spinal cord development. This effect was observed in the form of pathological changes in the spinal cord of rat pups, and it may be that these pathological changes led to an increase in rat pups' motor activities.

(NE) Ogawa K, Nabae K, Wang J, Wake K, Watanabe S, Kawabe M, Fujiwara O, Takahashi S, Ichihara T, Tamano S, Shirai T. Effects of gestational exposure to 1.95-GHz W-CDMA signals for IMT-2000 cellular phones: Lack of embryotoxicity and teratogenicity in rats. Bioelectromagnetics. 30(3):205-212, 2009. (AS, CE, DE)

The present study was designed to evaluate whether gestational exposure to an EMF targeting the head region, similar to that from cellular phones, might affect embryogenesis in rats. A 1.95-GHz wide-band code division multiple access (W-CDMA) signal, which is one applied for the International Mobile Telecommunication 2000 (IMT-2000) system and used for the freedom of mobile multimedia access (FOMA), was employed for exposure to the heads of four groups of pregnant CD(SD) IGS rats (20 per group) for gestational days 7-17. The exposure was performed for 90 min/day in the morning. The spatial average specific absorption rate (SAR) for individual brains was designed to be 0.67 and 2.0 W/kg with peak brain SARs of 3.1 and 7.0 W/kg for low (group 3) and high (group 4) exposures, respectively, and a whole-body average SAR less than 0.4 W/kg so as not to cause thermal effects due to temperature elevation. Control and sham exposure groups were also included. At gestational day 20, all dams were killed and fetuses were taken out by cesarean section. There were no differences in maternal body weight gain. No adverse effects of EMF exposure were observed on any reproductive and embryotoxic parameters such as number of live (243-271 fetuses), dead or resorbed embryos, placental weights, sex ratios, weights or external, visceral or skeletal abnormalities of live fetuses.

(NE) Okano T, Terao Y, Furubayashi T, Yugeta A, Hanajima R, Ugawa Y. The effect of electromagnetic field emitted by a mobile phone on the inhibitory control of saccades. Clin Neurophysiol. 121(4):603-611, 2010. (HU, PE)

OBJECTIVE: To investigate whether exposure to a pulsed high-frequency electromagnetic field (pulsed EMF) emitted by a mobile phone has short-term effects on the inhibitory control of saccades. **METHODS:** A double-blind, counterbalanced crossover study design was employed. We assessed the performance of 10 normal subjects on antisaccade (AS) and cued saccade (CUED) tasks as well as two types of overlap saccade (OL1, OL2) task before and after 30 min of exposure to EMF emitted by a mobile phone or sham exposure. **RESULTS:** After EMF or sham exposure, we observed a slight but significant shortening of latency in the CUED and OL2 tasks. AS amplitude decreased as well as the saccade velocities in the AS, CUED, and OL1 tasks after exposure. These changes occurred regardless of whether exposure was real or sham. The frequencies of pro-saccades in the AS task, saccades to cue in the CUED task, and prematurely initiated saccades in the overlap (OL2) task did not change significantly after real or sham EMF exposure. **CONCLUSIONS:** Thirty minutes of mobile phone exposure has no significant short-term effect on the inhibitory control of saccades. **SIGNIFICANCE:** The cortical processing responsible for saccade inhibition is not affected by exposure to EMF emitted by a mobile phone.

(E) Panda NK, Jain R, Bakshi J, Munjal S. Audiologic disturbances in long-term mobile phone users. J Otolaryngol Head Neck Surg. 39(1):5-11, 2010. (HU, CE, PE)

INTRODUCTION: There is general concern regarding the possible hazardous health effects of exposure to radiofrequency electromagnetic radiation emitted from mobile

phones. This study aimed to assess the effects of chronic exposure to electromagnetic waves emitted from Global System for Mobile Communication (GSM) mobile phones on auditory functions. **MATERIAL AND METHODS:** A retrospective, cross-sectional, randomized, case control study was carried out in a tertiary care hospital. One hundred twelve subjects who were long-term mobile phone users (more than 1 year) and 50 controls who had never used a mobile phone underwent a battery of audiologic investigations including pure-tone audiometry (both speech and high frequency), tympanometry, distortion product otoacoustic emissions, auditory brain responses, and middle latency responses. Changes in the various parameters were studied in the mobile phone- and non-mobile phone-using ears of subjects and corresponding ears of the controls to ascertain the effects of electromagnetic exposure. **RESULTS:** There was no significant difference between users and controls for any of the audiologic parameters. However, trends for audiologic abnormalities were seen within the users. High-frequency loss and absent distortion product otoacoustic emissions were observed with an increase in the duration of mobile phone use, excessive use of mobile phones, and age more than 30 years. Additionally, users with some complaints during mobile phone use demonstrated absent distortion product otoacoustic emissions and abnormalities in auditory brainstem response. **CONCLUSION:** Long-term and intensive mobile phone use may cause inner ear damage. A large sample size would be required to reach definitive conclusions.

(E) Panda NK, Modi R, Munjal S, Virk RS. Auditory changes in mobile users: is evidence forthcoming? Otolaryngol Head Neck Surg. 144(4):581-585, 2011. (HU, CE, PE)

OBJECTIVE: Genuine concerns are being raised as to the potential health risks posed by electromagnetic frequency exposure secondary to mobile phone usage. This study was undertaken to assess and compare potential changes in hearing function at the level of the inner ear and central auditory pathway due to chronic exposure to electromagnetic waves from both global system for mobile communications (GSM) and code division multiple access (CDMA) mobile phone usage. DESIGN: Cohort study. SETTING: Tertiary referral center. SUBJECTS AND METHODS: One hundred twenty-five subjects who were long-term mobile phone users (more than 1 year; 63 GSM and 62 CDMA) and 58 controls who had never used mobile phones underwent audiological investigations including pure tone audiometry (250-12 kHz), tympanometry, distortion product otoacoustic emissions (DPOAE), auditory brain responses (ABR), and middle latency responses (MLRs). The changes in various parameters were studied in mobile-using and non-mobile-using ears of both GSM and CDMA subjects and corresponding ears of the controls to ascertain the effects of electromagnetic exposure. RESULTS: GSM and CDMA users were found to be at a significantly higher risk of having DPOAE absent as compared with controls ($P < .05$). They were found to have higher speech frequency thresholds and lower MLR wave and Na and Pa amplitudes. More than 3 years of mobile phone usage emerged as a risk factor ($P < .05$). The damage done was bilateral, with the quantum of damage being the same for both GSM and CDMA. CONCLUSION: Long-term

and intensive GSM and CDMA mobile phone use may cause damage to cochlea as well as the auditory cortex.

(E) Papageorgiou CC, Hountala CD, Maganioti AE, Kyprianou MA, Rabavilas AD, Papadimitriou GN, Capsalis CN. Effects of wi-fi signals on the p300 component of event-related potentials during an auditory hayling task. J Integr Neurosci. 10(2):189-202, 2011 (HU, EE)

The P300 component of event-related potentials (ERPs) is believed to index attention and working memory (WM) operation of the brain. The present study focused on the possible gender-related effects of Wi-Fi (Wireless Fidelity) electromagnetic fields (EMF) on these processes. Fifteen male and fifteen female subjects, matched for age and education level, were investigated while performing a modified version of the Hayling Sentence Completion test adjusted to induce WM. ERPs were recorded at 30 scalp electrodes, both without and with the exposure to a Wi-Fi signal. P300 amplitude values at 18 electrodes were found to be significantly lower in the response inhibition condition than in the response initiation and baseline conditions. Independent of the above effect, within the response inhibition condition there was also a significant gender X radiation interaction effect manifested at 15 leads by decreased P300 amplitudes of males in comparison to female subjects only at the presence of EMF. In conclusion, the present findings suggest that Wi-Fi exposure may exert gender-related alterations on neural activity associated with the amount of attentional resources engaged during a linguistic test adjusted to induce WM.

(NE) Papparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (AS, CH)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Parazzini M, Ravazzani P, Tognola G, Thuróczy G, Molnar FB, Sacchetti A, Ardesi G, Mainardi LT. Electromagnetic fields produced by GSM cellular phones and heart rate variability. Bioelectromagnetics. 28(2):122-129, 2007. (HU, PE)

In this study, 26 healthy young volunteers were submitted to 900 MHz (2 W) GSM cellular phone exposure and to sham exposure in separate sessions. The study was designed to assess cardiac regulatory mechanism in different autonomic nervous system (ANS) states during exposure to low-intensity EMF. Rest-to-stand protocol was applied to evaluate ANS in quiet condition (rest, vagal prevalence) and after a sympathetic activation (stand). The procedure is conducted twice in a double-blind design: once with a genuine EMF exposure and once with a sham exposure (at least 24 h apart). During each session three-leads electrocardiograms were recorded and RR series extracted off-line. Time domain and frequency domain HRV parameters were calculated in every phase of the protocol and during different exposures. The analysis of the data show there was no statistically significant effect due to EMF exposure both on main (i.e., RR mean) and most of the other HRV parameters. A weak interaction between some HRV parameters (i.e., SDNN, TINN, and triangular index in time domain and LF power in frequency domain analysis) and RF exposure was observed and this effect seems to be gathered around the sympathetic response to stand.

(NE) Parazzini M, Sibella F, Lutman ME, Mishra S, Moulin A, Sliwinska-Kowalska M, Woznicka E, Poltanski P, Zmyslony M, Thuroczy G, Molnár F, Kubinyi G, Tavartkiladze G, Bronyakin S, Uloziene I, Uloza V, Gradauskiene E, Ravazzani P. Effects of UMTS cellular phones on human hearing: results of the European project EMFnEAR. Radiat Res. 172(2):244-251, 2009. (HU, PE)

The European project EMFnEAR was undertaken to assess potential changes in human auditory function after a short-term exposure to radiofrequency (RF) radiation produced by UMTS (Universal Mobile Telecommunication System) mobile phones. Participants were healthy young adults with no hearing or ear disorders. Auditory function was assessed immediately before and after exposure to radiofrequency radiation, and only the exposed ear was tested. Tests for the assessment of auditory function were hearing threshold level (HTL), distortion product otoacoustic emissions (DPOAE), contralateral suppression of transiently evoked otoacoustic emission (CAS effect on TEOAE), and auditory evoked potentials (AEP). The exposure consisted of speech at a typical conversational level delivered via an earphone to one ear, plus genuine or sham RF-radiation exposure produced by a commercial phone controlled by a personal computer. Results from 134 participants did not show any consistent pattern of effects on the auditory system after a 20-min UMTS exposure at the maximum output of the phone with 69 mW/kg SAR in the cochlea region in a double blind comparison of genuine and sham exposure. An isolated effect on the hearing threshold at high frequencies was identified, but this was statistically nonsignificant after correction for multiple comparisons. It is concluded that UMTS short-term exposure at the maximum output of consumer mobile phones does not cause measurable immediate effects on the human auditory system.

(E) Partsvania B, Sulaberidze T, Shoshiashvili L, Modebadze Z. Acute effect of exposure of mollusk single neuron to 900-MHz mobile phone radiation. Electromagn Biol Med. 30(3):170-179, 2011. (CS, EE)

The goal of the present work was to explore the influence of commercially available cell phone irradiation on the single neuron excitability and memory processes. A Transverse Electromagnetic Cell (TEM Cell) was used to expose single neurons of mollusk to the electromagnetic field. Finite-Difference Time-Domain (FDTD) method was used for modeling the TEM Cell and the electromagnetic field interactions with living nerve ganglion and neurons. Neuron electrophysiology was investigated using standard microelectrode technique. The specific absorption rate (SAR) deposited into the single neuron was calculated to be 0.63 W/kg with a temperature increment of 0.1°C. After acute exposure, average firing threshold of the action potentials was not changed. However, the average latent period was significantly decreased. This indicates that together with latent period the threshold and the time of habituation might be altered during exposure. However, these alterations are transient and only latent period remains on the changed level.

(E) Pelletier A, Delanaud S, Décima P, Thuroczy G, de Seze R, Cerri M, Bach V, Libert JP, Loos N. Effects of chronic exposure to radiofrequency electromagnetic fields on energy balance in developing rats. Environ Sci Pollut Res Int. 2012 Nov 10. [Epub ahead of print] (AS, LI, CE, BE, PE, SL)

The effects of radiofrequency electromagnetic fields (RF-EMF) on the control of body energy balance in developing organisms have not been studied, despite the involvement of energy status in vital physiological functions. We examined the effects of chronic RF-EMF exposure (900 MHz, 1 V m⁻¹) on the main functions involved in body energy homeostasis (feeding behaviour, sleep and thermoregulatory processes). Thirteen juvenile male Wistar rats were exposed to continuous RF-EMF for 5 weeks at 24 °C of air temperature (T (a)) and compared with 11 non-exposed animals. Hence, at the beginning of the 6th week of exposure, the functions were recorded at T (a) of 24 °C and then at 31 °C. We showed that the frequency of rapid eye movement sleep episodes was greater in the RF-EMF-exposed group, independently of T (a) (+42.1 % at 24 °C and +31.6 % at 31 °C). The other effects of RF-EMF exposure on several sleep parameters were dependent on T (a). At 31 °C, RF-EMF-exposed animals had a significantly lower subcutaneous tail temperature (-1.21 °C) than controls at all sleep stages; this suggested peripheral vasoconstriction, which was confirmed in an experiment with the vasodilator prazosin. Exposure to RF-EMF also increased daytime food intake (+0.22 g h⁻¹). Most of the observed effects of RF-EMF exposure were dependent on T (a). Exposure to RF-EMF appears to modify the functioning of vasomotor tone by acting peripherally through α-adrenoceptors. The elicited vasoconstriction may restrict body

cooling, whereas energy intake increases. Our results show that RF-EMF exposure can induce energy-saving processes without strongly disturbing the overall sleep pattern.

(NE) Perentos N, Croft RJ, McKenzie RJ, Cvetkovic D, Cosic I. Comparison of the effects of continuous and pulsed mobile phone like RF exposure on the human EEG. Australas Phys Eng Sci Med. 30(4):274-280, 2007. (HU, EE)

It is not clear yet whether Global System for Mobiles (GSM) mobile phone radiation has the ability to interfere with normal resting brain function. There have been reports that GSM exposure increases alpha band power, and does so only when the signal is modulated at low frequencies (Huber, R., Treyer, V., Borbely, A. A., Schuderer, J., Gottselig, J. M., Landolt, H.P., Werth, E., Berthold, T., Kuster, N., Buck, A and Achermann, P. Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking EEG. J Sleep Res 11, 289-295, 2002.) However, as that research employed exposure distributions that are not typical of normal GSM handset usage (deep brain areas were overexposed), it remains to be determined whether a similar result patterning would arise from a more representative exposure. In this fully counterbalanced cross-over design, we recruited 12 participants and tried to replicate the modulation linked post exposure alpha band power increase described above, but with an exposure source (dipole antenna) more closely resembling that of a real GSM handset. Exposures lasted for 15 minutes. No changes to alpha power were found for either modulated or unmodulated radiofrequency fields, and thus we failed to replicate the above results. Possible reasons for this failure to replicate are discussed, with the main reason argued to be the lower and more representative exposure distribution employed in the present study. In addition we investigated the possible GSM exposure related effects on the non-linear features of the resting electroencephalogram using the Approximate Entropy (ApEn) method of analysis. Again, no effect was demonstrated for either modulated or unmodulated radiofrequency exposures.

(NE) Platano D, Mesirca P, Paffi A, Pellegrino M, Liberti M, Apollonio F, Bersani F, Aicardi G.

Acute exposure to low-level CW and GSM-modulated 900 MHz radiofrequency does not affect Ba²⁺ currents through voltage-gated calcium channels in rat cortical neurons. Bioelectromagnetics. 28(8):599-607, 2007. (CS, EE)

We have studied the non-thermal effects of radiofrequency (RF) electromagnetic fields (EMFs) on Ba²⁺ currents (I_{Ba²⁺}) through voltage-gated calcium channels (VGCC), recorded in primary cultures of rat cortical neurons using the patch-clamp technique. To assess whether low-level acute RF field exposure could modify the amplitude and/or the voltage-dependence of I_{Ba²⁺}, Petri dishes containing cultured neurons were exposed for 1-3 periods of 90 s to 900 MHz RF-EMF continuous wave (CW) or amplitude-modulated according to global system mobile communication standard (GSM) during whole-cell recording. The specific absorption rates (SARs) were 2 W/kg for CW and 2 W/kg (time average value) for GSM-modulated signals, respectively. The results

obtained indicate that single or multiple acute exposures to either CW or GSM-modulated 900 MHz RF-EMFs do not significantly alter the current amplitude or the current-voltage relationship of I Ba 2+, through VGCC.

(NE) Poulletier de Gannes F, Haro E, Hurtier A, Taxile M, Ruffié G, Billaudel B, Veyret B, Lagroye I. Effect of exposure to the edge signal on oxidative stress in brain cell models. Radiat Res. 175(2):225-230, 2011. (CS, OX)

In this study we investigated the effect of the Enhanced Data rate for GSM Evolution (EDGE) signal on cells of three human brain cell lines, SH-SY5Y, U87 and CHME5, used as models of neurons, astrocytes and microglia, respectively, as well as on primary cortical neuron cultures. SXC-1800 waveguides (IT'IS-Foundation, Zürich, Switzerland) were modified for in vitro exposure to the EDGE signal radiofrequency (RF) radiation at 1800 MHz. Four exposure conditions were tested: 2 and 10 W/kg for 1 and 24 h. The production of reactive oxygen species (ROS) was measured by flow cytometry using the dichlorofluorescein diacetate (DCFH-DA) probe at the end of the 24-h exposure or 24 h after the 1-h exposure. Rotenone treatment was used as a positive control. All cells tested responded to rotenone treatment by increasing ROS production. These findings indicate that exposure to the EDGE signal does not induce oxidative stress under these test conditions, including 10 W/kg. Our results are in agreement with earlier findings that RF radiation alone does not increase ROS production.

(NE) Prochnow N, Gebing T, Ladage K, Krause-Finkeldey D, El Ouardi A, Bitz A, Streckert J, Hansen V, Dermietzel R. Electromagnetic field effect or simply stress? Effects of UMTS exposure on hippocampal longterm plasticity in the context of procedure related hormone release. PLoS One. 6(5):e19437, 2011. (AS, EE)

Harmful effects of electromagnetic fields (EMF) on cognitive and behavioural features of humans and rodents have been controversially discussed and raised persistent concern about adverse effects of EMF on general brain functions. In the present study we applied radio-frequency (RF) signals of the Universal Mobile Telecommunications System (UMTS) to full brain exposed male Wistar rats in order to elaborate putative influences on stress hormone release (corticosteron; CORT and adrenocorticotrophic hormone; ACTH) and on hippocampal derived synaptic long-term plasticity (LTP) and depression (LTD) as electrophysiological hallmarks for memory storage and memory consolidation. Exposure was computer controlled providing blind conditions. Nominal brain-averaged specific absorption rates (SAR) as a measure of applied mass-related dissipated RF power were 0, 2, and 10 W/kg over a period of 120 min. Comparison of cage exposed animals revealed, regardless of EMF exposure, significantly increased CORT and ACTH levels which corresponded with generally decreased field potential slopes and amplitudes in hippocampal LTP and LTD. Animals following SAR exposure of 2 W/kg (averaged over the whole brain of 2.3 g tissue mass) did not differ from the sham-exposed group in LTP and LTD experiments. In contrast, a significant reduction in LTP and LTD was observed at the high power rate of SAR (10 W/kg). The results demonstrate

that a rate of 2 W/kg displays no adverse impact on LTP and LTD, while 10 W/kg leads to significant effects on the electrophysiological parameters, which can be clearly distinguished from the stress derived background. Our findings suggest that UMTS exposure with SAR in the range of 2 W/kg is not harmful to critical markers for memory storage and memory consolidation, however, an influence of UMTS at high energy absorption rates (10 W/kg) cannot be excluded.

(E) Qin F, Yuan H, Nie J, Cao Y, Tong J. [Effects of nano-selenium on cognition performance of mice exposed in 1800 MHz radiofrequency fields]. Wei Sheng Yan Jiu. 43(1):16-21, 2014. [Article in Chinese] (AS, CE, BE, CH, OX)

OBJECTIVE: To study the effects of nano-selenium (NSe) on cognition performance of mice exposed to 1800 MHz radiofrequency fields (RF). METHODS: Male mice were randomly divided into four groups, control and nano-Se low, middle and high dose groups (L, M, H). Each group was sub-divided into three groups, RF 0 min, RF 30 min and RF 120 min. Nano-se solution (2, 4 and 8 microg/ml) were administered to mice of L, M, H groups by intra-gastric injection respectively, 0.5 ml/d for 50 days, the control group was administered with distilled water. At the 21st day, the mice in RF subgroup were exposed to 208 microW/cm² 1800 MHz radiofrequency fields (0, 30 and 120 min/d respectively) for 30 days. The cognitive ability of the mice were tested with Y-maze. Further, the levels of MDA, GABA, Glu, Ach and the activities of CAT and GSH-Px in cerebra were measured. RESULTS: Significant impairments in learning and memory ($P < 0.05$) were observed in the RF 120 min group, and with reduction of the Ach level and the activities of CAT and GSH-Px and increase of the content of GABA, Glu and MDA in cerebrum. NSe enhanced cognitive performance of RF mice, decreased GABA, Glu and MDA levels, increased Ach levels, GSH-Px and CAT activities. CONCLUSION: NSe could improve cognitive impairments of mice exposed to RF, the mechanism of which might involve the increasing antioxidation, decreasing free radical content and the changes of cerebra neurotransmitters.

(NE) Rağbetli MC, Aydinlioğlu A, Koyun N, Rağbetli C, Karayel M. Effect of prenatal exposure to mobile phone on pyramidal cell numbers in the mouse hippocampus: a stereological study. Int J Neurosci. 119(7):1031-1041, 2009. (AS, ME, DE)

Because of the possible risk factor for the health, World Health Organization (WHO) recommended the study with animals on the developing nervous system concerning the exposure to radiofrequency (RF) field. A few studies related to hippocampal exposure are available, which indicate the impact of RF field in some parameters. The present study investigated the effect of exposure to mobile phone on developing hippocampus. Male and female Swiss albino mice were housed as control and mobile phone exposed groups. The pregnant animals in tested group were exposed to the effects of mobile phone in a room possessing the exposure system. The left hemispheres of the brains were processed by frozen microtome. The sections obtained were stained with

Hematoxylin & Eosin. For cell counting by the optical fractionator method, a pilot study was first performed. Hippocampal areas were analyzed using Axiovision software running on a personal computer. The optical dissector, systematically and randomly spaced, was focused to the widest profile of the pyramidal cell nucleus. No significant difference in pyramidal cell number of total Cornu Ammonis (CA) sectors of hippocampus was found between the control and the mobile phone exposed groups ($p > .05$). It was concluded that further study is needed in this field due to popular use of mobile telephones and relatively high exposure to the developing brain.

(E) Rağbetli MC, Aydinlioğlu A, Koyun N, Rağbetli C, Bektas S, Ozdemir S. The effect of mobile phone on the number of Purkinje cells: a stereological study. Int J Radiat Biol. 86(7):548-554, 2010. (AS, ME, DE)

PURPOSE: The World Health Organisation proposed an investigation concerning the exposure of animals to radiofrequency fields because of the possible risk factor for health. At power frequencies there is evidence to associate both childhood leukaemia and brain tumours with magnetic field exposures. There is also evidence of the effect of mobile phone exposure on both cognitive functions and the cerebellum. Purkinje cells of the cerebellum are also sensitive to high dose microwave exposure in rats. The present study investigated the effect of exposure to mobile phone on the number of Purkinje and granule neurons in the developing cerebellum. **MATERIAL AND METHODS:** Male and female Swiss albino mice were housed as control and mobile phone-exposed groups. Pregnant animals in the experimental group were exposed to Global System for Mobile Communication (GSM) mobile phone radiation at 890-915 MHz at 0.95 W/Kg specific absorption rate (SAR). The cerebella were processed by frozen microtome. The sections obtained were stained with Haematoxylin-eosin and cresyl violet. For cell counting by the optical fractionator method, a pilot study was firstly performed. Cerebellar areas were analysed by using Axiovision software running on a personal computer. The optical dissectors were systematically spaced at random, and focused to the widest profile of the neuron cell nucleus. **RESULTS:** A significant decrease in the number of Purkinje cells and a tendency for granule cells to increase in cerebellum was found. **CONCLUSION:** Further studies in this area are needed due to the popular use of mobile telephones and relatively high exposure on developing brain.

(E) Razavinasab M, Moazzami K, Shabani M. Maternal mobile phone exposure alters intrinsic electrophysiological properties of CA1 pyramidal neurons in rat offspring. Toxicol Ind Health. 2014 Mar 6. [Epub ahead of print] (AS, CE, BE, DE, EE)

Some studies have shown that exposure to electromagnetic field (EMF) may result in structural damage to neurons. In this study, we have elucidated the alteration in the hippocampal function of offspring Wistar rats ($n = 8$ rats in each group) that were chronically exposed to mobile phones during their gestational period by applying behavioral, histological, and electrophysiological tests. Rats in the EMF group were exposed to 900 MHz pulsed-EMF irradiation for 6 h/day. Whole cell recordings in

hippocampal pyramidal cells in the mobile phone groups did show a decrease in neuronal excitability. Mobile phone exposure was mostly associated with a decrease in the number of action potentials fired in spontaneous activity and in response to current injection in both male and female groups. There was an increase in the amplitude of the afterhyperpolarization (AHP) in mobile phone rats compared with the control. The results of the passive avoidance and Morris water maze assessment of learning and memory performance showed that phone exposure significantly altered learning acquisition and memory retention in male and female rats compared with the control rats. Light microscopy study of brain sections of the control and mobile phone-exposed rats showed normal morphology. Our results suggest that exposure to mobile phones adversely affects the cognitive performance of both female and male offspring rats using behavioral and electrophysiological techniques.

(E) Redmayne M, Smith E, and Abramson MJ. The relationship between adolescents' well-being and their wireless phone use: a cross-sectional study. Environmental Health 12(1):90, 2013. (HU, BE)

Background. The exposure of young people to radiofrequency electromagnetic fields (RF-EMFs) has increased rapidly in recent years with their increased use of cellphones and use of cordless phones and WiFi. We sought to ascertain associations between New Zealand early-adolescents' subjective well-being and self-reported use of, or exposure to, wireless telephone and internet technology. Methods. In this cross-sectional survey, participants completed questionnaires in class about their cellphone and cordless phone use, their self-reported well-being, and possible confounding information such as whether they had had influenza recently or had a television in the bedroom. Parental questionnaires provided data on whether they had WiFi at home and cordless phone ownership and model. Data were analysed with Ordinal Logistic Regression adjusting for common confounders. Odds ratios (OR) and 95% confidence intervals were calculated. Results. The number and duration of cellphone and cordless phone calls were associated with increased risk of headaches (>6 cellphone calls over 10 minutes weekly, adjusted OR 2.4, CI 1.2-4.8; >15 minutes cordless use daily adjusted OR 1.74, CI 1.1-2.9). Texting and extended use of wireless phones was related to having a painful 'texting' thumb). Using a wired cellphone headset was associated with tinnitus (adjusted OR 1.8, CI 1.0-3.3), while wireless headsets were associated with headache (adjusted OR 2.2, CI 1.1-4.5), feeling down/depressed (adjusted OR 2.0, CI 1.1-3.8), and waking in the night (adjusted OR 2.4, CI 1.2-4.8). Several cordless phone frequencies bands were related to tinnitus, feeling down/depressed and sleepiness at school, while the last of these was also related to modulation. Waking nightly was less likely for those with WiFi at home (adjusted OR 0.7, CI 0.4-0.99). Being woken at night by a cellphone was strongly related to tiredness at school (OR 4.1, CI 2.2-7.7). Conclusions . There were more

statistically significant associations (36%) than could be expected by chance (5%).

Several were dose-dependent relationships. To safeguard young people's well-being, we suggest limiting their use of cellphones and cordless phones to less than 15 minutes daily, and employing a speaker-phone device for longer daily use. We recommend parental measures are taken to prevent young people being woken by their cellphones.

(E) Regel SJ, Tinguely G, Schuderer J, Adam M, Kuster N, Landolt HP, Achermann P. Pulsed radio-frequency electromagnetic fields: dose-dependent effects on sleep, the sleep EEG and cognitive performance. J Sleep Res. 16(3):253-258, 2007. (HU, EE, BE, SL)

To establish a dose-response relationship between the strength of electromagnetic fields (EMF) and previously reported effects on the brain, we investigated the influence of EMF exposure by varying the signal intensity in three experimental sessions. The head of 15 healthy male subjects was unilaterally exposed for 30 min prior to sleep to a pulse-modulated EMF (GSM handset like signal) with a 10 g-averaged peak spatial specific absorption rate of (1) 0.2 W kg⁻¹, (2) 5 W kg⁻¹, or (3) sham exposed in a double-blind, crossover design. During exposure, subjects performed two series of three computerized cognitive tasks, each presented in a fixed order [simple reaction time task, two-choice reaction time task (CRT), 1-, 2-, 3-back task]. Immediately after exposure, night-time sleep was polysomnographically recorded for 8 h. Sleep architecture was not affected by EMF exposure. Analysis of the sleep electroencephalogram (EEG) revealed a dose-dependent increase of power in the spindle frequency range in non-REM sleep. Reaction speed decelerated with increasing field intensity in the 1-back task, while accuracy in the CRT and N-back task were not affected in a dose-dependent manner. In summary, this study reveals first indications of a dose-response relationship between EMF field intensity and its effects on brain physiology as demonstrated by changes in the sleep EEG and in cognitive performance.

(NE) Riddervold IS, Pedersen GF, Andersen NT, Pedersen AD, Andersen JB, Zachariae R, Mølhav L, Sigsgaard T, Kjaergaard SK. Cognitive function and symptoms in adults and adolescents in relation to rf radiation from UMTS base stations. Bioelectromagnetics. 29(4):257-267, 2008. (HU, BE)

There is widespread public concern about the potential adverse health effects of mobile phones in general and their associated base stations in particular. This study was designed to investigate the acute effects of radio frequency (RF) electromagnetic fields (EMF) emitted by the Universal Mobile Telecommunication System (UMTS) mobile phone base stations on human cognitive function and symptoms. Forty adolescents (15-16 years) and 40 adults (25-40 years) were exposed to four conditions: (1) sham, (2) a Continuous Wave (CW) at 2140 MHz, (3) a signal at 2140 MHz modulated as UMTS and (4) UMTS at 2140 MHz including all control features in a randomized, double blinded cross-over design. Each exposure lasted 45 min. During exposure the participants performed different cognitive tasks with the Trail Making B (TMB) test as the main

outcome and completed a questionnaire measuring self reported subjective symptoms. No statistically significant differences between the UMTS and sham conditions were found for performance on TMB. For the adults, the estimated difference between UMTS and sham was -3.2% (-9.2%; 2.9%) and for the adolescents 5.5% (-1.1%; 12.2%). No significant changes were found in any of the cognitive tasks. An increase in 'headache rating' was observed when data from the adolescents and adults were combined ($P = 0.027$), an effect that may be due to differences at baseline. In conclusion, the primary hypothesis that UMTS radiation reduces general performance in the TMB test was not confirmed. However, we suggest that the hypothesis of subjective symptoms and EMF exposure needs further research.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (CS, CH)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(E) Sarapultseva EI, Igolkina JV, Tikhonov VN, Dubrova YE. THE IN VIVO EFFECTS OF LOW-INTENSITY RADIOFREQUENCY FIELDS ON THE MOTOR ACTIVITY OF PROTOZOA. Int J Radiat Biol. 2013 Nov 25. [Epub ahead of print] (AS, BE, LI)

Purpose: To analyze the direct and transgenerational effects of exposure to low-dose 1 GHz (mobile phone/wireless telecommunication range) and 10 GHz (radar/satellite communication range) radiofrequency electromagnetic fields (RF-EMF) on the motility of ciliates *Spirostomum ambiguum*. Materials and Methods: *S. ambiguum* were exposed to 1 GHz and 10 GHz RF-EMF with power flux densities (PD) ranging from 0.05 to 0.5 W/m^2 over a period of time from 0.05 to 10 h. The motility of directly exposed ciliates and their non-exposed progeny across 10-15 generations was measured. Results: Exposure to 0.1 W/m^2 of either 1 or 10 GHz RF-EMF resulted in a significant decrease in the motility. The dose of exposure capable of altering the mobility of ciliates was inversely correlated with the flux density of RF-EMF. The motility of the non-exposed progeny of ciliates irradiated with 0.1 W/m^2 of 10 GHz RF-EMF remained significantly compromised, at least, across 10-15 generations, thus indicating the presence of transgenerational effects. Conclusions: The results of our study show that low-dose exposure to RF-EMF can significantly affect the motility of irradiated ciliates and their

non-exposed offspring, thus providing further insights into the unknown mechanisms underlying the in vivo effects of RF-EMF.

(NE) Sauter C, Dorn H, Bahr A, Hansen ML, Peter A, Bajbouj M, Danker-Hopfe H. Effects of exposure to electromagnetic fields emitted by GSM 900 and WCDMA mobile phones on cognitive function in young male subjects. *Bioelectromagnetics*. 32(3):179-190, 2011. (HU, BE)

Results of studies on the possible effects of electromagnetic fields emitted by mobile phones on cognitive functions are contradictory, therefore, possible effects of long-term (7 h 15 min) electromagnetic field (EMF) exposure to handset-like signals of Global System for Mobile Communications (GSM) 900 and Wideband Code-Division Multiple Access (WCDMA) on attention and working memory were studied. The sample comprised 30 healthy male subjects (mean \pm SD: 25.3 \pm 2.6 years), who were tested on nine study days in which they were exposed to three exposure conditions (sham, GSM 900 and WCDMA) in a randomly assigned and balanced order. All tests were presented twice (morning and afternoon) on each study day within a fixed timeframe. Univariate comparisons revealed significant changes when subjects were exposed to GSM 900 compared to sham, only in the vigilance test. In the WCDMA exposure condition, one parameter in the vigilance and one in the test on divided attention were altered compared to sham. Performance in the selective attention test and the n-back task was not affected by GSM 900 or WCDMA exposure. Time-of-day effects were evident for the tests on divided and selective attention, as well as for working memory. After correction for multiple testing, only time-of-day effects remained significant in two tests, resulting in faster reactions in the afternoon trials. The results of the present study do not provide any evidence of an EMF effect on human cognition, but they underline the necessity to control for time of day.

(E) Schmid MR, Loughran SP, Regel SJ, Murbach M, Bratic Grunauer A, Rusterholz T, Bersagliere A, Kuster N, Achermann P. Sleep EEG alterations: effects of different pulse-modulated radio frequency electromagnetic fields. *J Sleep Res*. 21(1):50-58, 2012a. (HU, EE, BE, SL, WS)

Previous studies have observed increases in electroencephalographic power during sleep in the spindle frequency range (approximately 11-15 Hz) after exposure to mobile phone-like radio frequency electromagnetic fields (RF EMF). Results also suggest that pulse modulation of the signal is crucial to induce these effects. Nevertheless, it remains unclear which specific elements of the field are responsible for the observed changes. We investigated whether pulse-modulation frequency components in the range of sleep spindles may be involved in mediating these effects. Thirty young healthy men were exposed, at weekly intervals, to three different conditions for 30 min directly prior to an 8-h sleep period. Exposure consisted of a 900-MHz RF EMF, pulse modulated at 14 Hz or 217 Hz, and a sham control condition. Both active conditions had a peak spatial specific absorption rate of 2 W kg⁻¹. During exposure subjects

performed three different cognitive tasks (measuring attention, reaction speed and working memory), which were presented in a fixed order. Electroencephalographic power in the spindle frequency range was increased during non-rapid eye movement sleep (2nd episode) following the 14-Hz pulse-modulated condition. A similar but non-significant increase was also observed following the 217-Hz pulse-modulated condition. Importantly, this exposure-induced effect showed considerable individual variability. Regarding cognitive performance, no clear exposure-related effects were seen. Consistent with previous findings, our results provide further evidence that pulse-modulated RF EMF alter brain physiology, although the time-course of the effect remains variable across studies. Additionally, we demonstrated that modulation frequency components within a physiological range may be sufficient to induce these effects.

(E) Schmid MR, Murbach M, Lustenberger C, Maire M, Kuster N, Achermann P, Loughran SP. Sleep EEG alterations: effects of pulsed magnetic fields versus pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 21(6):620-629, 2012b. (HU, EE, SL)

Studies have repeatedly shown that electroencephalographic power during sleep is enhanced in the spindle frequency range following radio frequency electromagnetic field exposures pulse-modulated with fundamental frequency components of 2, 8, 14 or 217 Hz and combinations of these. However, signals used in previous studies also had significant harmonic components above 20 Hz. The current study aimed: (i) to determine if modulation components above 20 Hz, in combination with radio frequency, are necessary to alter the electroencephalogram; and (ii) to test the demodulation hypothesis, if the same effects occur after magnetic field exposure with the same pulse sequence used in the pulse-modulated radio frequency exposure. In a randomized double-blind crossover design, 25 young healthy men were exposed at weekly intervals to three different conditions for 30 min before sleep. Cognitive tasks were also performed during exposure. The conditions were a 2-Hz pulse-modulated radio frequency field, a 2-Hz pulsed magnetic field, and sham. Radio frequency exposure increased electroencephalogram power in the spindle frequency range. Furthermore, delta and theta activity (non-rapid eye movement sleep), and alpha and delta activity (rapid eye movement sleep) were affected following both exposure conditions. No effect on sleep architecture and no clear impact of exposure on cognition was observed. These results demonstrate that both pulse-modulated radio frequency and pulsed magnetic fields affect brain physiology, and the presence of significant frequency components above 20 Hz are not fundamental for these effects to occur. Because responses were not identical for all exposures, the study does not support the hypothesis that effects of radio frequency exposure are based on demodulation of the signal only.

(E) Sharma A, Sisodia R, Bhatnagar D, Saxena VK. Spatial memory and learning performance and its relationship to protein synthesis of Swiss albino mice exposed to

10 GHz microwaves. Int J Radiat Biol. 2013 Aug 19. [Epub ahead of print] (AS, CE, BE, CH)

Purpose: To study the possible role of microwave (MW) exposure on spatial memory of Swiss albino mice and its relationship to protein concentration in whole brain. Materials and methods: Mice were exposed to 10 GHz (Giga Hertz) microwaves with the power density of 0.25 mW/cm² (milliwatt per centimeter square) with average whole body specific absorption rate (SAR) 0.1790 W/kg daily for 2 hours per day (h/day) for 30 days. After exposure mice were tested for spatial memory performance using Morris water maze test (MWT). For this purpose mice (6-8 weeks old) were divided into two groups (i) sham exposed and, (ii) microwaves exposed. After initial training for two days, MWT was performed for another 6 days. Protein was estimated 48 hours after exposure and immediately after completion of MWT. Results: Both sham exposed and microwave exposed animals showed a significant decrease in escape time with training. Microwave exposed animals had statistically significant higher mean latency to reach the target quadrant compared to sham exposed. A concurrent decrease in protein levels was estimated in whole brain of the exposed mice compared to sham exposed mice. Conclusions: It can be concluded from the current study that exposure to microwave radiation caused decrements in the ability of mice to learn the special memory task, this may be due to simultaneous decrease in protein levels in the brain of mice.

(E) Sirav B, Seyhan N. Effects of radiofrequency radiation exposure on blood-brain barrier permeability in male and female rats. Electromagn Biol Med. 30(4):253-260, 2011. (AS, ME)

During the last several decades, numerous studies have been performed aiming at the question of whether or not exposure to radiofrequency radiation (RFR) influences the permeability of the blood-brain barrier (BBB). The objective of this study was to investigate the effect of RFR on the permeability of BBB in male and female Wistar albino rats. Right brain, left brain, cerebellum, and total brain were analyzed separately in the study. Rats were exposed to 0.9 and 1.8 GHz continuous-wave (CW) RFR for 20 min (at SARs of 4.26 mW/kg and 1.46 mW/kg, respectively) while under anesthesia. Control rats were sham-exposed. Disruption of BBB integrity was detected spectrophotometrically using the Evans-blue dye, which has been used as a BBB tracer and is known to be bound to serum albumin. Right brain, left brain, cerebellum, and total brain were evaluated for BBB permeability. In female rats, no albumin extravasation was found in the brain after RFR exposure. A significant increase in albumin was found in the brains of the RF-exposed male rats when compared to sham-exposed male brains. These results suggest that exposure to 0.9 and 1.8 GHz CW RFR at levels below the international limits can affect the vascular permeability in the brain of male rats. The possible risk of RFR exposure in humans is a major concern for the society. Thus, this topic should be investigated more thoroughly in the future.

(E) Söderqvist F, Carlberg M, Hardell L. Mobile and cordless telephones, serum transthyretin and the blood-cerebrospinal fluid barrier: a cross-sectional study. Environ Health. 21; 8:19, 2009. (HU, PE)

BACKGROUND: Whether low-intensity radiofrequency radiation damages the blood-brain barrier has long been debated, but little or no consideration has been given to the blood-cerebrospinal fluid barrier. In this cross-sectional study we tested whether long-term and/or short-term use of wireless telephones was associated with changes in the serum transthyretin level, indicating altered transthyretin concentration in the cerebrospinal fluid, possibly reflecting an effect of radiation. **METHODS:** One thousand subjects, 500 of each sex aged 18-65 years, were randomly recruited using the population registry. Data on wireless telephone use were assessed by a postal questionnaire and blood samples were analyzed for serum transthyretin concentrations determined by standard immunonephelometric techniques on a BN Prospec instrument. **RESULTS:** The response rate was 31.4%. Logistic regression of dichotomized TTR serum levels with a cut-point of 0.31 g/l on wireless telephone use yielded increased odds ratios that were statistically not significant. Linear regression of time since first use overall and on the day that blood was withdrawn gave different results for males and females: for men significantly higher serum concentrations of TTR were seen the longer an analogue telephone or a mobile and cordless desktop telephone combined had been used, and in contrast, significantly lower serum levels were seen the longer an UMTS telephone had been used. Adjustment for fractions of use of the different telephone types did not modify the effect for cumulative use or years since first use for mobile telephone and DECT, combined. For women, linear regression gave a significant association for short-term use of mobile and cordless telephones combined, indicating that the sooner blood was withdrawn after the most recent telephone call, the higher the expected transthyretin concentration. **CONCLUSION:** In this hypothesis-generating descriptive study time since first use of mobile telephones and DECT combined was significantly associated with higher TTR levels regardless of how much each telephone type had been used. Regarding short-term use, significantly higher TTR concentrations were seen in women the sooner blood was withdrawn after the most recent telephone call on that day.

(E) Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. Exposure to an 890-MHz mobile phone-like signal and serum levels of S100B and transthyretin in volunteers. Toxicol Lett. 189(1):63-66, 2009. (HU, PE)

Whether low-intensity non-thermal microwave radiation alters the integrity of the blood-brain barrier has been debated since the late 1970s, yet no experimental study has been carried out on humans. The aim of this study was to test, using peripheral markers, whether exposure to a mobile phone-like signal alters the integrity of the human blood-brain and blood-cerebrospinal fluid barriers. A provocation study was carried out that exposed 41 volunteers to a 30 min GSM 890 MHz signal with an average specific energy absorption rate distribution of 1.0 W/kg in the temporal area of the head

as measured over any 1g of contiguous tissue. The outcome was assessed by changes in serum concentrations of two putative markers of brain barrier integrity, S100B and transthyretin. Repeated blood sampling before and after the provocation showed no statistically significant increase in the serum levels of S100B, while for transthyretin a statistically significant increase was seen in the final blood sample 60 min after the end of the provocation as compared to the prior sample taken immediately after provocation (p=0.02). The clinical significance of this finding, if any, is unknown. Further randomized studies with use of additional more brain specific markers are needed.

(NE) Söderqvist F, Carlberg M, Hardell L. Use of wireless telephones and serum S100B levels: a descriptive cross-sectional study among healthy Swedish adults aged 18-65 years. Sci Total Environ. 407(2):798-805, 2009. (HU, PE)

BACKGROUND: Since the late 1970s, experimental animal studies have been carried out on the possible effects of low-intensive radiofrequency fields on the blood-brain barrier (BBB), but no epidemiological study has been published to date. **OBJECTIVE:** Using serum S100B as a putative marker of BBB dysfunction we performed a descriptive cross-sectional study to investigate whether protein levels were higher among frequent than non-frequent users of mobile and cordless desktop phones. **METHOD:** One thousand subjects, 500 of each sex aged 18-65 years, were randomly recruited using the population registry. Data on wireless phone use were assessed by a postal questionnaire and blood samples were analyzed for S100B. **RESULTS:** The response rate was 31.4%. The results from logistic and linear regression analyses were statistically insignificant, with one exception: the linear regression analysis of latency for UMTS use, which after stratifying on gender remained significant only for men ($p = 0.01$; $n = 31$). A low p-value (0.052) was obtained for use of cordless phone ($n = 98$) prior to giving the blood samples indicating a weak negative association. Total use of mobile and cordless phones over time yielded odds ratio (OR) 0.8 and 95% confidence interval (CI) 0.3-2.0 and use on the same day as giving blood yielded $OR=1.1$, $CI=0.4-2.8$. **CONCLUSIONS:** This study failed to show that long- or short-term use of wireless telephones was associated with elevated levels of serum S100B as a marker of BBB integrity. The finding regarding latency of UMTS use may be interesting but it is based on small numbers. Generally, S100B levels were low and to determine whether this association - if causal - is clinically relevant, larger studies with sufficient follow-up are needed.

(E) Söderqvist F, Hardell L, Carlberg M, Mild KH. Radiofrequency fields, transthyretin, and Alzheimer's disease. J Alzheimers Dis. 20(2):599-606, 2010. (HU, PE, MA)

Radiofrequency field (RF) exposure provided cognitive benefits in an animal study. In Alzheimer's disease (AD) mice, exposure reduced brain amyloid-beta (Abeta) deposition through decreased aggregation of Abeta and increase in soluble Abeta levels. Based on our studies on humans on RF from wireless phones, we propose that transthyretin (TTR) might explain the findings. In a cross-sectional study on 313 subjects, we used serum TTR as a marker of cerebrospinal fluid TTR. We found a statistically significantly positive

beta coefficient for TTR for time since first use of mobile phones and desktop cordless phones combined ($P=0.03$). The electromagnetic field parameters were similar for the phone types. In a provocation study on 41 persons exposed for 30 min to an 890-MHz GSM signal with specific absorption rate of 1.0 Watt/kg to the temporal area of the brain, we found statistically significantly increased serum TTR 60 min after exposure. In our cross-sectional study, use of oral snuff also yielded statistically significantly increased serum TTR concentrations and nicotine has been associated with decreased risk for AD and to upregulate the TTR gene in choroid plexus but not in the liver, another source of serum TTR. TTR sequesters Abeta, thereby preventing the formation of Abeta plaques in the brain. Studies have shown that patients with AD have lowered TTR concentrations in the cerebrospinal fluid and have attributed the onset of AD to insufficient sequestering of Abeta by TTR. We propose that TTR might be involved in the findings of RF exposure benefit in AD mice.

(E) Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G, Krstic D, Cvetkovic T, Pavlovic V. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. J Radiat Res. 49(6):579-586, 2008. (AS, CE, CH, OX)

PURPOSE: The aim of the study was to evaluate the intensity of oxidative stress in the brain of animals chronically exposed to mobile phones and potential protective effects of melatonin in reducing oxidative stress and brain injury. **MATERIALS AND METHODS:** Experiments were performed on Wistar rats exposed to microwave radiation during 20, 40 and 60 days. Four groups were formed: I group (control)- animals treated by saline, intraperitoneally (i.p.) applied daily during follow up, II group (Mel)- rats treated daily with melatonin (2 mg kg⁻¹ body weight i.p.), III group (MWs)- microwave exposed rats, IV group (MWs + Mel)- MWs exposed rats treated with melatonin (2 mg kg⁻¹ body weight i.p.). The microwave radiation was produced by a mobile test phone (SAR = 0.043-0.135 W/kg). **RESULTS:** A significant increase in the brain tissue malondialdehyde (MDA) and carbonyl group concentration was registered during exposure. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of exposure to mobile phones. Melatonin treatment significantly prevented the increase in the MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. **CONCLUSION:** We demonstrated two important findings; that mobile phones caused oxidative damage biochemically by increasing the levels of MDA, carbonyl groups, XO activity and decreasing CAT activity; and that treatment with the melatonin significantly prevented oxidative damage in the brain.

(E) Sokolovic D, Djordjevic B, Kocic G, Babovic P, Ristic G, Stanojkovic Z, Sokolovic DM, Veljkovic A, Jankovic A, Radovanovic Z. The effect of melatonin on body mass and behaviour of rats during an exposure to microwave radiation from mobile phone. Bratisl Lek Listy. 113(5):265-269, 2012. (AS, CE, PE, BE)

BACKGROUND: Microwave radiation (MW) produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may cause various disorders in human organism. On the other hand, melatonin is a potent antioxidant, immunostimulator and neuromodulator. The aim of this research was to determine body mass and behaviour changes in rats after a chronic microwave exposure, as well as to determine the effects of melatonin on body mass and behaviour in irradiated rats. **METHODS:** Wistar rats were divided into the four experimental groups: I group (control) - rats treated with 0,9 % saline, II group (Mel) - rats treated with melatonin (2 mg/kg), III group (MW) - rats exposed to MW radiation (4 h/day), IV group (MW+Mel) - rats, which were both exposed to MW radiation and received melatonin premedication (2 mg/kg). **RESULTS:** A significant body mass reduction was noted in animals exposed to MW radiation when compared to controls after 20, 40 and 60 days ($p < 0.001$). Furthermore, body weight was significantly increased ($p < 0.05$) in irradiated rats, which received melatonin pretreatment (MW+Mel) in comparison to irradiated group (MW) after 20 days. Microwave radiation exposed animals showed an anxiety related behaviour (agitation, irritability) after 10 days of exposure. After the radiation source removal, changes in behaviour were less noticeable. Melatonin administration to irradiated rats caused a decrease in the stress induced behaviour. **CONCLUSION:** Microwave radiation causes body mass decrease and anxiety related behaviour in rats, however melatonin causes a reverse of those effects on both body weight and behaviour of irradiated animals (Fig. 2, Ref. 32).

(E) Sonmez OF, Odaci E, Bas O, Kaplan S. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. Brain Res. 1356:95-101, 2010. (AS, CE, ME)

The biological effects of electromagnetic field (EMF) exposure from mobile phones have growing concern among scientists since there are some reports showing increased risk for human health, especially in the use of mobile phones for a long duration. In the presented study, the effects on the number of Purkinje cells in the cerebellum of 16-week (16 weeks) old female rats were investigated following exposure to 900 MHz EMF. Three groups of rats, a control group (CG), sham exposed group (SG) and an electromagnetic field exposed group (EMFG) were used in this study. While EMFG group rats were exposed to 900 MHz EMF (1h/day for 28 days) in an exposure tube, SG was placed in the exposure tube but not exposed to EMF (1h/day for 28 days). The specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). The CG was not placed into the exposure tube nor was it exposed to EMF during the study period. At the end of the experiment, all of the female rats were sacrificed and the number of Purkinje cells was estimated using a stereological counting technique. Histopathological evaluations were also done on sections of the cerebellum. Results showed that the total number of Purkinje cells in the cerebellum of the EMFG was significantly lower than those of CG ($p < 0.004$) and SG ($p < 0.002$). In addition, there was no significant difference at the 0.05 level between the rats' body and brain weights

in the EMFG and CG or SG. Therefore, it is suggested that long duration exposure to 900 MHz EMF leads to decreases of Purkinje cell numbers in the female rat cerebellum.

(E) Spichtig S, Scholkmann F, Chin L, Lehmann H, Wolf M. Assessment of intermittent UMTS electromagnetic field effects on blood circulation in the human auditory region using a near-infrared system. Bioelectromagnetics. 33(1):40-54, 2012. (HU, PE)

The aim of the present study was to assess the potential effects of intermittent Universal Mobile Telecommunications System electromagnetic fields (UMTS-EMF) on blood circulation in the human head (auditory region) using near-infrared spectroscopy (NIRS) on two different timescales: short-term (effects occurring within 80 s) and medium-term (effects occurring within 80 s to 30 min). For the first time, we measured potential immediate effects of UMTS-EMF in real-time without any interference during exposure. Three different exposures (sham, 0.18 W/kg, and 1.8 W/kg) were applied in a controlled, randomized, crossover, and double-blind paradigm on 16 healthy volunteers. In addition to oxy-, deoxy-, and total haemoglobin concentrations ([O(2) Hb], [HHb], and [tHb], respectively), the heart rate (HR), subjective well-being, tiredness, and counting speed were recorded. During exposure to 0.18 W/kg, we found a significant short-term increase in Δ [O(2) Hb] and Δ [tHb], which is small (\approx 17%) compared to a functional brain activation. A significant decrease in the medium-term response of Δ [HHb] at 0.18 and 1.8 W/kg exposures was detected, which is in the range of physiological fluctuations. The medium-term Δ HR was significantly higher (+1.84 bpm) at 1.8 W/kg than for sham exposure. The other parameters showed no significant effects. Our results suggest that intermittent exposure to UMTS-EMF has small short- and medium-term effects on cerebral blood circulation and HR.

(NE) Stefanics G, Kellényi L, Molnár F, Kubinyi G, Thuróczy G, Hernádi I. Short GSM mobile phone exposure does not alter human auditory brainstem response. BMC Public Health. 7:325, 2007. (HU, EE)

BACKGROUND: There are about 1.6 billion GSM cellular phones in use throughout the world today. Numerous papers have reported various biological effects in humans exposed to electromagnetic fields emitted by mobile phones. The aim of the present study was to advance our understanding of potential adverse effects of the GSM mobile phones on the human hearing system. **METHODS:** Auditory Brainstem Response (ABR) was recorded with three non-polarizing Ag-AgCl scalp electrodes in thirty young and healthy volunteers (age 18-26 years) with normal hearing. ABR data were collected before, and immediately after a 10 minute exposure to 900 MHz pulsed electromagnetic field (EMF) emitted by a commercial Nokia 6310 mobile phone. Fifteen subjects were exposed to genuine EMF and fifteen to sham EMF in a double blind and counterbalanced order. Possible effects of irradiation was analyzed by comparing the latency of ABR waves I, III and V before and after genuine/sham EMF exposure. **RESULTS:** Paired sample t-test was conducted for statistical analysis. Results revealed

no significant differences in the latency of ABR waves I, III and V before and after 10 minutes of genuine/sham EMF exposure. **CONCLUSION:** The present results suggest that, in our experimental conditions, a single 10 minute exposure of 900 MHz EMF emitted by a commercial mobile phone does not produce measurable immediate effects in the latency of auditory brainstem waves I, III and V.

(NE) Stefanics G, Thuróczy G, Kellényi L, Hernádi I. Effects of twenty-minute 3G mobile phone irradiation on event related potential components and early gamma synchronization in auditory oddball paradigm. Neuroscience. 157(2):453-462, 2008. (HU, EE)

We investigated the potential effects of 20 min irradiation from a new generation Universal Mobile Telecommunication System (UMTS) 3G mobile phone on human event related potentials (ERPs) in an auditory oddball paradigm. In a double-blind task design, subjects were exposed to either genuine or sham irradiation in two separate sessions. Before and after irradiation subjects were presented with a random series of 50 ms tone burst (frequent standards: 1 kHz, P=0.8, rare deviants: 1.5 kHz, P=0.2) at a mean repetition rate of 1500 ms while electroencephalogram (EEG) was recorded. The subjects' task was to silently count the appearance of targets. The amplitude and latency of the N100, N200, P200 and P300 components for targets and standards were analyzed in 29 subjects. We found no significant effects of electromagnetic field (EMF) irradiation on the amplitude and latency of the above ERP components. In order to study possible effects of EMF on attentional processes, we applied a wavelet-based time-frequency method to analyze the early gamma component of brain responses to auditory stimuli. We found that the early evoked gamma activity was insensitive to UMTS RF exposition. Our results support the notion, that a single 20 min irradiation from new generation 3G mobile phones does not induce measurable changes in latency or amplitude of ERP components or in oscillatory gamma-band activity in an auditory oddball paradigm.

(NE) Stovner LJ, Oftedal G, Straume A, Johnsson A. Nocebo as headache trigger: evidence from a sham-controlled provocation study with RF fields. Acta Neurol Scand Suppl. 188:67-71, 2008. (HU, PE)

BACKGROUND: A large proportion of the population in Norway has experienced headache in connection with mobile phone use, but several double-blind provocation studies with radiofrequency (RF) and sham exposures have shown no relation between headache and mobile phone RF fields. **AIMS:** To investigate the type and location of headache experienced by participants in one provocation study in order to gain insight into possible causes and mechanisms of the headaches. **METHOD:** Questionnaire about headache, indication on figure of location of headache after exposure, interview with neurologist about headache features to make headache diagnoses. **RESULTS:** The 17 participants went through 130 trials (sham or RF exposure). No significant difference existed in headache type, laterality or location between the headaches experienced

with the two exposures types. In most participants, the headache was compatible with tension-type headache. DISCUSSION: As participants experienced their typical 'mobile phone headache' both with and without RF exposure, and since the experiment did not involve the stress or the arm/head position of mobile phone use, the most likely explanation is that the headache in this situation is caused by negative expectations (nocebo). CONCLUSION: This and other similar studies indicate that headache occurring in connection with mobile phone use is not related to RF fields, and that a nocebo effect is important for this and possibly other headache triggers.

(E) Sudan M, Kheifets L, Arah OA, Olsen J. Cell phone exposures and hearing loss in children in the Danish National Birth Cohort. Paediatr Perinat Epidemiol. 27(3):247-257, 2013. (HU, BE)

BACKGROUND: Children today are exposed to cell phones early in life, and may be the most vulnerable if exposure is harmful to health. We investigated the association between cell phone use and hearing loss in children. METHODS: The Danish National Birth Cohort (DNBC) enrolled pregnant women between 1996 and 2002. Detailed interviews were conducted during gestation, and when the children were 6 months, 18 months and 7 years of age. We used multivariable-adjusted logistic regression, marginal structural models (MSM) with inverse-probability weighting, and doubly robust estimation (DRE) to relate hearing loss at age 18 months to cell phone use at age 7 years, and to investigate cell phone use reported at age 7 in relation to hearing loss at age 7. RESULTS: Our analyses included data from 52 680 children. We observed weak associations between cell phone use and hearing loss at age 7, with odds ratios and 95% confidence intervals from the traditional logistic regression, MSM and DRE models being 1.21 [95% confidence interval [CI] 0.99, 1.46], 1.23 [95% CI 1.01, 1.49] and 1.22 [95% CI 1.00, 1.49], respectively. CONCLUSIONS: Our findings could have been affected by various biases and are not sufficient to conclude that cell phone exposures have an effect on hearing. This is the first large-scale epidemiologic study to investigate this potentially important association among children, and replication of these findings is needed.

(NE) Terao Y, Okano T, Furubayashi T, Yugeta A, Inomata-Terada S, Ugawa Y. Effects of thirty-minute mobile phone exposure on saccades. Clin Neurophysiol. 118(7):1545-1556, 2007. (HU PE)

OBJECTIVE: To investigate whether exposure to pulsed high-frequency electromagnetic field (pulsed EMF) emitted by a mobile phone has short-term effects on saccade performances. METHODS: A double blind, counterbalanced crossover design was employed. In 10 normal subjects, we studied the performance of visually guided saccade (VGS), gap saccade (GAP), and memory guided saccade (MGS) tasks before and after exposure to EMF emitted by a mobile phone for thirty minutes or sham exposure. We also implemented a hand reaction time (RT) task in response to a visual signal. RESULTS:

With the exception of VGS and MGS latencies, the parameters of VGS, GAP and MGS tasks were unchanged before and after real or sham EMF exposure. In addition, the latencies of VGS and MGS did not change differently after real and sham exposure. The hand RT shortened with the repetition of trials, but again this trend was of similar magnitude for real and sham exposures. CONCLUSIONS: Thirty minutes of mobile phone exposure has no significant short-term effect on saccade performances. SIGNIFICANCE: This is the first study to investigate saccade performance in relation to mobile phone exposure. No significant effect of mobile phone use was demonstrated on the performance of various saccade tasks, suggesting that the cortical processing for saccades and attention is not affected by exposure to EMF emitted by a mobile phone.

(NE) Thomas S, Benke G, Dimitriadis C, Inyang I, Sim MR, Wolfe R, Croft RJ, Abramson MJ. Use of mobile phones and changes in cognitive function in adolescents. Occup Environ Med. 67(12):861-866, 2010a. (HU, BE)

BACKGROUND: Several studies have investigated the impact of mobile phone exposure on cognitive function in adults. However, children and adolescents are of special interest due to their developing nervous systems. METHODS: Data were derived from the Australian Mobile Radiofrequency Phone Exposed Users' Study (MoRPhEUS) which comprised a baseline examination of year 7 students during 2005/2006 and a 1-year follow-up. Sociodemographic and exposure data were collected with a questionnaire. Cognitive functions were assessed with a computerised test battery and the Stroop Color-Word test. RESULTS: 236 students participated in both examinations. The proportion of mobile phone owners and the number of voice calls and short message services (SMS) per week increased from baseline to follow-up. Participants with more voice calls and SMS at baseline showed less reductions in response times over the 1-year period in various computerised tasks. Furthermore, those with increased voice calls and SMS exposure over the 1-year period showed changes in response time in a simple reaction and a working memory task. No associations were seen between mobile phone exposure and the Stroop test. CONCLUSIONS: We have observed that some changes in cognitive function, particularly in response time rather than accuracy, occurred with a latency period of 1 year and that some changes were associated with increased exposure. However, the increased exposure was mainly applied to those who had fewer voice calls and SMS at baseline, suggesting that these changes over time may relate to statistical regression to the mean, and not be the effect of mobile phone exposure.

(E) Thomas S, Heinrich S, von Kries R, Radon K. Exposure to radio-frequency electromagnetic fields and behavioural problems in Bavarian children and adolescents. Eur J Epidemiol. 25(2):135-141, 2010b. (HU, BE)

Only few studies have so far investigated possible health effects of radio-frequency electromagnetic fields (RF EMF) in children and adolescents, although experts discuss a potential higher vulnerability to such fields. We aimed to investigate a possible association between measured exposure to RF EMF fields and behavioural problems in

children and adolescents. 1,498 children and 1,524 adolescents were randomly selected from the population registries of four Bavarian (South of Germany) cities. During an Interview data on participants' mental health, socio-demographic characteristics and potential confounders were collected. Mental health behaviour was assessed using the German version of the Strengths and Difficulties Questionnaire (SDQ). Using a personal dosimeter, we obtained radio-frequency EMF exposure profiles over 24 h. Exposure levels over waking hours were expressed as mean percentage of the reference level. Overall, exposure to radiofrequency electromagnetic fields was far below the reference level. Seven percent of the children and 5% of the adolescents showed an abnormal mental behaviour. In the multiple logistic regression analyses measured exposure to RF fields in the highest quartile was associated to overall behavioural problems for adolescents (OR 2.2; 95% CI 1.1-4.5) but not for children (1.3; 0.7-2.6). These results are mainly driven by one subscale, as the results showed an association between exposure and conduct problems for adolescents (3.7; 1.6-8.4) and children (2.9; 1.4-5.9). As this is one of the first studies that investigated an association between exposure to mobile telecommunication networks and mental health behaviour more studies using personal dosimetry are warranted to confirm these findings.

(E) Thomée S, Härenstam A, Hagberg M. Mobile phone use and stress, sleep disturbances, and symptoms of depression among young adults--a prospective cohort study. BMC Public Health. 11:66, 2011. (HU, BE) (Effects may not be caused by RFR exposure.)

BACKGROUND: Because of the quick development and widespread use of mobile phones, and their vast effect on communication and interactions, it is important to study possible negative health effects of mobile phone exposure. The overall aim of this study was to investigate whether there are associations between psychosocial aspects of mobile phone use and mental health symptoms in a prospective cohort of young adults. METHODS: The study group consisted of young adults 20-24 years old (n = 4156), who responded to a questionnaire at baseline and 1-year follow-up. Mobile phone exposure variables included frequency of use, but also more qualitative variables: demands on availability, perceived stressfulness of accessibility, being awakened at night by the mobile phone, and personal overuse of the mobile phone. Mental health outcomes included current stress, sleep disorders, and symptoms of depression. Prevalence ratios (PRs) were calculated for cross-sectional and prospective associations between exposure variables and mental health outcomes for men and women separately. RESULTS: There were cross-sectional associations between high compared to low mobile phone use and stress, sleep disturbances, and symptoms of depression for the men and women. When excluding respondents reporting mental health symptoms at baseline, high mobile phone use was associated with sleep disturbances and symptoms of depression for the men and symptoms of depression for the women at 1-year follow-up. All qualitative variables had cross-sectional associations with mental health outcomes. In prospective analysis, overuse was associated with stress and sleep disturbances for women, and high accessibility stress was associated with stress, sleep

disturbances, and symptoms of depression for both men and women. **CONCLUSIONS:** High frequency of mobile phone use at baseline was a risk factor for mental health outcomes at 1-year follow-up among the young adults. The risk for reporting mental health symptoms at follow-up was greatest among those who had perceived accessibility via mobile phones to be stressful. Public health prevention strategies focusing on attitudes could include information and advice, helping young adults to set limits for their own and others' accessibility.

(E) Tombini M, Pellegrino G, Pasqualetti P, Assenza G, Benvenga A, Fabrizio E, Rossini PM Mobile phone emissions modulate brain excitability in patients with focal epilepsy. Brain Stimul. 2012 Aug 9. [Epub ahead of print] (HU, EE, MA)

BACKGROUND: Electromagnetic fields (EMFs) emitted by mobile phones had been shown to increase cortical excitability in healthy subjects following 45 min of continuous exposure on the ipsilateral hemisphere. **OBJECTIVE:** Using Transcranial Magnetic Stimulation (TMS), the current study assessed the effects of acute exposure to mobile phone EMFs on the cortical excitability in patients with focal epilepsy. **METHODS:** Ten patients with cryptogenic focal epilepsy originating outside the primary motor area (M1) were studied. Paired-pulse TMS were applied to the M1 of both the hemisphere ipsilateral (IH) and contralateral (CH) to the epileptic focus before and immediately after real/sham exposure to the GSM-EMFs (45 min). The TMS study was carried out in all subjects in three different experimental sessions (IH and CH exposure, sham), 1 week apart, according to a crossover, double-blind and counter-balanced paradigm. **RESULTS:** The present study clearly demonstrated that an acute and relatively prolonged exposure to GSM-EMFs modulates cortical excitability in patients affected by focal epilepsy; however, in contrast to healthy subjects, these effects were evident only after EMFs exposure over the hemisphere contralateral to the epileptic focus (CH). They were characterized by a significant cortical excitability increase in the exposed hemisphere paired with slight excitability decrease in the other one (IH). Both sham and real EMFs exposure of the IH did not affect brain excitability. **CONCLUSION:** Present results suggest a significant interaction between the brain excitability changes induced by EMFs and the epileptic focus, which eliminated the excitability enhancing effects of EMFs evident only in the CH.

(E) Tong J, Chen S, Liu XM, Hao DM. [Effect of electromagnetic radiation on discharge activity of neurons in the hippocampus CA1 in rats]. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 29(5):423-427, 2013. [Article in Chinese] (AS, CE, EE)

OBJECTIVE: In order to explore effect of electromagnetic radiation on learning and memory ability of hippocampus neuron in rats, the changes in discharge patterns and overall electrical activity of hippocampus neuron after electromagnetic radiation were observed. **METHODS:** Rat neurons discharge was recorded with glass electrode extracellular recording technology and a polygraph respectively. Radiation frequency of

electromagnetic wave was 900 MHz and the power was 10 W/m². In glass electrode extracellular recording, the rats were separately irradiated for 10, 20, 30, 40, 50 and 60 min, every points repeated 10 times and updated interval of 1h, observing the changes in neuron discharge and spontaneous discharge patterns after electromagnetic radiation. In polygraph recording experiments, irradiation group rats for five days a week, 6 hours per day, repeatedly for 10 weeks, memory electrical changes in control group and irradiation group rats when they were feeding were repeatedly monitored by the implanted electrodes, observing the changes in peak electric digits and the largest amplitude in hippocampal CA1 area, and taking some electromagnetic radiation sampling sequence for correlation analysis. RESULTS: (1) Electromagnetic radiation had an inhibitory role on discharge frequency of the hippocampus CA1 region neurons. After electromagnetic radiation, discharge frequency of the hippocampus CA1 region neurons was reduced, but the changes in scale was not obvious. (2) Electromagnetic radiation might change the spontaneous discharge patterns of hippocampus CA1 region neurons, which made the explosive discharge pattern increased obviously. (3) Peak potential total number within 5 min in irradiation group was significantly reduced, the largest amplitude was less than that of control group. (4) Using mathematical method to make the correlation analysis of the electromagnetic radiation sampling sequence, that of irradiation group was less than that of control group, indicating that there was a tending to be inhibitory connection between neurons in irradiation group after electromagnetic radiation. CONCLUSION: Electromagnetic radiation may cause structure and function changes of transfer synaptic in global, make hippocampal CA1 area neurons change in the overall discharge characteristic and discharge patterns, thus lead to decrease in the ability of learning and memory.

(E) Trosić I, Pavčić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol. 35(4):1259-1264, 2011. (AS, CE, CH)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m², whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in

comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Trunk A, Stefanics G, Zentai N, Kovács-Bálint Z, Thuróczy G, Hernádi I. No effects of a single 3G UMTS mobile phone exposure on spontaneous EEG activity, ERP correlates, and automatic deviance detection. Bioelectromagnetics. 2012 Jun 4. doi: 10.1002/bem.21740. [Epub ahead of print] (HU, EE)

Potential effects of a 30 min exposure to third generation (3G) Universal Mobile Telecommunications System (UMTS) mobile phone-like electromagnetic fields (EMFs) were investigated on human brain electrical activity in two experiments. In the first experiment, spontaneous electroencephalography (sEEG) was analyzed (n = 17); in the second experiment, auditory event-related potentials (ERPs) and automatic deviance detection processes reflected by mismatch negativity (MMN) were investigated in a passive oddball paradigm (n = 26). Both sEEG and ERP experiments followed a double-blind protocol where subjects were exposed to either genuine or sham irradiation in two separate sessions. In both experiments, electroencephalograms (EEG) were recorded at midline electrode sites before and after exposure while subjects were watching a silent documentary. Spectral power of sEEG data was analyzed in the delta, theta, alpha, and beta frequency bands. In the ERP experiment, subjects were presented with a random series of standard (90%) and frequency-deviant (10%) tones in a passive binaural oddball paradigm. The amplitude and latency of the P50, N100, P200, MMN, and P3a components were analyzed. We found no measurable effects of a 30 min 3G mobile phone irradiation on the EEG spectral power in any frequency band studied. Also, we found no significant effects of EMF irradiation on the amplitude and latency of any of the ERP components. In summary, the present results do not support the notion that a 30 min unilateral 3G EMF exposure interferes with human sEEG activity, auditory evoked potentials or automatic deviance detection indexed by MMN.

(NE) Unterlechner M, Sauter C, Schmid G, Zeitlhofer J. No effect of an UMTS mobile phone-like electromagnetic field of 1.97 GHz on human attention and reaction time. Bioelectromagnetics. 29(2):145-153, 2008. (HU, BE)

Several studies in the past reported influences of electromagnetic emissions of GSM phones on reaction time in humans. However, there are currently only a few studies available dealing with possible effects of the electromagnetic fields emitted by UMTS mobile phones. In our study, 40 healthy volunteers (20 female, 20 male), aged 26.0 years (range 21-30 years) underwent four different computer tests measuring reaction time and attention under three different UMTS mobile phone-like exposure conditions (two exposure levels plus sham exposure). Exposure of the subjects was accomplished by small helical antennas operated close to the head and fed by a generic signal

representing the emissions of a UMTS mobile phone under constant receiving conditions as well as under a condition of strongly varying transmit power. In the high exposure condition the resulting peak spatial average exposure of the test subjects in the cortex of the left temporal lobe of the brain was 0.63 W/kg (min. 0.25 W/kg, max. 1.49 W/kg) in terms of 1 g averaged SAR and 0.37 W/kg (min. 0.16 W/kg, max. 0.84 W/kg) in terms of 10 g averaged SAR, respectively. Low exposure condition was one-tenth of high exposure and sham was at least 50 dB below low exposure. Statistical analysis of the obtained test parameters showed that exposure to the generic UMTS signal had no statistically significant immediate effect on attention or reaction. Therefore, this study does not provide any evidence that exposure of UMTS mobiles interferes with attention under short-term exposure conditions.

(E) Vácha M, Puzová T, Kvíčalová M. Radio frequency magnetic fields disrupt magnetoreception in American cockroach. J Exp Biol. 212(Pt 21):3473-3477, 2009. (AS, LI, BE)

The sense that allows birds to orient themselves by the Earth's magnetic field can be disabled by an oscillating magnetic field whose intensity is just a fraction of the geomagnetic field intensity and whose oscillations fall into the medium or high frequency radio wave bands. This remarkable phenomenon points very clearly at one of two existing alternative magnetoreception mechanisms in terrestrial animals, i.e. the mechanism based on the radical pair reactions of specific photosensitive molecules. As the first such study in invertebrates, our work offers evidence that geomagnetic field reception in American cockroach is sensitive to a weak radio frequency field. Furthermore, we show that the 'deafening' effect at Larmor frequency 1.2 MHz is stronger than at different frequencies. The parameter studied was the rise in locomotor activity of cockroaches induced by periodic changes in the geomagnetic North positions by 60 deg. The onset of the disruptive effect of a 1.2 MHz field was found between 12 nT and 18 nT whereas the threshold of a doubled frequency field 2.4 MHz fell between 18 nT and 44 nT. A 7 MHz field showed no impact even in maximal 44 nT magnetic flux density. The results indicate resonance effects rather than non-specific bias of procedure itself and suggest that insects may be equipped with the same magnetoreception system as the birds.

(E) Vecchio F, Babiloni C, Ferreri F, Curcio G, Fini R, Del Percio C, Rossini PM. Mobile phone emission modulates interhemispheric functional coupling of EEG alpha rhythms. Eur J Neurosci. 25(6):1908-1913, 2007. (HU, EE)

We tested the working hypothesis that electromagnetic fields from mobile phones (EMFs) affect interhemispheric synchronization of cerebral rhythms, an important physiological feature of information transfer into the brain. Ten subjects underwent two electroencephalographic (EEG) recordings, separated by 1 week, following a crossover double-blind paradigm in which they were exposed to a mobile phone signal (global system for mobile communications; GSM). The mobile phone was held on the left side

of the subject head by a modified helmet, and orientated in the normal position for use over the ear. The microphone was orientated towards the corner of the mouth, and the antenna was near the head in the parietotemporal area. In addition, we positioned another similar phone (but without battery) on the right side of the helmet, to balance the weight and to prevent the subject localizing the side of GSM stimulation (and consequently lateralizing attention). In one session the exposure was real (GSM) while in the other it was Sham; both sessions lasted 45 min. Functional interhemispheric connectivity was modelled using the analysis of EEG spectral coherence between frontal, central and parietal electrode pairs. Individual EEG rhythms of interest were delta (about 2-4 Hz), theta (about 4-6 Hz), alpha 1 (about 6-8 Hz), alpha 2 (about 8-10 Hz) and alpha 3 (about 10-12 Hz). Results showed that, compared to Sham stimulation, GSM stimulation modulated the interhemispheric frontal and temporal coherence at alpha 2 and alpha 3 bands. The present results suggest that prolonged mobile phone emission affects not only the cortical activity but also the spread of neural synchronization conveyed by interhemispherical functional coupling of EEG rhythms.

(E) Vecchio F, Buffo P, Sergio S, Iacoviello D, Rossini PM, Babiloni C. Mobile phone emission modulates event-related desynchronization of α rhythms and cognitive-motor performance in healthy humans. Clin Neurophysiol. 123(1):121-128, 2012a. (HU, EE, BE)

OBJECTIVES: It has been shown that electromagnetic fields of Global System for Mobile Communications phone (GSM-EMFs) affect human brain rhythms (Vecchio et al., 2007, 2010), but it is not yet clear whether these effects are related to alterations of cognitive functions. **METHODS:** Eleven healthy adults underwent two electroencephalographic (EEG) sessions separated by 1 week, following a cross-over, placebo-controlled, double-blind paradigm. In both sessions, they performed a visual go/no-go task before real exposure to GSM-EMFs or after a sham condition with no EMF exposure. In the GSM real session, temporal cortex was continuously exposed to GSM-EMFs for 45 min. In the sham session, the subjects were not aware that the EMFs had been switched off for the duration of the experiment. In the go/no-go task, a central fixation stimulus was followed by a green (50% of probability) or red visual stimulus. Subjects had to press the mouse button after the green stimuli (go trials). With reference to a baseline period, power decrease of low- (about 8-10 Hz) and high-frequency (about 10-12 Hz) alpha rhythms indexed the cortical activity. **RESULTS:** It was found less power decrease of widely distributed high-frequency alpha rhythms and faster reaction time to go stimuli in the post- than pre-exposure period of the GSM session. No effect was found in the sham session. **CONCLUSIONS:** These results suggest that the peak amplitude of alpha ERD and the reaction time to the go stimuli are modulated by the effect of the GSM-EMFs on the cortical activity. **SIGNIFICANCE:** Exposure to GSM-EMFs for 45 min may enhance human cortical neural efficiency and simple cognitive-motor processes in healthy adults.

(E) Vecchio F, Tombini M, Buffo P, Assenza G, Pellegrino G, Benvenega A, Babiloni C, Rossini PM. Mobile phone emission increases inter-hemispheric functional coupling of electroencephalographic alpha rhythms in epileptic patients. Int J Psychophysiol. 84(2):164-171, 2012b. (HU, EE, MA)

It has been reported that GSM electromagnetic fields (GSM-EMFs) of mobile phones modulate - after a prolonged exposure - inter-hemispheric synchronization of temporal and frontal resting electroencephalographic (EEG) rhythms in normal young and elderly subjects (Vecchio et al., 2007, 2010). Here we tested the hypothesis that this can be even more evident in epileptic patients, who typically suffer from abnormal mechanisms governing synchronization of rhythmic firing of cortical neurons. Eyes-closed resting EEG data were recorded in ten patients affected by focal epilepsy in real and sham exposure conditions. These data were compared with those obtained from 15 age-matched normal subjects of the previous reference studies. The GSM device was turned on (45 min) in the "GSM" condition and was turned off (45 min) in the other condition ("sham"). The mobile phone was always positioned on the left side in both patients and control subjects. Spectral coherence evaluated the inter-hemispheric synchronization of EEG rhythms at the following frequency bands: delta (about 2-4 Hz), theta (about 4-6 Hz), alpha1 (about 6-8 Hz), alpha2 (about 8-10 Hz), and alpha3 (about 10-12 Hz). The effects on the patients were investigated comparing the inter-hemispheric EEG coherence in the epileptic patients with the control group of subjects evaluated in the previous reference studies. Compared with the control subjects, epileptic patients showed a statistically significant higher inter-hemispheric coherence of temporal and frontal alpha rhythms (about 8-12 Hz) in the GSM than "Sham" condition. These results suggest that GSM-EMFs of mobile phone may affect inter-hemispheric synchronization of the dominant (alpha) EEG rhythms in epileptic patients. If confirmed by future studies on a larger group of epilepsy patients, the modulation of the inter-hemispheric alpha coherence due to the GSM-EMFs could have clinical implications and be related to changes in cognitive-motor function.

(E) Vecchio F, Babiloni C, Ferreri F, Buffo P, Cibelli G, Curcio G, van Dijkman S, Melgari JM, Giambattistelli F, Rossini PM. Mobile phone emission modulates inter-hemispheric functional coupling of EEG alpha rhythms in elderly compared to young subjects. Clin Neurophysiol. 121(2):163-171, 2010. (HU, EE, AD)

OBJECTIVE: It has been reported that GSM electromagnetic fields (GSM-EMFs) of mobile phones modulate--after a prolonged exposure--inter-hemispheric synchronization of temporal and frontal resting electroencephalographic (EEG) rhythms in normal young subjects [Vecchio et al., 2007]. Here we tested the hypothesis that this effect can vary on physiological aging as a sign of changes in the functional organization of cortical neural synchronization. **METHODS:** Eyes-closed resting EEG data were recorded in 16 healthy elderly subjects and 5 young subjects in the two conditions of the previous reference study. The GSM device was turned on (45 min) in one condition and was turned off (45 min) in the other condition. Spectral coherence evaluated the inter-

hemispheric synchronization of EEG rhythms at the following bands: delta (about 2-4 Hz), theta (about 4-6 Hz), alpha 1 (about 6-8 Hz), alpha 2 (about 8-10 Hz), and alpha 3 (about 10-12 Hz). The aging effects were investigated comparing the inter-hemispheric EEG coherence in the elderly subjects vs. a young group formed by 15 young subjects (10 young subjects of the reference study; Vecchio et al., 2007). **RESULTS:** Compared with the young subjects, the elderly subjects showed a statistically significant ($p < 0.001$) increment of the inter-hemispheric coherence of frontal and temporal alpha rhythms (about 8-12 Hz) during the GSM condition. **CONCLUSIONS:** These results suggest that GSM-EMFs of a mobile phone affect inter-hemispheric synchronization of the dominant (alpha) EEG rhythms as a function of the physiological aging. **SIGNIFICANCE:** This study provides further evidence that physiological aging is related to changes in the functional organization of cortical neural synchronization.

(E) Vecsei Z, Csathó A, Thuróczy G, Hernádi I. Effect of a single 30 min UMTS mobile phone-like exposure on the thermal pain threshold of young healthy volunteers. Bioelectromagnetics. 2013 Jun 20. doi: 10.1002/bem.21801. [Epub ahead of print] (HU, BE)

One of the most frequently investigated effects of radiofrequency electromagnetic fields (RF EMFs) on the behavior of complex biological systems is pain sensitivity. Despite the growing body of evidence of EMF-induced changes in pain sensation, there is no currently accepted experimental protocol for such provocation studies for the healthy human population. In the present study, therefore, we tested the effects of third generation Universal Mobile Telecommunications System (UMTS) RF EMF exposure on the thermal pain threshold (TPT) measured on the surface of the fingers of 20 young adult volunteers. The protocol was initially validated with a topical capsaicin treatment. The exposure time was 30 min and the genuine (or sham) signal was applied to the head through a patch antenna, where RF EMF specific absorption rate (SAR) values were controlled and kept constant at a level of 1.75 W/kg. Data were obtained using randomized, placebo-controlled trials in a double-blind manner. Subjective pain ratings were tested blockwise on a visual analogue rating scale (VAS). Compared to the control and sham conditions, the results provide evidence for intact TPT but a reduced desensitization effect between repeated stimulations within the individual blocks of trials, observable only on the contralateral side for the genuine UMTS exposure. Subjective pain perception (VAS) data indicated marginally decreased overall pain ratings in the genuine exposure condition only. The present results provide pioneering information about human pain sensation in relation to RF EMF exposure and thus may contribute to cover the existing gap between safety research and applied biomedical science targeting the potential biological effects of environmental RF EMFs.

(E) Velayutham P, Govindasamy GK, Raman R, Prepageran N, Ng KH. High-frequency hearing loss among mobile phone users. Indian J Otolaryngol Head Neck Surg. 2014 Jan;66(Suppl 1):169-72. doi: 10.1007/s12070-011-0406-4. Epub 2011 Dec 15. (HU, BE)

The objective of this study is to assess high frequency hearing (above 8 kHz) loss among prolonged mobile phone users in a tertiary Referral Center. Prospective single blinded study. This is the first study that used high-frequency audiometry. The wide usage of mobile phone is so profound that we were unable to find enough non-users as a control group. Therefore we compared the non-dominant ear to the dominant ear using audiometric measurements. The study was a blinded study wherein the audiologist did not know which was the dominant ear. A total of 100 subjects were studied. Of the subjects studied 53% were males and 47% females. Mean age was 27. The left ear was dominant in 63%, 22% were dominant in the right ear and 15% did not have a preference. This study showed that there is significant loss in the dominant ear compared to the non-dominant ear ($P < 0.05$). Chronic usage mobile phone revealed high frequency hearing loss in the dominant ear (mobile phone used) compared to the non dominant ear.

(E) Volkow ND, Tomasi D, Wang GJ, Vaska P, Fowler JS, Telang F, Alexoff D, Logan J, Wong C.

Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. JAMA. 305(8):808-813, 2011. (HU, PE)

CONTEXT: The dramatic increase in use of cellular telephones has generated concern about possible negative effects of radiofrequency signals delivered to the brain. However, whether acute cell phone exposure affects the human brain is unclear.

OBJECTIVE: To evaluate if acute cell phone exposure affects brain glucose metabolism, a marker of brain activity. **DESIGN, SETTING, AND PARTICIPANTS:** Randomized crossover study conducted between January 1 and December 31, 2009, at a single US laboratory among 47 healthy participants recruited from the community. Cell phones were placed on the left and right ears and positron emission tomography with ((18)F)fluorodeoxyglucose injection was used to measure brain glucose metabolism twice, once with the right cell phone activated (sound muted) for 50 minutes ("on" condition) and once with both cell phones deactivated ("off" condition). Statistical parametric mapping was used to compare metabolism between on and off conditions using paired t tests, and Pearson linear correlations were used to verify the association of metabolism and estimated amplitude of radiofrequency-modulated electromagnetic waves emitted by the cell phone. Clusters with at least 1000 voxels (volume $>8 \text{ cm}^3$) and $P < .05$ (corrected for multiple comparisons) were considered significant. **MAIN OUTCOME MEASURE:** Brain glucose metabolism computed as absolute metabolism ($\mu\text{mol}/100 \text{ g per minute}$) and as normalized metabolism (region/whole brain). **RESULTS:** Whole-brain metabolism did not differ between on and off conditions. In contrast, metabolism in the region closest to the antenna (orbitofrontal cortex and temporal pole) was significantly higher for on than off conditions (35.7 vs 33.3 $\mu\text{mol}/100 \text{ g per minute}$; mean difference, 2.4 [95% confidence interval, 0.67-4.2]; $P = .004$). The increases were significantly correlated with the estimated electromagnetic field amplitudes both for absolute metabolism ($R = 0.95$, $P < .001$) and normalized

metabolism ($R = 0.89$; $P < .001$). **CONCLUSIONS:** In healthy participants and compared with no exposure, 50-minute cell phone exposure was associated with increased brain glucose metabolism in the region closest to the antenna. This finding is of unknown clinical significance.

(NE) Wallace D, Eltiti S, Ridgewell A, Garner K, Russo R, Sepulveda F, Walker S, Quinlan T, Dudley S, Maung S, Deeble R, Fox E. Cognitive and physiological responses in humans exposed to a TETRA base station signal in relation to perceived electromagnetic hypersensitivity. Bioelectromagnetics. 33(1):23-39, 2012. (HU, BE)

Terrestrial Trunked Radio (TETRA) technology ("Airwave") has led to public concern because of its potential interference with electrical activity in the brain. The present study is the first to examine whether acute exposure to a TETRA base station signal has an impact on cognitive functioning and physiological responses. Participants were exposed to a 420 MHz TETRA signal at a power flux density of 10 mW/m² as well as sham (no signal) under double-blind conditions. Fifty-one people who reported a perceived sensitivity to electromagnetic fields as well as 132 controls participated in a double-blind provocation study. Forty-eight sensitive and 132 control participants completed all three sessions. Measures of short-term memory, working memory, and attention were administered while physiological responses (blood volume pulse, heart rate, skin conductance) were monitored. After applying exclusion criteria based on task performance for each aforementioned cognitive measure, data were analyzed for 36, 43, and 48 sensitive participants for these respective tasks and, likewise, 107, 125, and 129 controls. We observed no differences in cognitive performance between sham and TETRA exposure in either group; physiological response also did not differ between the exposure conditions. These findings are similar to previous double-blind studies with other mobile phone signals (900-2100 MHz), which could not establish any clear evidence that mobile phone signals affect health or cognitive function.

(E) Wang H, Peng R, Zhou H, Wang S, Gao Y, Wang L, Yong Z, Zuo H, Zhao L, Dong J, Xu X, Su Z. Impairment of long-term potentiation induction is essential for the disruption of spatial memory after microwave exposure. Int J Radiat Biol. 2013 Jul 24. [Epub ahead of print] (AS, BE, ME, EE)

Purpose: To assess the impact of microwave exposure on learning and memory and to explore the underlying mechanisms. Materials and methods: 100 Wistar rats were exposed to a 2.856 GHz pulsed microwave field at average power densities of 0 mW/cm², 5 mW/cm², 10 mW/cm² and 50 mW/cm² for 6 min. The spatial memory was assessed by the Morris Water Maze (MWM) task. An in vivo study was conducted soon after microwave exposure to evaluate the changes of population spike (PS) amplitudes of long-term potentiation (LTP) in the medial perforant path (MPP)-dentate gyrus (DG) pathway. The structure of the hippocampus was observed by the light microscopy and the transmission electron microscopy (TEM) at 7 d after microwave exposure. Results:

Our results showed that the rats exposed in 10 mW/cm² and 50 mW/cm² microwave displayed significant deficits in spatial learning and memory at 6 h, 1 d and 3 d after exposure. Decreased PS amplitudes were also found after 10 mW/cm² and 50 mW/cm² microwave exposure. In addition, varying degrees of degeneration of hippocampal neurons, decreased synaptic vesicles and blurred synaptic clefts were observed in the rats exposed in 10 mW/cm² and 50 mW/cm² microwave. Compared with the sham group, the rats exposed in 5 mW/cm² microwave showed no difference in the above experiments. Conclusions: This study suggested that impairment of LTP induction and the damages of hippocampal structure, especially changes of synapses, might contribute to cognitive impairment after microwave exposure.

(NE) Watilliaux A, Edeline JM, L  v  que P, Jay TM, Mallat M. Effect of exposure to 1,800 MHz electromagnetic fields on heat shock proteins and glial cells in the brain of developing rats. Neurotox Res. 20(2):109-119, 2011. (AS, DE, CC)

The increasing use of mobile phones by children raise issues about the effects of electromagnetic fields (EMF) on the immature Central Nervous System (CNS). In the present study, we quantified cell stress and glial responses in the brain of developing rats one day after a single exposure of 2 h to a GSM 1,800 MHz signal at a brain average Specific Absorption Rate (SAR) in the range of 1.7 to 2.5 W/kg. Young rats, exposed to EMF on postnatal days (P) 5 (n = 6), 15 (n = 5) or 35 (n = 6), were compared to pseudo-exposed littermate rats (n = 6 at all ages). We used western blotting to detect heat shock proteins (HSPs) and cytoskeleton- or neurotransmission-related proteins in the developing astroglia. The GSM signal had no significant effect on the abundance of HSP60, HSC70 or HSP90, of serine racemase, glutamate transporters including GLT1 and GLAST, or of glial fibrillary acid protein (GFAP) in either total or soluble tissue extracts. Immunohistochemical detection of CD68 antigen in brain sections from pseudo-exposed and exposed animals did not reveal any differences in the morphology or distribution of microglial cells. These results provide no evidence for acute cell stress or glial reactions indicative of early neural cell damage, in developing brains exposed to 1,800 MHz signals in the range of SAR used in our study.

(E) Wiholm C, Lowden A, Kuster N, Hillert L, Arnetz BB, Akerstedt T, Moffat SD. Mobile phone exposure and spatial memory. Bioelectromagnetics. 30(1):59-65, 2009. (HU, BE)

Radiofrequency (RF) emission during mobile phone use has been suggested to impair cognitive functions, that is, working memory. This study investigated the effects of a 2 1/2 h RF exposure (884 MHz) on spatial memory and learning, using a double-blind repeated measures design. The exposure was designed to mimic that experienced during a real-life mobile phone conversation. The design maximized the exposure to the left hemisphere. The average exposure was peak spatial specific absorption rate (psSAR10g) of 1.4 W/kg. The primary outcome measure was a "virtual" spatial navigation task modeled after the commonly used and validated Morris Water Maze.

The distance traveled on each trial and the amount of improvement across trials (i.e., learning) were used as dependent variables. The participants were daily mobile phone users, with and without symptoms attributed to regular mobile phone use. Results revealed a main effect of RF exposure and a significant RF exposure by group effect on distance traveled during the trials. The symptomatic group improved their performance during RF exposure while there was no such effect in the non-symptomatic group. Until this new finding is further investigated, we can only speculate about the cause.

(E) Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res. 1311:189-196, 2010. (CS, CH, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is particularly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24 h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Concomitant with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient antioxidant in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (AS, CE, CH)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yang XS, He GL, Hao YT, Xiao Y, Chen CH, Zhang GB, Yu ZP. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. Brain Res Bull. 88(4):371-378, 2012. (AS, CH)

The issue of possible neurobiological effects of the electromagnetic field (EMF) exposure is highly controversial. To determine whether electromagnetic field exposure could act as an environmental stimulus capable of producing stress responses, we employed the hippocampus, a sensitive target of electromagnetic radiation, to assess the changes in its stress-related gene and protein expression after EMF exposure. Adult male Sprague-Dawley rats with body restrained were exposed to a 2.45 GHz EMF at a specific absorption rate (SAR) of 6 W/kg or sham conditions. cDNA microarray was performed to examine the changes of gene expression involved in the biological effects of electromagnetic radiation. Of 2048 candidate genes, 23 upregulated and 18 downregulated genes were identified. Of these differential expression genes, two heat shock proteins (HSP), HSP27 and HSP70, are notable because expression levels of both proteins are increased in the rat hippocampus. Result from immunocytochemistry revealed that EMF caused intensive staining for HSP27 and HSP70 in the hippocampus, especially in the pyramidal neurons of cornu ammonis 3 (CA3) and granular cells of dentate gyrus (DG). The gene and protein expression profiles of HSP27 and HSP70 were further confirmed by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. Our data provide direct evidence that exposure to electromagnetic fields elicits a stress response in the rat hippocampus.

(NE) Yilmaz F, Dasdag S, Akdag MZ, Kilinc N. Whole-body exposure of radiation emitted from 900 MHz mobile phones does not seem to affect the levels of anti-apoptotic bcl-2 protein. Electromagn Biol Med. 27(1):65-72, 2008. (AS, CH)

The purpose of the present study was to investigate the anti-apoptotic bcl-2 protein in rat brain and testes after whole-body exposure to radiation emitted from 900 MHz cellular phones. Two groups (sham and experimental) of Sprague-Dawley rats of eight rats each were used in the study. Exposure began approximately 10 min after transferring into the exposure cages, a period of time when rats settled down to a prone position and selected a fixed location inside the cage spontaneously. For the experimental group, the phones were in the speech condition for 20 min per day for 1 month. The same procedure was applied to the sham group rats, but the phones were turned off. Immunohistochemical staining of bcl-2 was performed according to the standardized avidin-biotin complex method. The results of this study showed that 20 min of the radiation emitted from 900 MHz cellular phones did not alter anti-apoptotic bcl-2 protein in the brain and testes of rats. We speculate that bcl-2 may not be involved in the effects of radiation on the brain and testes of rats.

***(E) Yuan K, Qin W, Wang G, Zeng F, Zhao L, Yang X, Liu P, Liu J, Sun J, von Deneen KM, Gong Q, Liu Y, Tian J. Microstructure abnormalities in adolescents with internet**

addiction disorder. PLoS One. 6(6):e20708, 2011. (HU, ME) (*Effects observed probably not caused by exposure to RFR.)

BACKGROUND: Recent studies suggest that internet addiction disorder (IAD) is associated with structural abnormalities in brain gray matter. However, few studies have investigated the effects of internet addiction on the microstructural integrity of major neuronal fiber pathways, and almost no studies have assessed the microstructural changes with the duration of internet addiction. **METHODOLOGY/PRINCIPAL FINDINGS:** We investigated the morphology of the brain in adolescents with IAD (N = 18) using an optimized voxel-based morphometry (VBM) technique, and studied the white matter fractional anisotropy (FA) changes using the diffusion tensor imaging (DTI) method, linking these brain structural measures to the duration of IAD. We provided evidences demonstrating the multiple structural changes of the brain in IAD subjects. VBM results indicated the decreased gray matter volume in the bilateral dorsolateral prefrontal cortex (DLPFC), the supplementary motor area (SMA), the orbitofrontal cortex (OFC), the cerebellum and the left rostral ACC (rACC). DTI analysis revealed the enhanced FA value of the left posterior limb of the internal capsule (PLIC) and reduced FA value in the white matter within the right parahippocampal gyrus (PHG). Gray matter volumes of the DLPFC, rACC, SMA, and white matter FA changes of the PLIC were significantly correlated with the duration of internet addiction in the adolescents with IAD. **CONCLUSIONS:** Our results suggested that long-term internet addiction would result in brain structural alterations, which probably contributed to chronic dysfunction in subjects with IAD. The current study may shed further light on the potential brain effects of IAD.

(E) Zareen N, Khan MY, Ali Minhas L. Derangement of chick embryo retinal differentiation caused by radiofrequency electromagnetic fields. Congenit Anom (Kyoto). 49(1):15-19, 2009. (AS, CE, ME, DE)

The possible adverse effects of radiofrequency electromagnetic fields (EMF) emitted from mobile phones present a major public concern. Biological electrical activities of the human body are vulnerable to interference from oscillatory aspects of EMF, which affect fundamental cellular activities, in particular, the highly active development process of embryos. Some studies highlight the possible health hazards of EMF, while others contest the hypothesis of biological impact of EMF. The present study was designed to observe the histomorphological effects of EMF emitted by a mobile phone on the retinae of developing chicken embryos. Fertilized chicken eggs were exposed to a ringing mobile set on silent tone placed in the incubator at different ages of development. After exposure for the scheduled duration the retinae of the embryos were dissected out and processed for histological examination. The control and experimental embryos were statistically compared for retinal thickness and epithelial pigmentation grades. Contrasting effects of EMF on the retinal histomorphology were noticed, depending on the duration of exposure. The embryos exposed for 10 post-incubation days exhibited decreased retinal growth and mild pigmentation of the

epithelium. Growth retardation reallocated to growth enhancement on increasing EMF exposure for 15 post-incubation days, with a shift of pigmentation grade from mild to intense. We conclude that EMF emitted by a mobile phone cause derangement of chicken embryo retinal differentiation.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (CS, CH, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. METHODS: Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative reverse transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). RESULTS: Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhang Y, She F, Li L, Chen C, Xu S, Luo X, Li M, He M, Yu Z. p25/CDK5 is partially involved in neuronal injury induced by radiofrequency electromagnetic field exposure. Int J Radiat Biol. 2013 Jul 29. [Epub ahead of print] (CS, CC)

Purpose: Several studies suggest that radiofrequency electromagnetic field (RF-EMF) exposure can induce neuronal injury. The aim of the present work was to investigate whether the cyclin-dependent kinase 5 (CDK5) pathway is involved in neuronal injury induced by RF-EMF exposure. Materials and methods: Newborn Sprague-Dawley rats' primary cultured cortical neurons were exposed to pulsed 2.45 GHz RF-EMF for 10 min. The cellular viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The apoptosis was assessed by Hoechst 33342 and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling co-staining. The protein expressions of CDK5, p35, p25, and phosphorylated tau at Ser⁴⁰⁴ were examined by Western blot analysis. The CDK5 activity was detected using a histone-H1 kinase assay. Results: The cellular viability of neurons was significantly decreased ($p < 0.01$, Partial Eta Squared [η_p^2]: 0.554), and the percentage of apoptotic nuclei ($p < 0.01$, $\eta_p^2 = 0.689$), activity of CDK5 ($p < 0.05$, $\eta_p^2 = 0.589$), ratio of p25 and p35 ($p < 0.05$, $\eta_p^2 = 0.670$), levels of tau phosphorylation at Ser⁴⁰⁴ ($p < 0.01$, $\eta_p^2 = 0.896$) were significantly increased after RF-EMF exposure. No significant change was detected in CDK5 expression after RF-EMF exposure. Pretreatment with Roscovitine (a CDK5 inhibitor) significantly blocked the RF-EMF-induced decrease of cellular viability ($p < 0.05$, $\eta_p^2 = 0.398$) and tau hyperphosphorylation at Ser⁴⁰⁴ ($p < 0.01$, $\eta_p^2 = 0.917$), but did not significantly block the RF-EMF-induced apoptosis ($p > 0.05$, $\eta_p^2 = 0.130$). Conclusions: These results suggest that abnormal activity of p25/CDK5 is partially involved in primary cultured cortical neuron injury induced by RF-EMF exposure.

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology. 235(3):167-175, 2007. (CS, CH)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (CS, CH)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

Literature on neurological effects of extremely-low frequency electromagnetic fields (2007-2014)

Keys: (E) - effect observed; (NE) -no significant effect observed.

AS- animal study; CS- cell/in vitro study; CE- chronic/repeated exposure; AE- acute exposure; HU- human study; MC- morphological changes; CC- chemical changes; FC- functional changes; EE- electrophysiological changes; BE- changes in behavior; OX- oxidative changes; DE- development; MA- possible medical application; ND- neurodegenerative disease; EF- electric field.

(E) Ahmed Z, Wieraszko A. The mechanism of magnetic field-induced increase of excitability in hippocampal neurons. Brain Res. 1221:30-40, 2008. (CS, AE, EE)

The influence of a pulsed magnetic field (PMF) on hippocampal evoked potentials has been investigated in vitro. The exposure to PMF (0.16 Hz, 15 mT) applied for 30 min amplified the population spike and the slope of EPSP recorded from stratum pyramidale and stratum radiatum respectively. This amplification was additive to previously induced LTP and occurred in an NMDA-independent way. The increase in the activity of electrical synapses accompanied PMF-induced amplification of evoked potentials. Since PMF exposure modified paired-pulse facilitation and paired-pulse inhibition, it was concluded that it modifies excitatory and inhibitory processes in the hippocampus. Control experiments revealed that observed effects were exclusively related to PMF exposure. The results support and extend our previous research indicating a significant influence of magnetic fields on hippocampal physiology.

(E) Akdag MZ, Dasdag S, Ulukaya E, Uzunlar AK, Kurt MA, Taşkin A. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. Biol Trace Elem Res. 138(1-3):238-249, 2010. (OX, AS, CC, CE)

This study was aimed to investigate the effect of extremely low-frequency magnetic field (ELF-MF) on apoptosis and oxidative stress values in the brain of rat. Rats were exposed to 100 and 500 μ T ELF-MF, which are the safety standards of public and occupational exposure for 2 h/day for 10 months. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3 in order to measure the apoptotic index by a semi-quantitative scoring system. In addition, the levels of catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in rat brain. Final score of apoptosis and MPO activity were not significantly different between the groups. CAT activity decreased in both exposure groups ($p < 0.05$), while TAC was found to be lower in ELF 500 group than those in ELF-100 and sham groups ($p < 0.05$). MDA, TOS, and OSI values were found to be higher in ELF-500 group than those in ELF-100 and sham groups ($p < 0.05$). In conclusion, apoptosis was not changed by long-term ELF-MF exposure, while both 100 and 500 μ T ELF-MF

exposure induced toxic effect in the rat brain by increasing oxidative stress and diminishing antioxidant defense system.

(E) Akdag MZ, Dasdag S, Cakir DU, Yokus B, Kizil G, Kizil M. Do 100- and 500- μ T ELF magnetic fields alter beta-amyloid protein, protein carbonyl and malondialdehyde in rat brains? *Electromagn Biol Med.* 32(3):363-372, 2013. (AS, CE, CC, OX)

Several studies still state that presently accepted safety standards for extremely low-frequency magnetic fields (ELF-MFs) do not provide adequate protection, and therefore the standards are still open to question. To help resolve this question, the aim of this study was to illuminate the interaction between biomolecules and ELF-MFs by investigating the effect of ELF-MFs on beta-amyloid protein (BAP), protein carbonyl (PC) and malondialdehyde (MDA) in rat brain. For this study, 30 adult male Sprague-Dawley rats were used, which were divided into two experimental groups and a sham exposed group. Rats in two experimental groups were exposed to 100- and 500- μ T ELF-MFs (50 Hz) for 2 h/day for 10 months, which are the generally accepted safety standards for public and occupational exposures. The same procedures were applied to the rats in the sham group, but with the generator turned off. The results of this study showed that neither ELF-MFs used in this study altered BAP level significantly ($p > 0.05$). However, PC and MDA levels were increased by the exposure to 100- and 500- μ T ELF-MFs ($p < 0.0001$). In conclusion, both PC and MDA levels were altered by long-term exposure to either 100 or 500 μ T ELF-MF. However, many further and more comprehensive studies will be required to elucidate the interaction mechanisms between ELF-MFs exposure and living organisms.

(E) Akpinar D, Ozturk N, Ozen S, Agar A, Yargicoglu P. The effect of different strengths of extremely low-frequency electric fields on antioxidant status, lipid peroxidation, and visual evoked potentials. *Electromagn Biol Med.* 31(4):436-448, 2012. (AS, CE, OX, EE)

The aim of the study was to investigate the effects of extremely low-frequency electric field (ELF EF) on visual evoked potential (VEP), thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), total oxidant status (TOS), and oxidant stress index (OSI). Thirty female Wistar rats, aged 3 months, were divided into three equal groups: Control (C), the group exposed to EF at 12 kV/m strength (E12), and the group exposed to EF at 18 kV/m strength (E18). Electric field was applied to the E12 and E18 groups for 14 days (1 h/day). Brain and retina TBARS, TOS, and OSI were significantly increased in the E12 and E18 groups with respect to the control group. Also, TBARS levels were significantly increased in the E18 group compared with the E12 group. Electric fields significantly decreased TAS levels in both brain and retina in E12 and E18 groups with respect to the control group. All VEP components were significantly prolonged in rats exposed to electric fields compared to control group. In addition, all latencies of VEP components were increased in the E18 group with respect to the E12

group. It is conceivable to suggest that EF-induced lipid peroxidation may play an important role in changes of VEP parameters.

(NE) Aldinucci C, Carretta A, Maiorca SM, Leoncini S, Signorini C, Ciccoli L, Pessina GP. Effects of 50 Hz electromagnetic fields on rat cortical synaptosomes. Toxicol Ind Health. 25(4-5):249-252, 2009. (CS, CC, AE)

Nerve cells are very responsive to weak pulsed electromagnetic fields (EMFs). Such non-ionizing radiation, with frequencies of 0-300 Hz and 0.1-100 mT, can affect several cellular activities, with unusual dose-response characteristics. The present study examined the effect of a 2-h exposure of synaptosomes on a system generating a peak magnetic field of 2 mT. We evaluated the changes of the synaptosomal mitochondrial respiration rate and ATP production, membrane potential, intrasynaptosomal Ca²⁺ concentration, and the release of free iron and F₂-isoprostanes. O₂ consumption and ATP production remained unchanged in exposed synaptosomes. The intrasynaptosomal Ca²⁺ concentration decreased slowly and no depolarization of the synaptosomal membrane was detected. Finally, the release of free iron and F₂-isoprostanes by synaptosomal suspensions also remained unchanged after EMF exposure. These results indicate that the physiological behavior of cortical synaptosomes was unaffected by weak pulsed EMFs.

(E) Amirifalah Z, Firoozabadi SM, Shafiei SA. Local Exposure of Brain Central Areas to a Pulsed ELF Magnetic Field for a Purposeful Change in EEG. Clin EEG Neurosci. 44(1):44-52, 2013. (HU, EE)

This study examines the simultaneous exposure of 2 brain areas in the location of central electrodes (C3 and C4) to a weak and pulsed extremely low-frequency magnetic field (ELF-MF) on the electroencephalogram (EEG). The intent is to change the EEG for a therapeutic application, such as neurofeedback, by inducing the "resonance effect." A total of 10 healthy women received 9 minutes of ELF-MF (intensity 200 µT) and sham in a counterbalanced design. ELF-MF exposure frequencies were 10, 14, and 18 Hz. The paired t test revealed that local pulsed ELF-MF significantly decreases beta (15-25 Hz), sensorimotor rhythm (13-15 Hz), and theta (4-8 Hz) powers at a frequency of 10 Hz in C3 and C4 regions (12.0%-26.6%) after exposure, in comparison with that achieved during the exposure (P < .05). Variations during the exposure were transient and different from those after. The resonance effect was observed nowhere around the regions. The study suggests that this technique may be applied in the treatment of anxiety; however, further investigation is needed.

(NE) Azanza MJ, del Moral A, Calvo AC, Pérez-Bruzón RN, Junquera C. Synchronization dynamics induced on pairs of neurons under applied weak alternating magnetic fields. Comp Biochem Physiol A Mol Integr Physiol. 166(4):603-618, 2013.(CS, AE, EE, MC)

Pairs of *Helix aspersa* neurons show an alternating magnetic field dependent frequency synchronization (AMFS) when exposed to a weak (amplitude B_0 between 0.2 and 150 Gauss (G)) alternating magnetic field (AMF) of extremely low frequency (ELF, $f_M = 50$ Hz). We have compared the AMFS patterns of discharge with: i) the synaptic activity promoted by glutamate and acetylcholine; ii) the activity induced by caffeine; iii) the bioelectric activity induced on neurons interconnected by electric synapses. AMFS activity reveals several specific features: i) a tight coincidence in time of the pattern and frequency, f , of discharge; ii) it is induced in the time interval of field application; iii) it is dependent on the intensity of the sinusoidal applied magnetic field; iv) elicited biphasic responses (excitation followed by inhibition) run in parallel for the pair of neurons; and v) some neuron pairs either spontaneously or AMF synchronized can be desynchronized under applied higher AMF. Our electron microscopy studies reveal gap-like junctions confirming our immunocytochemistry results about expression of connexin 26 (Cx26) in 4.7% of *Helix* neurons. AMF and carbenoxolone did not induce any significant effect on spontaneous synchronization through electric synapses.

(E) Bai WF, Xu WC, Feng Y, Huang H, Li XP, Deng CY, Zhang MS. Fifty-Hertz electromagnetic fields facilitate the induction of rat bone mesenchymal stromal cells to differentiate into functional neurons. *Cytotherapy*. 15(8):961-970, 2013. (CS, CE, MC, MA, ND)

BACKGROUND AIMS: Research results have shown that bone mesenchymal stromal cells (BMSC) can differentiate into neural cells. Electromagnetic fields (EMF) play a role in regulating cell proliferation and differentiation, but the mechanisms behind this are unknown. In the present study, we explored the efficacy of EMF on the induction of rat BMSC differentiation into neurons in vitro. **METHODS:** First, rat BMSC were induced in a nerve cell culture environment and divided into three groups: an EMF induction treatment group (frequency of 50 Hz, magnetic induction of 5 mT, 60 min per day for 12 days), an induction-only group and a control group. Second, we observed cell phenotypes in a confocal microscope, tested gene expression through the use of reverse transcriptase-polymerase chain reaction, and detected postsynaptic currents by means of a cell patch-clamp. We analyzed the cell cycles and the portion of cells expressing neural cell markers with the use of flow cytometry. **RESULTS:** The results indicated that EMF can facilitate BMSC differentiation into neural cells, which expressed neuronal-specific markers and genes; they formed synaptic junctions and pulsed excitatory postsynaptic currents. At the same time, the G0-G1 phase ratio recorded by means of flow cytometry gradually decreased under the EMF treatment, whereas there was an increase of S-phase ratio, and the portion of cells expressing neuronal-specific markers increased. **CONCLUSIONS:** Given that a noninvasive treatment of 50-Hz EMF could significantly facilitate BMSC to differentiate into functional neurons, EMF appears to be a promising clinical option for stem cell transplantation therapies to combat central nervous system diseases.

(E) Balassa T, Szemerszky R, Bárdos G. Effect of short-term 50 Hz electromagnetic field exposure on the behavior of rats. Acta Physiol Hung. 96(4):437-448, 2009. (AS, BE, AE)

Extremely low-frequency electromagnetic field generated by transformer stations located within buildings has been suspected to initiate non-specific health problems. This possibility was examined in model experiments in rats. Following short-term exposure (50 Hz, 500 μ T, 20 min), situational and social anxiety as well as locomotor activity pattern were examined by several different tests (elevated plus-maze, novel object exploration, social interaction and territoriality). Based on our results having obtained so far, it seems that these field parameters (that equals the official reference limit for workers) may cause some kind of discomfort, may influence behavior, increase passivity and situational anxiety, but has no verified effect on the social and territorial behavior.

(E) Balassa T, Varró P, Elek S, Drozdovszky O, Szemerszky R, Világi I, Bárdos G. Changes in synaptic efficacy in rat brain slices following extremely low-frequency magnetic field exposure at embryonic and early postnatal age. Int J Dev Neurosci. 31(8):724-730, 2013. (AS, CE, EE, DE)

An earlier study demonstrated changes in synaptic efficacy and seizure susceptibility in adult rat brain slices following extremely low-frequency magnetic field (ELF-MF) exposure. The developing embryonic and early postnatal brain may be even more sensitive to MF exposure. The aim of the present study was to determine the effects of a long-term ELF-MF (0.5 and 3 mT, 50 Hz) exposure on synaptic functions in the developing brain. Rats were treated with chronic exposure to MF during two critical periods of brain development, i.e. in utero during the second gestation week or as newborns for 7 days starting 3 days after birth, respectively. Excitability and plasticity of neocortical and hippocampal areas were tested on brain slices by analyzing extracellular evoked field potentials. We demonstrated that the basic excitability of hippocampal slices (measured as amplitude of population spikes) was increased by both types of treatment (fetal 0.5 mT, newborn 3 mT). Neocortical slices seemed to be responsive mostly to the newborn treatment, the amplitude of excitatory postsynaptic potentials was increased. Fetal ELF-MF exposure significantly inhibited the paired-pulse depression (PPD) and there was a significant decrease in the efficacy of LTP (long-term potentiation induction) in neocortex, but not in hippocampus. On the other hand, neonatal treatment had no significant effect on plasticity phenomena. Results demonstrated that ELF-MF has significant effects on basic neuronal functions and synaptic plasticity in brain slice preparations originating from rats exposed either in fetal or in newborn period.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (CS, AE, CC)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH₂ methylene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO₂- stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β -sheet contents in amide I, and around the 1740 cm⁻¹ band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β -sheet contents as to α -helix components of amide I region, as well.

(E) Calabrò E, Condello S, Currò M, Ferlazzo N, Vecchio M, Caccamo D, Magazù S, Ientile R. 50 Hz Electromagnetic Field Produced Changes in FTIR Spectroscopy Associated with Mitochondrial Transmembrane Potential Reduction in Neuronal-Like SH-SY5Y Cells. Oxid Med Cell Longev. 2013;2013:414393. doi: 10.1155/2013/414393. Epub 2013 Jul 16. (CS, AE, EE)

SH-SY5Y neuroblastoma cells were used as an experimental model to study the effects of 50 Hz electromagnetic field, in the range from 50 μ T to 1.4 mT. Fourier transform infrared spectroscopy analysis evidenced a reduction in intensity of the amide A band and a slight increase of vibration bands at 2921 cm⁻¹ and 2853 cm⁻¹ corresponding to methylene groups. A further increase of the magnetic field intensity of exposure up to 0.8 mT and 1.4 mT produced a clear increase in intensity of CH₂ vibration bands. Moreover, it has been observed some alterations in the amide I region, such as a shifted peak of the amide I band to a smaller wavenumber, probably due to protein conformational changes. These results suggested that exposure to extremely low electromagnetic fields influenced lipid components of cellular membrane and the N-H in-plane bending and C-N stretching vibrations of peptide linkages, modifying the secondary structures of α -helix and β -sheet contents and producing unfolding process in cell membrane proteins. The observed changes after exposure to 50 Hz electromagnetic field higher than 0.8 mT were associated with a significant reduction of cell viability and reduced mitochondrial transmembrane potential.

(NE) Canseven AG, Keskil ZA, Keskil S, Seyhan N. Pentylentetrazol-induced seizures are not altered by pre- or post-drug exposure to a 50 Hz magnetic field. Radiat Biol. 83(4):231-235, 2007. (AS, AE, BE)

PURPOSE: To investigate whether pre- and post-drug magnetic field (MF) exposure of 50 Hz, 0.2 mT has any significant effect on pentylentetrazol (PTZ)-induced seizures in

mice. MATERIAL AND METHODS: MF was generated by a pair of Helmholtz coils. Seizures were induced by PTZ injection intraperitoneally (i.p.) at a dose of 60 mg/kg. A total of 48 locally bred adult female mice 25-35 g in weight were used. Latency to seizure, total seizure duration, and mortality were recorded for each mouse. RESULTS: Neither pre- nor post-drug exposure to a 50 Hz, 0.2 mT MF was found to have any effect on PTZ-induced epileptic seizures or mortality rates in mice. CONCLUSION: The present study failed to provide any support for a therapeutic potential of a 50 Hz, 0.2 mT MF for epilepsy.

(E) Capone F, Dileone M, Profice P, Pilato F, Musumeci G, Minicuci G, Ranieri F, Cadossi R, Setti S, Tonali PA, Di Lazzaro V. Does exposure to extremely low frequency magnetic fields produce functional changes in human brain? J Neural Transm. 116(3):257-265, 2009. (HU, FC)

Behavioral and neurophysiological changes have been reported after exposure to extremely low frequency magnetic fields (ELF-MF) both in animals and in humans. The physiological bases of these effects are still poorly understood. In vitro studies analyzed the effect of ELF-MF applied in pulsed mode (PEMFs) on neuronal cultures showing an increase in excitatory neurotransmission. Using transcranial brain stimulation, we studied noninvasively the effect of PEMFs on several measures of cortical excitability in 22 healthy volunteers, in 14 of the subjects we also evaluated the effects of sham field exposure. After 45 min of PEMF exposure, intracortical facilitation produced by paired pulse brain stimulation was significantly enhanced with an increase of about 20%, while other parameters of cortical excitability remained unchanged. Sham field exposure produced no effects. The increase in paired-pulse facilitation, a physiological parameter related to cortical glutamatergic activity, suggests that PEMFs exposure may produce an enhancement in cortical excitatory neurotransmission. This study suggests that PEMFs may produce functional changes in human brain.

(E) Carrubba S, Frilot C 2nd, Chesson AL Jr, Marino AA. Mobile-phone pulse triggers evoked potentials. Neurosci Lett. 469(1):164-168, 2010. (HU, EE)

If mobile-phone electromagnetic fields (EMFs) are hazardous, as suggested in the literature, processes or mechanisms must exist that allow the body to detect the fields. We hypothesized that the low-frequency pulses produced by mobile phones (217 Hz) were detected by sensory transduction, as evidenced by the ability of the pulses to trigger evoked potentials (EPs). Electroencephalograms (EEGs) were recorded from six standard locations in 20 volunteers and analyzed to detect brain potentials triggered by a pulse of the type produced by mobile phones. Evoked potentials having the expected latency were found in 90% of the volunteers, as assessed using a nonlinear method of EEG analysis. Evoked potentials were not detected when the EEG was analyzed using time averaging. The possibility of systematic error was excluded by sham-exposure analyses. The results implied that mobile-phones trigger EP at the rate of 217 Hz during

ordinary phone use. Chronic production of the changes in brain activity might be pertinent to the reports of health hazards among mobile-phone users.

(E) Carrubba S, Frilot C, Chesson AL, Marino AA. Nonlinear EEG activation evoked by low-strength low-frequency magnetic fields. *Neurosci Lett.* 417(2):212-216, 2007. (HU, AE, EE)

Recent electrophysiological evidence suggested the existence of a human magnetic sense, but the kind of dynamical law that governed the stimulus-response relationship was not established. We tested the hypothesis that brain potentials evoked by the onset of a weak, low-frequency magnetic field were nonlinearly related to the stimulus. A field of 1G, 60 Hz was applied for 2s, with a 5s inter-stimulus period, and brain potentials were recorded from occipital electrodes in eight subjects, each of whom were measured twice, with at least 1 week between measurements. The recorded signals were subjected to nonlinear (recurrence analysis) and linear (time averaging) analyses. Using recurrence analysis, magnetosensory evoked potentials (MEPs) were detected in each subject in both the initial and replicate studies, with one exception. All MEPs exhibited the expected latency but differed in dynamical characteristics, indicating that they were nonlinearly related to the stimulus. MEPs were not detected using time averaging, thereby further confirming their nonlinearity. Evolutionarily conditioned structures that help mediate linear field-transduction in lower life forms may be expressed and functionally utilized in humans, but in a role where they facilitate vulnerability to man-made environmental fields.

(E) Celik MS, Guven K, Akpolat V, Akdag MZ, Naziroglu M, Gul-Guven R, Celik MY, Erdogan S. Extremely low-frequency magnetic field induces manganese accumulation in brain, kidney and liver of rats. *Toxicol Ind Health.* 2013 Feb 28. [Epub ahead of print] (AS, CE, CC)

The aim of the present study was to determine the effects of extremely low-frequency magnetic field (ELF-MF) on accumulation of manganese (Mn) in the kidney, liver and brain of rats. A total of 40 rats were randomly divided into eight groups. Four control groups received 0, 3.75, 15 and 60 mg Mn per kg body weight orally every 2 days for 45 days, respectively. The remaining four groups received same concentrations of Mn and were also exposed to ELF-MF (1.5 mT; 50 Hz) for 4 h for 5 days a week during 45 days. Following the last exposure, kidney, liver and brain were taken from all rats and they were analyzed for Mn accumulation levels using an inductively coupled plasma-optical emission spectrometer. In result of the current study, we observed that Mn levels in brain, kidney and liver were higher in Mn groups than in control groups. Mn levels in brain, kidney and liver were also higher in Mn plus ELF-MF groups than in Mn groups. In conclusion, result of the current study showed that the ELF-MF induced manganese accumulation in kidney, liver and brain of rats.

(E) Che Y, Sun H, Cui Y, Zhou D, Ma Y. Effects of exposure to 50 Hz magnetic field of 1 mT on the performance of detour learning task by chicks. Brain Res Bull. 74(1-3):178-182, 2007. (AS, CE, BE)

In the present study, we examined the effects of exposure to an extremely low-frequency magnetic field of 1 mT intensity on learning and memory in Lohmann brown domestic chicks using detour learning task. These results show that 20 h/day exposure to a low-frequency magnetic field induces a significant impairment in detour learning but 50 min/day exposure has no effect.

(E) Cho H, Seo YK, Yoon HH, Kim SC, Kim SM, Song KY, Park JK. Neural stimulation on human bone marrow-derived mesenchymal stem cells by extremely low frequency electromagnetic fields (ELF-EMFs). Biotechnol Prog. 2012 Jul 31. doi: 10.1002/btpr.1607. [Epub ahead of print] (CS, CE, MC, DE, MA)

Adult stem cells are considered to be multipotent. Especially, human bone marrow-derived mesenchymal stem cells (hBM-MSCs) have the potential to differentiate into nerve type cells. Electromagnetic fields (EMFs) are widely distributed in the environment, and recently there have been many reports on the biological effects of EMFs. hBM-MSCs are weak and sensitive pluripotent stem cells, therefore extremely low frequency- electromagnetic fields (ELF-EMFs) could be affect the changes of biological functions within the cells. In our experiments, ELF-EMFs inhibited the growth of hBM-MSCs in 12 days exposure. Their gene level was changed and expression of the neural stem cell marker like nestin was decreased but the neural cell markers like MAP2, NEUROD1, NF-L and Tau were induced. In immunofluorescence study, we confirmed the expression of each protein of neural cells. And also both oligodendrocyte and astrocyte related proteins like O4 and GFAP were expressed by ELF-EMFs. **We suggest that EMFs can induce neural differentiation in BM-MSCs without any chemicals or differentiation factors.**

(E) Cho SI, Nam YS, Chu LY, Lee JH, Bang JS, Kim HR, Kim HC, Lee YJ, Kim HD, Sul JD, Kim D, Chung YH, Jeong JH. Extremely low-frequency magnetic fields modulate nitric oxide signaling in rat brain. Bioelectromagnetics. 33(7):568-574, 2012. (AS, CE, CC, OX)

Our previous study has shown that an extremely low-frequency magnetic field (ELF-MF) induces nitric oxide (NO) synthesis by Ca(2+) -dependent NO synthase (NOS) in rat brain. The present study was designed to confirm that ELF-MF affects neuronal NOS (nNOS) in several brain regions and to investigate the correlation between NO and nNOS activation. The exposure of rats to a 2 mT, 60 Hz ELF-MF for 5 days resulted in increases of NO levels in parallel with cGMP elevations in the cerebral cortex, striatum, and hippocampus. Cresyl violet staining and electron microscopic evaluation revealed that there were no significant differences in the morphology and number of neurons in the cerebral cortex, striatum, and hippocampus. Differently, the numbers of nNOS-immunoreactive (IR) neurons were significantly increased in those cerebral areas in ELF-

MF-exposed rats. These data suggest that the increase in NO could be due to the increased expression and activation of nNOS in cells. Based on NO signaling in physiological and pathological states, ELF-MF created by electric power systems may induce various physiological changes in modern life.

(E) Chu LY, Lee JH, Nam YS, Lee YJ, Park WH, Lee BC, Kim D, Chung YH, Jeong JH. Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum. Gen Physiol Biophys. 30(4):415-421, 2011. (AS, CE, OX)

We have investigated whether extremely low frequency magnetic field (ELF-MF) induces lipid peroxidation and reactive oxygen species in mouse cerebellum. After exposure to 60 Hz ELF-MF at 2.3 mT intensity for 3 hours, there was a significant increase in malondialdehyde level and hydroxyl radical. ELF-MF significantly induced concomitant increase in superoxide dismutase without alteration in glutathione peroxidase activity. While glutathione contents were not altered, ascorbic acid levels were significantly decreased by ELF-MF exposure. These results indicate that ELF-MF may induce oxidative stress in mouse cerebellum. However, the mechanism remains further to be characterized.

(E) Ciejka E, Kleniewska P, Skibska B, Goraca A. Effects of extremely low frequency magnetic field on oxidative balance in brain of rats. J Physiol Pharmacol. 62(6):657-661, 2011. (AS, CE, OX)

Extremely low frequency magnetic field (ELF-MF) may result in oxidative DNA damage and lipid peroxidation with an ultimate effect on a number of systemic disturbances and cell death. The aim of the study is to assess the effect of ELF-MF parameters most frequently used in magnetotherapy on reactive oxygen species generation (ROS) in brain tissue of experimental animals depending on the time of exposure to this field. The research material included adult male Sprague-Dawley rats, aged 3-4 months. The animals were divided into 3 groups: I - control (shame) group; II - exposed to the following parameters of the magnetic field: 7 mT, 40 Hz, 30 min/day, 10 days; III - exposed to the ELF-MF parameters of 7 mT, 40 Hz, 60 min/day, 10 days. The selected parameters of oxidative stress: thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H₂O₂), total free sulphhydryl groups (-SH groups) and protein in brain homogenates were measured after the exposure of rats to the magnetic field. ELF-MF parameters of 7 mT, 40 Hz, 30 min/day for 10 days caused a significant increase in lipid peroxidation and insignificant increase in H₂O₂ and free -SH groups. The same ELF-MF parameters but applied for 60 min/day caused a significant increase in free -SH groups and protein concentration in the brain homogenates indicating the adaptive mechanism. The study has shown that ELF-MF applied for 30 min/day for 10 days can affect free radical generation in the brain. Prolongation of the exposure to ELF-MF (60/min/day) caused adaptation to this field. The effect of ELF-MF irradiation on oxidative stress parameters depends on the time of animal exposure to magnetic field.

(E) Cook CM, Saucier DM, Thomas AW, Prato FS. Changes in human EEG alpha activity following exposure to two different pulsed magnetic field sequences. *Bioelectromagnetics*. 30(1):9-20, 2009. (AE, HU, EE)

The present study investigates the effects of a weak (± 200 microT(pk)), pulsed, extremely low frequency magnetic field (ELF MF) upon the human electroencephalogram (EEG). We have previously determined that exposure to pulsed ELF MFs can affect the EEG, notably the alpha frequency (8-13 Hz) over the occipital-parietal region of the scalp. In the present study, subjects ($n = 32$) were exposed to two different pulsed MF sequences (1 and 2, used previously) that differed in presentation rate, in order to examine the effects upon the alpha frequency of the human EEG. Results suggest that compared to sham exposure, alpha activity was lowered over the occipital-parietal regions of the brain during exposure to Sequence 1, while alpha activity over the same regions was higher after Sequence 2 exposure. These effects occurred after approximately 5 min of pulsed MF exposure. The results also suggest that a previous exposure to the pulsed MF sequence determined subjects' responses in the present experiment. This study supports our previous observation of EEG changes after 5 min pulsed ELF MF exposure. The results of this study are also consistent with existing EEG experiments of ELF MF and mobile phone effects upon the brain.

(E) Corbacio M, Brown S, Dubois S, Goulet D, Prato FS, Thomas AW, Legros A. Human cognitive performance in a 3 mT power-line frequency magnetic field. *Bioelectromagnetics*. 32(8):620-633, 2011. (HU, AE, BE)

Extremely low frequency (ELF, <300 Hz) magnetic fields (MF) have been reported to modulate cognitive performance in humans. However, little research exists with MF exposures comparable to the highest levels experienced in occupations like power line workers and industrial welders. This research aims to evaluate the impact of a 60 Hz, 3 mT MF on human cognitive performance. Ninety-nine participants completed the double-blind protocol, performing a selection of psychometric tests under two consecutive MF exposure conditions dictated by assignment to one of three groups (sham/sham, MF exposure/sham, or sham/MF exposure). Data were analyzed using a 3×2 mixed model analysis of variance. Performance between repetitions improved in 11 of 15 psychometric parameters (practice effect). A significant interaction effect on the digit span forward test ($F = 5.21, P < 0.05$) revealed an absence of practice effects for both exposure groups but not the control group. This memory test indicates MF-induced abolition of the improvement associated with practice. Overall, this study does not establish any clear MF effect on human cognition. It is speculated that an ELF MF may interfere with the neuropsychological processes responsible for this short-term learning effect supported by brain synaptic plasticity.

(E) Coşkun S, Balabanli B, Canseven A, Seyhan N. Effects of continuous and intermittent magnetic fields on oxidative parameters in vivo. *Neurochem Res*. 34(2):238-243, 2009. (AS, CE, CC, OX)

Continuous and intermittent 50 Hz, 1.5 mT magnetic field with the exposure period of 4 h/day for 4 days was used to investigate its possible effect on adult guinea pigs. Tissues and plasma specimens were assessed by biochemical parameters. Malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO) levels and myeloperoxidase activity (MPO) were examined in plasma, liver and brain tissues. All parameters were determined by spectrophotometer. While intermittent magnetic field was effective on plasma lipid peroxidation, continuous magnetic field was found to be effective on plasma MPO activity and NO levels. Augmentation of lipid peroxidation was also observed in liver tissue both intermittent and continuous magnetic field exposures. These results indicate that both the intermittent and continuous magnetic field exposures affect various tissues in a distinct manner because of having different tissue antioxidant status and responses.

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C.

Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Exp Neurol.* 226(1):173-182, 2010. (AS, CE, MC, MA)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel $\alpha(1C)$ subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Cui Y, Ge Z, Rizak JD, Zhai C, Zhou Z, Gong S, Che Y. Deficits in water maze performance and oxidative stress in the hippocampus and striatum induced by

extremely low frequency magnetic field exposure. PLoS One. 7(5):e32196, 2012. (AS, CE, BE, OX)

The exposures to extremely low frequency magnetic field (ELF-MF) in our environment have dramatically increased. Epidemiological studies suggest that there is a possible association between ELF-MF exposure and increased risks of cardiovascular disease, cancers and neurodegenerative disorders. Animal studies show that ELF-MF exposure may interfere with the activity of brain cells, generate behavioral and cognitive disturbances, and produce deficits in attention, perception and spatial learning. Although, many research efforts have been focused on the interaction between ELF-MF exposure and the central nervous system, the mechanism of interaction is still unknown. In this study, we examined the effects of ELF-MF exposure on learning in mice using two water maze tasks and on some parameters indicative of oxidative stress in the hippocampus and striatum. We found that ELF-MF exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and striatum and impaired hippocampal-dependent spatial learning and striatum-dependent habit learning. This study provides evidence for the association between the impairment of learning and the oxidative stress in hippocampus and striatum induced by ELF-MF exposure.

(E) Cvetkovic D, Cosic I. Alterations of human electroencephalographic activity caused by multiple extremely low frequency magnetic field exposures. Med Biol Eng Comput. 47(10):1063-1073, 2009. (HU, AE, EE, MA)

In the past, many studies have claimed that extremely low frequency (ELF) magnetic field (MF) exposures could alter the human electroencephalographic (EEG) activity. This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands. 33 human subjects were tested under a double-blind and counter-balanced conditions. The multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) analysis was employed followed by post-hoc t-tests and Bonferroni alpha-correction. The results from this study have shown that narrow alpha1 (7.5-9.5 Hz) and alpha2 (9-11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than control over the temporal and parietal regions within the 10-16 min of first MF exposure session and the MF exposures were significantly higher than control of the second session MF exposure (60-65 min from the commencement of testing). Also, it was found that the beta1 (12-14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region. The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at frequencies corresponding to those same bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders.

(E) Das S, Kumar S, Jain S, Avelev VD, Mathur R. Exposure to ELF- magnetic field promotes restoration of sensori-motor functions in adult rats with hemisection of thoracic spinal cord. Electromagn Biol Med. 31(3):180-194, 2012. (AS, CE, ME, BE, MA)

Clinically effective modalities of treatment for spinal cord injury (SCI) still remain unsatisfactory and are largely invasive in nature. There are reports of accelerated regeneration in injured peripheral nerves by extremely low-frequency pulsed electromagnetic field (ELF-EMF) in the rat. In the present study, the effect of (50 Hz), low-intensity (17.96 μ T) magnetic field (MF) exposure of rats after-hemisection of T13 spinal cord (hSCI) was investigated on sensori-motor and locomotor functions. Rats were divided into hSCI (sham-exposed) and hSCI+MF (MF: 2 h/d X 6 weeks) groups. Besides their general conditions, locomotor function by Basso, Beattie, and Brenahan (BBB) score; motor responses to noxious stimuli by threshold of tail flick (TTF), simple vocalization (TSV), tail flick latency (TFL), and neuronal excitability by H-reflex were noted. It is found that, in the hSCI+MF group, a statistically significant improvement over the hSCI control group was noted in BBB score from post-SCI wk2 and TFL and TTF by post-hSCI wk1 and wk3, respectively. Correspondingly, TSV gradually restored by post-hSCI wk5. The threshold of H-reflex was reduced on ipsilateral side vs. contralateral side in hSCI and hSCI+MF group. A complete bladder control was dramatically restored on post-hSCI day4 (vs. day7 of hSCI group) and the survival rate was 100% in the hSCI+MF group (vs. 90% of hSCI group). The results of our study suggest that extremely low-frequency (50 Hz), low-intensity (17.96 μ T) MF exposure for 2 h/d x 6wks promotes recovery of sensori-motor behavior including locomotion and bladder control both in terms of temporal pattern and magnitude in hemisection injury of (T13) spinal cord rats.

(E) Davanipour Z, Tseng C-C, Lee PJ, Markides KS, Sobel E. Severe Cognitive Dysfunction and Occupational Extremely Low Frequency Magnetic Field Exposure among Elderly Mexican Americans. Brit J Med Med Res 4 (8): 1641-1662, 2014. (HU, BE)

Aims: This report is the first study of the possible relationship between extremely low frequency (50-60 Hz, ELF) magnetic field (MF) exposure and severe cognitive dysfunction. Earlier studies investigated the relationships between MF occupational exposure and Alzheimer's disease (AD) or dementia. These studies had mixed results, depending upon whether the diagnosis of AD or dementia was performed by experts and upon the methodology used to classify MF exposure. Study Design: Population-based case-control. Place and Duration of Study: Neurology and Preventive Medicine, Keck School of Medicine, University of Southern California, 2 years. Methodology: The study population consisted of 3050 Mexican Americans, aged 65+, enrolled in Phase 1 of the Hispanic Established Population for the Epidemiologic Study of the Elderly (H-EPESE) study. Mini-Mental State Exam (MMSE) results, primary occupational history, and other data were collected. Severe cognitive dysfunction was defined as an MMSE score below 10. The MF exposure methodology developed and used in earlier studies was used.

Results: Univariate odds ratios (OR) were 3.4 ($P < .03$; 95% CI: 1.3-8.9) for high and 1.7 ($P = .27$; 95% CI: 0.7-4.1) for medium or high (M/H) MF occupations. In multivariate main effects models, the results were similar. When interaction terms were allowed in the models, the interactions between M/H or high occupational MF exposure and smoking history or age group were statistically significant, depending upon whether two (65-74, 75+) or three (65-74, 75-84, 85+) age groups were considered, respectively. When the analyses were limited to subjects aged 75+, the interactions between M/H or high MF occupations and a positive smoking history were statistically significant. Conclusion: The results of this study indicate that working in an occupation with high or M/H MF exposure may increase the risk of severe cognitive dysfunction. Smoking and older age may increase the deleterious effect of MF exposure.

(E) Del Giudice E, Facchinetti F, Nofrate V, Boccaccio P, Minelli T, Dam M, Leon A, Moschini G.

Fifty Hertz electromagnetic field exposure stimulates secretion of beta-amyloid peptide in cultured human neuroglioma. *Neurosci Lett.* 418(1):9-12, 2007. (CS, CE, ND)

Recent epidemiological studies raise the possibility that individuals with occupational exposure to low frequency (50-60 Hz) electromagnetic fields (LF-EMF), are at increased risk of Alzheimer's disease (AD). However, the mechanisms through which LF-EMF may affect AD pathology are unknown. We here tested the hypothesis that the exposure to LF-EMF may affect amyloidogenic processes. We examined the effect of exposure to 3.1 mT 50 Hz LF-EMF on A β secretion in H4 neuroglioma cells stably overexpressing human mutant amyloid precursor protein. We found that overnight exposure to LF-EMF induces a significant increase of amyloid-beta peptide (A β) secretion, including the isoform A β 1-42, without affecting cell survival. These findings show for the first time that exposure to LF-EMF stimulates A β secretion in vitro, thus alluding to a potential link between LF-EMF exposure and APP processing in the brain.

(E) Deng Y, Zhang Y, Jia S, Liu J, Liu Y, Xu W, Liu L. Effects of aluminum and extremely low frequency electromagnetic radiation on oxidative stress and memory in brain of mice. *Biol Trace Elem Res.* 156(1-3):243-252, 2013. (AS, CE, BE, OX)

This study was aimed to investigate the effect of aluminum and extremely low-frequency magnetic fields (ELF-MF) on oxidative stress and memory of SPF Kunming mice. Sixty male SPF Kunming mice were divided randomly into four groups: control group, ELF-MF group (2 mT, 4 h/day), load aluminum group (200 mg aluminum/kg, 0.1 ml/10 g), and ELF-MF + aluminum group (2 mT, 4 h/day, 200 mg aluminum/kg). After 8 weeks of treatment, the mice of three experiment groups (ELF-MF group, load aluminum group, and ELF-MF + aluminum group) exhibited firstly the learning memory impairment, appearing that the escaping latency to the platform was prolonged and percentage in the platform quadrant was reduced in the Morris water maze (MWM) task. Secondly are the pathologic abnormalities including neuronal cell loss and

overexpression of phosphorylated tau protein in the hippocampus and cerebral cortex. On the other hand, the markers of oxidative stress were determined in mice brain and serum. The results showed a statistically significant decrease in superoxide dismutase activity and increase in the levels of malondialdehyde in the ELF-MF group ($P < 0.05$ or $P < 0.01$), load aluminum group ($P < 0.01$), and ELF-MF + aluminum group ($P < 0.01$). However, the treatment with ELF-MF + aluminum induced no more damage than ELF-MF and aluminum did, respectively. In conclusion, both aluminum and ELF-MF could impact on learning memory and pro-oxidative function in Kunming mice. However, there was no evidence of any association between ELF-MF exposure with aluminum loading.

(E) Di Loreto S, Falone S, Caracciolo V, Sebastiani P, D'Alessandro A, Mirabilio A, Zimmitti V, Amicarelli F. Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. J Cell Physiol. 219(2):334-343, 2009. (CS, AE, CC, OX)

Large research activity has raised around the mechanisms of interaction between extremely low-frequency magnetic fields (ELF-MFs) and biological systems. ELF-MFs may interfere with chemical reactions involving reactive oxygen species (ROS), thus facilitating oxidative damages in living cells. Cortical neurons are particularly susceptible to oxidative stressors and are also highly dependent on the specific factors and proteins governing neuronal development, activity and survival. The aim of the present work was to investigate the effects of exposures to two different 50 Hz sinusoidal ELF-MFs intensities (0.1 and 1 mT) in maturing rat cortical neurons' major anti-oxidative enzymatic and non-enzymatic cellular protection systems, membrane peroxidative damage, as well as growth factor, and cytokine expression pattern. Briefly, our results showed that ELF-MFs affected positively the cell viability and concomitantly reduced the levels of apoptotic death in rat neuronal primary cultures, with no significant effects on the main anti-oxidative defences. Interestingly, linear regression analysis suggested a positive correlation between reduced glutathione (GSH) and ROS levels in 1 mT MF-exposed cells. On this basis, our hypothesis is that GSH could play an important role in the antioxidant defence towards the ELF-MF-induced redox challenge. Moreover, the GSH-based cellular response was achieved together with a brain-derived neurotrophic factor over-expression as well as with the interleukin 1beta-dependent regulation of pro-survival signaling pathways after ELF-MF exposure.

(E) Dimitrijević D, Savić T, Anđelković M, Prolić Z, Janać B. Extremely low frequency magnetic field (50 Hz, 0.5 mT) modifies fitness components and locomotor activity of Drosophila subobscura. Int J Radiat Biol. 2014 Mar19. [Epub ahead of print] (AS, AE, DE, BE)

Purpose: Extremely low frequency (ELF) magnetic fields are essential ecological factor which may induce changes in many organisms. The aim of this study was to examine the effects in *Drosophila subobscura* exposed for 48 h to ELF magnetic field (50 Hz, 0.5 mT)

at different developmental stages. Materials and methods: Egg-first instar larvae developmental stage of *D. subobscura* isofemale lines was exposed to ELF magnetic field, and fitness components (developmental time, developmental dynamics, viability and sex ratio) and locomotor activity of 3-days old males and females were monitored. Also, just eclosed *D. subobscura* isofemale adults were exposed to ELF magnetic field and their locomotor activity was monitored just after. Results: ELF magnetic field shortens developmental time, increases viability and does not affect sex ratio of *D. subobscura*. No matter which developmental stage is exposed, ELF magnetic field significantly decreases locomotor activity of adult flies, but after exposure of just eclosed adults observed change lasts longer. Conclusions: Applied ELF magnetic field modifies fitness components and locomotor activity of *D. subobscura*. Observed effects can be attributed to the influence of magnetic field on different stages of development where the hormonal and nervous systems play important role in the control of examined parameters.

(E) Duan Y, Wang Z, Zhang H, He Y, Lu R, Zhang R, Sun G, Sun X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. Food Funct. 4(8):1252-1262, 2013. (AS, CE, BE, OX)

The present study investigated the effects of lotus seedpod procyanidins (LSPCs) administered by oral gavage on the cognitive deficits and oxidative damage of mice at extremely low frequency electromagnetic field (ELF-EMF) exposure (50 Hz, 8 mT, 28 days). The results showed that 90 mg kg⁻¹ LSPCs treatment significantly increased body weight compared with the ELF-EMF group at ELF-EMF exposure and effectively maintained liver index, thymus index, kidney index and spleen index close to normal. A water maze test indicated that learning and memory abilities of the ELF-EMF group deteriorated significantly with ELF-EMF exposure when compared with the control group, but the ELF-EMF + LSPCs90 group had remarkably improved learning and memory abilities compared with the ELF-EMF group. Malondialdehyde (MDA), reactive oxygen species (ROS), nitric oxide (NO) and nitric oxide synthase (NOS) mostly exhibited significant increases, while the activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) decreased significantly under ELF-EMF exposure in the ELF-EMF group. LSPCs (especially 60, 90 mg kg⁻¹) administration decreased MDA, ROS, NO content and lowered NOS activity in LSPCs treatment groups. Furthermore, LSPCs (60, 90 mg kg⁻¹) treatment significantly augmented GPx, CAT, SOD activity in the hippocampus and serum. Pathological observation showed that number of pyramidal cells of the CA1 and CA3 regions of the hippocampus of the LSPCs treatment groups was significantly greater than the ELF-EMF group. All the data suggested that the LSPCs can effectively prevent learning and memory damage and oxidative damage caused by the ELF-EMF, most likely through the ability of LSPCs to scavenge oxygen free radicals and to stimulate antioxidant enzyme activity.

(E) El Gohary MI, Salama AA, El Saeid AA, El Sayed TM, Kotb HS. Influence of Magnetic Field on Brain Activity During Administration of Caffeine. Cell Biochem Biophys. 67(3):929-933, 2013. (AS, CE, EE)

The aim of the present work is to evaluate the effect of caffeine, the world's most popular psychoactive drug, on the electric activity of the rat's brain that exposed to extremely low-frequency magnetic field (ELF-MF), during 15 days. The obtained results showed that administration of caffeine in a group of rats by dose of 10 mg/kg (equivalent to human daily consumption) caused a reduction in the mean power amplitude of electroencephalogram (EEG) trace for almost all frequency bands especially α (8-12 Hz). It was observed that the influence of caffeine was more evident in motor cortex than in visual cortex. While the exposure of another group to ELF-MF of intensity 0.2 mT during the same period caused an enhancement in the mean power amplitude of most EEG frequency bands; this was more observed in the right hemisphere of the brain than that of the left hemisphere. The administration of caffeine while rats were exposed to ELF-MF, led, after 5 days of exposure, to a great increase in the mean power amplitude of α band at all places of recording electrodes. It may be concluded that caffeine administration was more effective in reducing the hazardous of ELF-MF in motor cortex than in visual cortex.

(E) Esmekaya MA, Acar SI, Kiran F, Canseven AG, Osmanagaoglu O, Seyhan N. Effects of ELF magnetic field in combination with Iron(III) chloride (FeCl₃) on cellular growth and surface morphology of Escherichia coli (E. coli). Appl Biochem Biotechnol. 169(8):2341-2349, 2013. (CS, AE, MC)

This study investigated the effects of extremely low frequency (ELF) magnetic field with/without iron(III) chloride (FeCl₃) on bacterial growth and morphology. The ELF exposures were carried out using a pair of Helmholtz coil-based ELF exposure system which was designed to generate 50 Hz sinusoidal magnetic field. The field was approximately uniform throughout the axis of the coil pair. The samples which were treated or non-treated with different concentrations FeCl₃ were exposed to 50 Hz, 2 millitesla (mT) magnetic field for 24 h. ELF effect on viability was assessed in terms of viable colony counts (in colony-forming unit per milliliter) with the standard plate count technique. Scanning electron microscopy was used to investigate the magnetic field effect on surface morphology of Escherichia coli. No significant results were seen in terms of cell viability between ELF and sham-exposed bacterial strains. Similarly, FeCl₃ treatment did not change cell viability of E. coli samples. However, we observed some morphological changes on E. coli cell surfaces. Pore formations and membrane destruction were seen on the surface of 24 h ELF field-exposed cells. We concluded that ELF magnetic field exposure at 2 mT does not affect cell viability; however, it may affect bacterial surface morphology.

(E) Falone S, Mirabilio A, Carbone MC, Zimmitti V, Di Loreto S, Mariggìo MA, Mancinelli R, Di Ilio C, Amicarelli F. Chronic exposure to 50Hz magnetic fields causes a

significant weakening of antioxidant defence systems in aged rat brain. Int J Biochem Cell Biol. 40(12):2762-2770, 2008. (AS, CE, CC, OX)

Several studies suggest that extremely low-frequency magnetic fields (ELF-MFs) may enhance the free radical endogenous production. It is also well known that one of the unavoidable consequences of ageing is an overall oxidative stress-based decline in several physiological functions and in the general resistance to stressors. On the basis of these assumptions, the aim of this study was to establish whether the ageing process can increase susceptibility towards widely present ELF-MF-mediated pro-oxidative challenges. To this end, female Sprague-Dawley rats were continuously exposed to a sinusoidal 50 Hz, 0.1 mT magnetic field for 10 days. Treatment-induced changes in the major antioxidant protection systems and in the neurotrophic support were investigated, as a function of the age of the subjects. All analyses were performed in brain cortices, due to the high susceptibility of neuronal cells to oxidative injury. Our results indicated that ELF-MF exposure significantly affects anti-oxidative capability, both in young and aged animals, although in opposite ways. Indeed, exposed young individuals enhanced their neurotrophic signalling and anti-oxidative enzymatic defence against a possible ELF-MF-mediated increase in oxygen radical species. In contrast, aged subjects were not capable of increasing their defences in response to ELF-MF treatment but, on the contrary, they underwent a significant decrease in the major antioxidant enzymatic activities. In conclusion, our data seem to suggest that the exposure to ELF-MFs may act as a risk factor for the occurrence of oxidative stress-based nervous system pathologies associated with ageing.

(E) Fournier NM, Mach QH, Whissell PD, Persinger MA. Neurodevelopmental anomalies of the hippocampus in rats exposed to weak intensity complex magnetic fields throughout gestation. Int J Dev Neurosci. 2012 Jul 31. [Epub ahead of print] (AS, CE, DE, BE, MC)

There has been increasing interest on the possible harmful effects of prenatal exposure to magnetic fields. To investigate the effect of weak intensity magnetic fields on the prenatal brain, pregnant Wistar rats were continuously exposed to one of four intensities (reference: 5-20nT; low 30-50nT; medium 90-580nT; high 590-1200nT) of a complex magnetic field sequence designed to interfere with brain development. As adults, rats exposed to the low-intensity (30-50nT) complex magnetic field displayed impairments in contextual fear learning and showed anomalies in the cytological and morphological development of the hippocampus. In particular, low-intensity exposures resulted in a reduction in overall hippocampal size and promoted subtle dysgenesis of the CA1 and CA3 regions. In contrast, exposure to weaker or stronger intensities of the same complex magnetic field pattern did not interfere with hippocampal development or fear behavior. These findings suggest that prenatal exposure to complex magnetic fields of a narrow intensity window during development can result in subtle but permanent alterations in hippocampal microstructure and function that can have lasting effects on behavior.

(E) Frilot C 2nd, Carrubba S, Marino AA. Transient and steady-state magnetic fields induce increased fluorodeoxyglucose uptake in the rat hindbrain. Synapse. 65(7):617-623, 2011. (HU, AE, CC)

We inquired into the biophysical basis of the ability of weak electromagnetic fields (EMFs) to trigger onset and offset evoked potentials, and to produce steady-state changes in the electroencephalogram (EEG). Rats were exposed to a 2.5-G, 60-Hz magnetic field and the neuroanatomical region of glucose activation associated with the effect of the field on the EEG was identified by positron emission tomography (PET) using fluorodeoxyglucose (FDG). Paired emission scans from the same animal with and without field treatment were differenced and averaged, and t values of the brain voxels computed using the pooled standard deviation were compared with a calculated critical t value to identify the field-activated voxels. Increased glucose utilization occurred in hindbrain voxels when the field was applied orthogonally to the sagittal plane, but not when the angle between the field and the sagittal plane varied randomly. Distinct FDG activation effects were observed in response to transient (both onset and offset) and steady-state magnetic stimuli. Observations of increased glucose utilization induced by magnetic stimuli and its dependence on the direction of the field suggested that signal transduction was mediated by a force detector and that the process and/or early post-transduction processing occurred in the hindbrain.

(E) Fu Y, Wang C, Wang J, Lei Y, Ma Y. Long-term exposure to extremely low-frequency magnetic fields impairs spatial recognition memory in mice. Clin Exp Pharmacol Physiol. 35(7):797-800, 2008. (AS, CE, BE)

In the present study, we investigated the short- and long-term effects of extremely low-frequency (ELF) magnetic fields on spatial recognition memory in mice by using a two-trial recognition Y-maze that is based on the innate tendency of rodents to explore novel environments. 2. Mice were exposed to 25 or 50 Hz electromagnetic fields for either 7 (short term) or 25 days (long term) and then tested in the Y-maze. 3. The results indicated that neither short- nor long-term exposure to magnetic fields affected the locomotor activity of mice in the Y-maze. However, long-term exposure to 50 Hz fields reduced recognition of the novel arm. 4. Our findings suggest that ELF magnetic fields impair spatial recognition memory in the Y-maze depending on the field strength and/or duration of exposure.

(NE) Gavoçi E, Zironi I, Remondini D, Virelli A, Castellani G, Del Re B, Giorgi G, Aicardi G, Bersani F. ELF magnetic fields tuned to ion parametric resonance conditions do not affect TEA-sensitive voltage-dependent outward K(+) currents in a human neural cell line. Bioelectromagnetics. 34(8):579-88, 2013. (CS, AE, CC)

Despite the experimental evidence of significant biological effects of extremely low frequency (ELF) magnetic fields (MFs), the underlying mechanisms are still unclear. Among the few mechanisms proposed, of particular interest is the so called "ion

parametric resonance (IPR)" hypothesis, frequently referred to as theoretical support for medical applications. We studied the effect of different combinations of static (DC) and alternating (AC) ELF MFs tuned on resonance conditions for potassium (K(+)) on TEA-sensitive voltage-dependent outward K(+) currents in the human neuroblastoma BE(2)C cell line. Currents through the cell membrane were measured by whole-cell patch clamp before, during, and after exposure to MF. No significant changes in K(+) current density were found. This study does not confirm the IPR hypothesis at the level of TEA-sensitive voltage-dependent outward K(+) currents in our experimental conditions. However, this is not a direct disprove of the hypothesis, which should be investigated on other ion channels and at single channel levels also.

(NE) Glover PM, Eldeghaidy S, Mistry TR, Gowland PA. Measurement of visual evoked potential during and after periods of pulsed magnetic field exposure. J Magn Reson Imaging. 26(5):1353-1356, 2007. (HU, EE)

PURPOSE: To study the effect of switched magnetic fields used in MR scanners on the visual evoked potential (VEP) in human subjects. MATERIALS AND METHODS: We have used an MRI gradient coil, remote from an MRI magnet to produce a time-varying magnetic field (0.5 kHz, peak field approximately 8.7 T/second) in the human brain without the confounding effects of static field exposure or accompanying acoustic noise. The VEP response to a 2-Hz reversal, 8 x 8 checkerboard, occupying 20 degrees of the visual field was recorded from occipital locations O1 and O2. VEP recordings were made every five minutes before, during, and after a 10-minute magnetic field exposure period for seven subjects. RESULTS: In contradiction to studies previously reported in the literature for fields of 50 Hz and 60 mT, no significant effects on the peak amplitude or latency of the VEP P100 O1 and O2 responses were found. CONCLUSION: Switched magnetic fields of a level and frequency comparable to those used in MRI do not have a significant effect on primary retinal or visual processing.

(E) Gulturk S, Demirkazik A, Kosar I, Cetin A, Dökmetas HS, Demir T. Effect of exposure to 50 Hz magnetic field with or without insulin on blood-brain barrier permeability in streptozotocin-induced diabetic rats. Bioelectromagnetics. 31(4):262-269, 2010. (AS, CE, ME)

We investigated the effect of long-term exposure to modulation magnetic field (MF), insulin, and their combination on blood-brain barrier (BBB) permeability in a diabetic rat model. Fifty-three rats were randomly assigned to one of six groups: sham, exposed to no MF; MF, exposed to MF; diabetes mellitus (DM), DM induced with streptozotocin (STZ); DM plus MF (DMMF); DM plus insulin therapy (DMI); and DM plus insulin therapy plus MF (DMIMF). All the rats underwent Evans blue (EB) measurement to evaluate the BBB 30 days after the beginning of experiments. The rats in MF, DMMF, and DMIMF groups were exposed to MF (B = 5 mT) for 165 min every day for 30 days. Mean arterial blood pressure (MABP), body mass, and serum glucose level of the study rats were recorded. The extravasation of brain EB of the MF, DM, DMMF, DMI, and DMIMF groups

was higher than that of the sham group and the extravasation of right hemisphere of the DMIMF group was highest ($P < 0.05$). The post-procedure body mass of the sham and MF groups were significantly higher than those of the DM and DMMF groups ($P < 0.05$). In the DM, DMMF, DMI, and DMIMF groups, the baseline glucose was significantly lower than the post-procedure glucose ($P < 0.05$). DM and MF increase BBB permeability; in combination, they cause more increase in BBB permeability, and insulin decreases their effect on BBB. Improved glucose metabolism may prevent body mass loss and the hypoglycemic effect of MF. DM increases MABP but MF causes no additional effect.

(E) Gutiérrez-Mercado YK, Cañedo-Dorantes L, Gómez-Pinedo U, Serrano-Luna G, Bañuelos-Pineda J, Feria-Velasco A. Increased vascular permeability in the circumventricular organs of adult rat brain due to stimulation by extremely low frequency magnetic fields. *Bioelectromagnetics*. 34(2):145-155, 2013. (AS, CE, MC)

It has been demonstrated that the exposure of biological systems to magnetic fields (MFs) can produce several beneficial effects: tissue recovery in chronic wounds, re-establishment of blood circulation after tissue ischemia or in necrotic tissues, improvement after epileptic episodes, angiogenesis, etc. In the current study, the effects of extremely low frequency (ELF) MF on the capillaries of some circumventricular organs (CVOs) are demonstrated; a vasodilator effect is reported as well as an increase in their permeability to non-liposoluble substances. For this study, 96 Wistar male rats (250 g body mass) were used and divided into three groups of 32 rats each: a control group (no treatment); a sham ELF-MF group; and an experimental group subjected to ELF-MF (120 Hz harmonic waves and 0.66 mT, root mean square) by the use of Helmholtz coils. All animals were administered colloidal carbon (CC) intravenously to study, through optical and transmission electron microscopy, the capillary permeability in CVOs and the blood-brain barrier (BBB) in brain areas. An increase in capillary permeability to CC was detected in the ELF-MF-exposed group as well as a significant increase in vascular area (capillary vasodilation); none of these effects were observed in individuals of the control and sham ELF-MF groups. It is important to investigate the mechanisms involved in the phenomena reported here in order to explain the effects of ELF-MF on brain vasculature.

(E) Harakawa S, Nedachi T, Hori T, Takahashi K, Tochio K, Inoue N. Effect of electric field in conditioned aversion response. *J Vet Med Sci*. 70(6):611-613, 2008. (AS, AE, BE, EF)

The aim of the present study was to estimate whether rat sense exogenous electric field (EF) including one used in our previous studies. Employing a conditioned place aversion response paradigm based on an aversive behavior against light environment, alteration in both voluntary behavior of Wistar rat to a 50 Hz sinusoidal EF was examined. Following conditioning without EF, the times spent in white place in rats was significantly shortened ($P < 0.05$). While, such changes were not shown in rats

conditioned with EF. Thus, it was considered that the aversion response to light environment was interfered by exposure to EF. An interference in recognition of brightness via EF induced effect to visual system or in learning system via direct effect to central nerve system was considerable as a factor for EF-induced effect. In addition, it was remained that rat possibly sense exposure to EF as preferable. In order to confirm which factor functioned, further studies are needed.

(E) He LH, Shi HM, Liu TT, Xu YC, Ye KP, Wang S. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. Chin Med J (Engl). 124(20):3362-3366, 2011. (AS, CE, BE)

BACKGROUND: As the widespread use of electric devices in modern life, human are exposed to extremely low frequency magnetic fields (ELF MF) much more frequently than ever. Over the past decades, a substantial number of epidemiological and experimental studies have demonstrated that ELF MF (50 Hz) exposure is associated with increased risk of various health effects. The present study examined the effects of chronic exposure to ELF MF on anxiety level and spatial memory of adult rats.

METHODS: The 50-Hz ELF MF was used during the whole experimental procedures and the value of magnetic field (MF) was set to 2 mT. Adult rats were divided randomly to control, MF 1 hour and MF 4 hours group. Anxiety-related behaviors were examined in the open field test and the elevated plus maze; changes in spatial learning and memory were determined in Morris water maze after 4 weeks of daily exposure. RESULTS: Rats in MF 4 hours group had increased anxiety-like behaviors with unaltered locomotor activity. In the Morris water maze test, rats had reduced latency to find the hidden platform and improved long-term memory of former location of platform without changes in short-term memory and locomotor activity. CONCLUSION: Chronic ELF MF exposure has anxiogenic effect on rats, and the promoting effects on spatial learning and long-term retention of spatial memory.

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(E) He YL, Liu DD, Fang YJ, Zhan XQ, Yao JJ, Mei YA. Exposure to extremely low-frequency electromagnetic fields modulates Na⁺ currents in rat cerebellar granule cells through increase of AA/PGE₂ and EP receptor-mediated cAMP/PKA pathway. PLoS One. 2013;8(1):e54376. doi: 10.1371/journal.pone.0054376. (CS, AE, CC, EE)

Although the modulation of Ca(2+) channel activity by extremely low-frequency electromagnetic fields (ELF-EMF) has been studied previously, few reports have addressed the effects of such fields on the activity of voltage-activated Na(+) channels (Na(v)). Here, we investigated the effects of ELF-EMF on Na(v) activity in rat cerebellar granule cells (GCs). Our results reveal that exposing cerebellar GCs to ELF-EMF for 10-60 min significantly increased Na(v) currents (I(Na)) by 30-125% in a time- and intensity-dependent manner. The Na(v) channel steady-state activation curve, but not the steady-state inactivation curve, was significantly shifted (by 5.2 mV) towards hyperpolarization by ELF-EMF stimulation. This phenomenon is similar to the effect of intracellular

application of arachidonic acid (AA) and prostaglandin E(2) (PGE(2)) on I(Na) in cerebellar GCs. Increases in intracellular AA, PGE(2) and phosphorylated PKA levels in cerebellar GCs were observed following ELF-EMF exposure. Western blottings indicated that the Na(V) 1.2 protein on the cerebellar GCs membrane was increased, the total expression levels of Na(V) 1.2 protein were not affected after exposure to ELF-EMF. Cyclooxygenase inhibitors and PGE(2) receptor (EP) antagonists were able to eliminate this ELF-EMF-induced increase in phosphorylated PKA and I(Na). In addition, ELF-EMF exposure significantly enhanced the activity of PLA(2) in cerebellar GCs but did not affect COX-1 or COX-2 activity. Together, these data demonstrate for the first time that neuronal I(Na) is significantly increased by ELF-EMF exposure via a cPLA2 AA PGE(2) EP receptors PKA signaling pathway.

(E) Hung CS, Anderson C, Horne JA, McEvoy P. Mobile phone 'talk-mode' signal delays EEG-determined sleep onset. *Neurosci Lett.* 421(1):82-86, 2007. (HU, AE, EE, BE)

Mobile phones signals are pulse-modulated microwaves, and EEG studies suggest that the extremely low-frequency (ELF) pulse modulation has sleep effects. However, 'talk', 'listen' and 'standby' modes differ in the ELF (2, 8, and 217Hz) spectral components and specific absorption rates, but no sleep study has differentiated these modes. We used a GSM900 mobile phone controlled by a base-station simulator and a test SIM card to simulate these three specific modes, transmitted at 12.5% (23dBm) of maximum power. At weekly intervals, 10 healthy young adults, sleep restricted to 6h, were randomly and single-blind exposed to one of: talk, listen, standby and sham (nil signal) modes, for 30 min, at 13:30 h, whilst lying in a sound-proof, lit bedroom, with a thermally insulated silent phone beside the right ear. Bipolar EEGs were recorded continuously, and subjective ratings of sleepiness obtained every 3 min (before, during and after exposure). After exposure the phone and base-station were switched off, the bedroom darkened, and a 90 min sleep opportunity followed. We report on sleep onset using: (i) visually scored latency to onset of stage 2 sleep, (ii) EEG power spectral analysis. There was no condition effect for subjective sleepiness. Post-exposure, sleep latency after talk mode was markedly and significantly delayed beyond listen and sham modes. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset. It is possible that 2, 8, 217Hz modulation may differentially affect sleep onset.

(E) Ishay JS, Plotkin M, Volynchik S, Shaked M, Schuss Z, Bergman DJ. Exposure to an additional alternating magnetic field affects comb building by worker hornets. *Physiol Chem Phys Med NMR.* 39(1):83-88, 2007. (AS, CE, BE)

Oriental hornet workers, kept in an Artificial Breeding Box (ABB) without a queen, construct within a few days brood combs of hexagonal cells with apertures facing down. These combs possess stems that fasten the former to the roof of the ABB. In an ABB with adult workers (more than 24 h after eclosion), exposed to an AC (50 Hz) magnetic field of a magnitude of B = 50-70 mGauss, the combs and cells are built differently from

those of a control ABB, subjected only to the natural terrestrial magnetic field. The effects of the additional magnetic field consist of (a) 35-55% smaller number of cells and fewer eggs in each comb, (b) disrupted symmetry of building, with many deformed and imperfectly hexagonal cells, and (c) more delicate and slender comb stems.

(E) Jadidi M, Firoozabadi SM, Rashidy-Pour A, Sajadi AA, Sadeghi H, Taherian AA. Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats. Neurobiol Learn Mem. 88(4):387-392, 2007. (AS, CE, BE)

This study was planned to evaluate the effect of an exposure to magnetic fields on consolidation and retrieval of hippocampus dependent spatial memory using a water maze. In Experiments 1 and 2, rats were trained in a hidden version (spatial) of water maze task with two blocks of four trials. The retention of spatial memory was evaluated 48 h later. Exposure to a 50 Hz 8 mT, but not 2 mT magnetic fields for 20 min immediately after training impaired retention performance. The same time exposure shortly before retention testing had no effect. In Experiment 3, rats were trained in a cued version of water maze with two blocks of four trials. Exposure to magnetic field at 8 mT for 20 min immediately after training did not impair retention performance. These findings indicate that acute exposure to a 50 Hz magnetic field at 8 mT for short time can impair consolidation of spatial memory.

(E) Janać B, Tovilović G, Tomić M, Prolić Z, Radenović L. Effect of continuous exposure to alternating magnetic field (50 Hz, 0.5 mT) on serotonin and dopamine receptors activity in rat brain. Gen Physiol Biophys. 28 Spec No:41-46, 2009. (AS, CE, FC)

External magnetic fields (MFs) have the ability to modify motor activity of animals, complex type of behaviour connected with dopaminergic and serotonergic neurotransmissions in the brain. Thus, the purpose of this study was to examine MF-induced changes in the activity of serotonin 5-HT_{2A} receptors in the prefrontal cortex, as well as dopamine D₁ and D₂ receptors in the striatum of adult Wistar rats, considering their involvement in motor behavior regulation. Experimental animals were continuously exposed to extremely low frequency MF (ELF-MF, 50 Hz, 0.5 mT) for 1, 3, and 7 days. Subsequently, binding properties (K_d and B_{max}) of receptors were determined by in vitro radioligand receptor binding assays. It was shown that the affinity of serotonin 5-HT_{2A} receptors decreased and their density increased in the prefrontal cortex of rats after ELF-MF exposure. Regarding affinity, this effect was duration-dependent and most prominent after 7-day of ELF-MF exposure. In contrast to serotonin 5-HT_{2A} receptors in the prefrontal cortex, ELF-MF had no significant effect on the affinity and density of dopamine D₁ and D₂ receptors in the striatum. We can conclude that continuous exposure to ELF-MF up to 7 days affects cortical serotonergic neurotransmission, whereby intensity of these changes depends on ELF-MF exposure duration.

(E) Janać B, Selaković V, Rauš S, Radenović L, Zrnić M, Prolić Z. Temporal patterns of extremely low frequency magnetic field-induced motor behavior changes in Mongolian gerbils of different age. Int J Radiat Biol. 88(4):359-366, 2012. (AS, CE, BE)

PURPOSE: The aim of this study was to investigate the influence of extremely low frequency magnetic field (ELF-MF) on different behavior parameters (locomotion, stereotypy, and immobility) in 3- and 10-month-old male Mongolian gerbils. **MATERIALS AND METHODS:** The animals were continuously exposed to ELF-MF (50 Hz; 0.1, 0.25 and 0.5 mT) for seven days. Their behavior was monitored for 60 min in the open field after the 1st, 2nd, 4th, and 7th day of exposure (immediate effect), and three days after ELF-MF exposure had been ceased (delayed effect). **RESULTS:** In 3-month-old gerbils, exposure to ELF-MF (0.1, 0.25 and 0.5 mT) increased motor behavior (locomotion and stereotypy), and consequently decreased immobility. Additionally, ELF-MF had delayed effect (except 0.25 mT) on stereotypy and immobility. In 10-month-old gerbils, ELF-MF of 0.1, 0.25 and 0.5 mT induced decrease, slight increase, and pronounced stimulation of motor behavior, respectively. Regardless of magnetic induction value, increased motor behavior was observed three days after ELF-MF exposure has been ceased (delayed effect). **CONCLUSIONS:** It can be proposed that the specific temporal patterns of ELF-MF-induced motor behavior changes in 3- and 10-month-old gerbils are a consequence of age-dependent morpho-functional differences in the brain structures responsible for a control of motor behavior.

(E) Kim HJ, Jung J, Park JH, Kim JH, Ko KN, Kim CW. Extremely low-frequency electromagnetic fields induce neural differentiation in bone marrow derived mesenchymal stem cells. Exp Biol Med (Maywood). 238(8):923-931, 2013. (CS, AE, MC, MA)

Extremely low-frequency electromagnetic fields (ELF-EMF) affect numerous biological functions such as gene expression, cell fate determination and even cell differentiation. To investigate the correlation between ELF-EMF exposure and differentiation, bone marrow derived mesenchymal stem cells (BM-MSCs) were subjected to a 50-Hz electromagnetic field during in vitro expansion. The influence of ELF-EMF on BM-MSCs was analysed by a range of different analytical methods to understand its role in the enhancement of neural differentiation. ELF-EMF exposure significantly decreased the rate of proliferation, which in turn caused an increase in neuronal differentiation. The ELF-EMF-treated cells showed increased levels of neuronal differentiation marker (MAP2), while early neuronal marker (Nestin) was down-regulated. In addition, eight differentially expressed proteins were detected in two-dimensional electrophoresis maps, and were identified using ESI-Q-TOF LC/MS/MS. Among them, ferritin light chain, thioredoxin-dependent peroxide reductase, and tubulin β -6 chain were up-regulated in the ELF-EMF-stimulated group. Ferritin and thioredoxin-dependent peroxide reductase are involved in a wide variety of functions, including Ca(2+) regulation, which is a critical component of neurodegeneration. We also observed that the intracellular Ca(2+)

content was significantly elevated after ELF-EMF exposure, which strengthens the modulatory role of ferritin and thioredoxin-dependent peroxide reductase, during differentiation. Notably, western blot analysis indicated significantly increased expression of the ferritin light chain in the ELF-EMF-stimulated group (0.60 vs. 1.08; $P < 0.01$). These proteins may help understand the effect of ELF-EMF stimulation on BM-MSCs during neural differentiation and its potential use as a clinically therapeutic option for treating neurodegenerative diseases.

(E) Kitaoka K, Kitamura M, Aoi S, Shimizu N, Yoshizaki K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. *Bioelectromagnetics*. 34(1):43-51, 2013. (AS, CE, BE, CC)

An extremely low-frequency magnetic field (ELF-MF) is generated by power lines and household electrical devices. Many studies have suggested an association between chronic ELF-MF exposure and anxiety and/or depression. The mechanism of these effects is assumed to be a stress response induced by ELF-MF exposure. However, this mechanism remains controversial. In the present study, we investigated whether chronic ELF-MF exposure (intensity, 3 mT; total exposure, 200 h) affected emotional behavior and corticosterone synthesis in mice. ELF-MF-treated mice showed a significant increase in total immobility time in a forced swim test and showed latency to enter the light box in a light-dark transition test, compared with sham-treated (control) mice. Corticosterone secretion was significantly high in the ELF-MF-exposed mice; however, no changes were observed in the amount of the adrenocorticotrophic hormone and the expression of genes related to stress response. Quantification of the mRNA levels of adrenal corticosteroid synthesis enzymes revealed a significant reduction in Cyp17a1 mRNA in the ELF-MF-exposed mice. Our findings suggest the possibility that high intensity and chronic exposure to ELF-MF induces an increase in corticosterone secretion, along with depression- and/or anxiety-like behavior, without enhancement of the hypothalamic-pituitary-adrenal axis.

(E) Korpınar MA, Kalkan MT, Tuncel H. The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats. *Bratisl Lek Listy*. 113(9):521-524, 2012. (AS, CE, BE)

Purpose: The purpose of this study was to investigate the behavioral changes induced by 50 Hz, 10 mT flux density Sinusoidal Magnetic Field (MF). Material and methods: Seventy-six young adult male Wistar albino rats were used in the study. They were separated into two groups: control group (C) n=38; MF group n=38. C animals were left under the same conditions with the MF group for 21 days but with prevented or avoided exposure to MF. Anxiety and stress-related behavioral changes were investigated by elevated plus-maze and hole-board systems. Just before being tested in the maze, each animal was tested by means of the hole-board method in order to separate the directed exploration behavior and locomotion activity changes from

anxiety-related behavior. Results: In the hole-board system parameters there were no statistically significant differences between the two groups. There was a statistically significant difference between MF and C groups when the ratio of time spent on open arms to the total time spent on all arms was evaluated (0.12 ± 0.08 and 0.34 ± 0.18 respectively and $p < 0.01$). Conclusion: Our results suggest that after 21 days, a continuous exposure to extremely low frequency of magnetic field (50 Hz, 10 mT) has no significant effect on activity and exploration activity but significantly induces stress and anxiety-related behavior in rats (Tab. 2, Fig. 9, Ref. 19).

(E) Kumar S, Jain S, Behari J, Avelev VD, Mathur R. Effect of magnetic field on food and water intake and body weight of spinal cord injured rats. Indian J Exp Biol. 48(10):982-986, 2010. (AS, CE, MA)

Chronic (2 h/d x 8 weeks) exposure to magnetic field (MF; 50 Hz, 17.9 microT) in complete spinal cord (T13) transected rats restored food intake (FI), water intake (WI) and body weight (BW) which were decreased in the spinal cord injured rats. The results suggest a significant beneficial effect of chronic exposure to magnetic field of paraplegic rats.

(E) Kumar S, Jain S, Velpandian T, Petrovich Gerasimenko Y, D Avelev V, Behari J, Behari M, Mathur R. Exposure to extremely low-frequency magnetic field restores spinal cord injury-induced tonic pain and its related neurotransmitter concentration in the brain. Electromagn Biol Med. 32(4):471-483, 2013. (AS, CE, BE, CC, MA)

Spinal cord injury (SCI) is unequivocally reported to produce hyperalgesia to phasic stimuli, while both hyper- and hypoalgesia to tonic stimuli. The former is spinally mediated and the latter centrally. Besides, its management is unsatisfactory. We report the effect of magnetic field (MF; 17.96 μ T, 50 Hz) on tonic pain behavior and related neurotransmitters in the brain of complete thoracic (T13) SCI rats at week 8. Adult male Wistar rats were divided into Sham, SCI and SCI+MF groups. Formalin-pain behavior was compared utilizing 5 min block pain rating (PR), 60 min session-PR, time spent in various categories of increasing pain (T0-T3) and flinch incidences. Serotonin (5-HT), dopamine (DA), norepinephrine (NE), gamma-aminobutyric acid (GABA), glutamate and glycine were estimated in brain tissue by liquid chromatography-mass spectrometry. Session-PR, block-PR and number of flinches were significantly lower, while time spent in categories 0-1 was higher in the SCI versus Sham group. These parameters were comparable in the SCI+MF versus Sham group. 5-HT concentration in cortex, remaining forebrain areas and brain stem (BS), was lower while GABA and NE were higher in BS of SCI, which were comparable with Sham in the SCI+MF group. The concentration of DA, glutamate and glycine was comparable amongst the groups. The data indicate significant hypoalgesia in formalin pain while increased in GABA, NE and decreased in 5-HT post-SCI, which were restored in the SCI+MF group. We suggest beneficial effect of chronic (2 h/day x 8 weeks) exposure to MF (50 Hz, 17.96 μ T) on tonic pain that is mediated by 5-HT, GABA and NE in complete SCI rats.

(E) Lahijani MS, Bigdeli MR, Kalantary S. Effects of sinusoidal electromagnetic fields on histopathology and structures of brains of preincubated white Leghorn chicken embryos. Electromagn Biol Med. 30(3):146-157, 2011. (AS, AE, MC, DE)

There are several reports indicating linkages between exposures to 50-60 Hz electromagnetic fields and abnormalities in the early stages of chicken embryonic development. Based on our previous published research carried out at the Department of Animal Sciences, Faculty of Biological Sciences, Shahid Beheshti University, effects of sinusoidal electromagnetic fields on histopathology and structures of brains of preincubated white leghorn hen eggs were investigated. Three hundred healthy fresh fertilized eggs (55-65 gr) were divided into three groups of experimental (n = 50), control (n = 75), and sham (n = 75). Experimental eggs (inside the coil) were exposed to 3 different intensities of 1.33, 2.66, and 7.32 mT and sham groups were located inside the same coil with no exposure, for 24 h before incubation. Control, sham, and experimental groups were all incubated in an incubator ($38 \pm 0.5(^{\circ})C$, 60% humidity) for 14 days. 14-day old chicken embryos were removed by C-sections, and the brains of all embryos of all groups were fixed in formalin(10%), stained with H&E and TUNEL assay, for studying the histopatholog and process of apoptosis. The brains of other embryos were prepared for Scanning Electeron Microscope. Results showed electromagnetic fields have toxic effects on brain cells by increasing the number of apoptotic cells and degeneration of brains' tissues of exposed chicken embryos. These findings suggest that the electromagnetic fields induce brain damages at different levels.

(E) Legros A, Corbacio M, Beuter A, Modolo J, Goulet D, Prato FS, Thomas AW. Neurophysiological and behavioral effects of a 60 Hz, 1,800 μ T magnetic field in humans. Eur J Appl Physiol. 112(5):1751-1762, 2012. (HU, AE, BE)

The effects of time-varying magnetic fields (MF) on humans have been actively investigated for the past three decades. One important unanswered question is the potential for MF exposure to have acute effects on human biology. Different strategies have been used to tackle this question using various physiological, neurophysiological and behavioral indicators. For example, researchers investigating electroencephalography (EEG) have reported that extremely low frequency (ELF, <300 Hz) MF can increase resting occipital alpha rhythm (8-12 Hz). Interestingly, other studies have demonstrated that human motricity can be modulated by ELF MF: a reduction of anteroposterior standing balance or a decrease of physiological tremor intensity have been reported as consequences of exposure. However, the main limitation in this domain lies in the lack of results replication, possibly originating from the large variety of experimental approaches employed. Therefore, the present study aimed to investigate the effects of a 60 Hz, 1,800 μ T MF exposure on neurophysiological (EEG) and neuromotor (standing balance, voluntary motor function, and physiological tremor) aspects in humans using a single experimental procedure. Though results from this study suggest a reduction of human standing balance with MF exposure, as well as an increase of physiological tremor amplitude within the frequency range associated with

central nervous system contribution, no exposure effect appeared on other investigated parameters (e.g., EEG or voluntary motor control). These results suggest that 1 h of 60 Hz, 1,800 μ T MF exposure may modulate human involuntary motor control without being detected in the cortical electrical activity.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (AS, CS, CE, AE, BE, CC, MA)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(NE) Li L, Xiong DF, Liu JW, Li ZX, Zeng GC, Li HL. No effects of power line frequency extremely low frequency electromagnetic field exposure on selected neurobehavior tests of workers inspecting transformers and distribution line stations versus controls. Australas Phys Eng Sci Med. 2013 Dec 31. [Epub ahead of print] (HU, BE, CE)

We aimed to evaluate the interference of 50 Hz extremely low frequency electromagnetic field (ELF-EMF) occupational exposure on the neurobehavior tests of workers performing tour-inspection close to transformers and distribution power lines. Occupational short-term "spot" measurements were carried out. 310 inspection workers and 300 logistics staff were selected as exposure and control. The

neurobehavior tests were performed through computer-based neurobehavior evaluation system, including mental arithmetic, curve coincide, simple visual reaction time, visual retention, auditory digit span and pursuit aiming. In 500 kV areas electric field intensity at 71.98 % of total measured 590 spots were above 5 kV/m (national occupational standard), while in 220 kV areas electric field intensity at 15.69 % of total 701 spots were above 5 kV/m. Magnetic field flux density at all the spots was below 1,000 μ T (ICNIRP occupational standard). The neurobehavior score changes showed no statistical significance. Results of neurobehavior tests among different age, seniority groups showed no significant changes. Neurobehavior changes caused by daily repeated ELF-EMF exposure were not observed in the current study.

(NE) Li Y, Zhang C, Song T. Disturbance of the magnetic field did not affect spatial memory. *Physiol Res.* 2014 Feb 24. [Epub ahead of print] (AS, CE, BE)

Extremely low-frequency magnetic field (ELF-MF) has been suggested to influence the cognitive capability and has to be dynamically evaluated in a longitudinal study. Previous training can affect performance, but the influence under magnetic field is unclear. This study aims to evaluate the effects of previous training and ELF-MF exposure on learning and memory using the Morris water maze (MWM). Sprague-Dawley rats were subjected to MWM training, ELF-MF exposure (50 Hz, 100 microT), or ELF-MF exposure combined with MWM training for 90 days. Normal rats were used as controls. The MWM was used to test. The data show that the rats exposed to training and ELF-MF with training performed better on spatial acquisition when re-tested. However, during the probe trial the rats showed no change between the training phase and the test phase. Compared with the control group, the ELF-MF group showed no significant differences. These results confirm that previous training can improve the learning and memory capabilities regarding spatial acquisition in the MWM and this effect can last for at least 90 days. However, this improvement in learning and memory capabilities was not observed during the probe trial. Furthermore, ELF-MF exposure did not interfere with the improvement in learning and memory capabilities.

(E) Liu T, Wang S, He L, Ye K. Anxiogenic effect of chronic exposure to extremely low frequency magnetic field in adult rats. *Neurosci Lett.* 434(1):12-17, 2008a. (AS, CE, BE)

Previous study has suggested some relations between extremely low frequency magnetic field (ELF MF) and the emotional state of human beings and animals. The aim of the present study was to investigate whether the anxiety level could be affected by repeated ELF MF exposure of different daily durations. Adult SD rats were submitted to no exposure, MF exposure 1h/day or 4h/day for 25 days. Anxiety-related behaviors were examined in the open field test (OFT), the elevated plus maze (EPM), and light/dark box on the 21th, 23th and 25th exposure day, respectively. Results demonstrated that MF exposure 4h/day increased the anxiety-like behaviors in rats in the open field test and the elevated plus maze test, without altering their locomotor activity, but had no effect in the light/dark box test. Moreover, MF exposure 1h/day had

no effect in any test. These findings indicate that chronic ELF MF exposure has anxiogenic effect in rats, which is dependent on the daily exposure duration and it is more sensitive to void space than to strong light.

(E) Liu T, Wang S, He L, Ye K. Chronic exposure to low-intensity magnetic field improves acquisition and maintenance of memory. Neuroreport. 19(5):549-552, 2008b. (AS, CE, BE)

Although past research has suggested that acute exposure to extremely low-frequency magnetic field (ELF MF) impairs learning and memory function, data on chronic exposure remain scarce. In this study, we examined the changes in spatial learning and memory by the Morris water maze test after 4 weeks of daily exposure of rats to a 50-Hz magnetic field of 2 mT for either 1 or 4 h. We found that chronic exposure to ELF MF reduced the latency to find the hidden platform and improved long-term memory of former location of platform without affecting the short-term memory and motor activity. These findings for the first time indicate that chronic exposure to ELF MF exerts a positive effect on the acquisition and maintenance of spatial memory.

(E) Maestú C, Blanco M, Nevado A, Romero J, Rodríguez-Rubio P, Galindo J, Bautista Lorite J, de las Morenas F, Fernández-Argüelles P. Reduction of pain thresholds in fibromyalgia after very low-intensity magnetic stimulation: a double-blinded, randomized placebo-controlled clinical trial. Pain Res Manag. 18(6):e101-106, 2013. (HU, BE, MA)

BACKGROUND: Exposure to electromagnetic fields has been reported to have analgesic and antinociceptive effects in several organisms. Objective: To test the effect of very low-intensity transcranial magnetic stimulation on symptoms associated with fibromyalgia syndrome. METHODS: A double-blinded, placebo-controlled clinical trial was performed in the Sagrado Corazón Hospital, Seville, Spain. Female fibromyalgia patients (22 to 50 years of age) were randomly assigned to either a stimulation group or a sham group. The stimulation group (n=28) was stimulated using 8 Hz pulsed magnetic fields of very low intensity, while the sham group (n=26) underwent the same protocol without stimulation. Pressure pain thresholds before and after stimulation were determined using an algometer during the eight consecutive weekly sessions of the trial. In addition, blood serotonin levels were measured and patients completed questionnaires to monitor symptom evolution. RESULTS: A repeated-measures ANOVA indicated statistically significant improvement in the stimulation group compared with the control group with respect to somatosensory pain thresholds, ability to perform daily activities, perceived chronic pain and sleep quality. While improvement in pain thresholds was apparent after the first stimulation session, improvement in the other three measures occurred after the sixth week. No significant between-group differences were observed in scores of depression, fatigue, severity of headaches or serotonin levels. No adverse side effects were reported in any of the patients. CONCLUSIONS:

Very low-intensity magnetic stimulation may represent a safe and effective treatment for chronic pain and other symptoms associated with fibromyalgia.

(E) Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya RS, Sashidhar RB, Subramanyam C. Influence of extremely low frequency magnetic fields on Ca²⁺ signaling and NMDA receptor functions in rat hippocampus. *Neurosci Lett.* 413(2):145-149, 2007. (AS, CE, CC)

Extremely low frequency (ELF<300Hz) electromagnetic fields affect several neuronal activities including memory. Because ELF magnetic fields cause altered Ca(2+) homeostasis in neural tissues, we examined their influence on Ca(2+) signaling enzymes in hippocampus and related them with NMDA receptor functions. Hippocampal regions were obtained from brains of 21-day-old rats that were exposed for 90 days to 50Hz magnetic fields at 50 and 100 microT intensities. In comparison to controls, ELF exposure caused increased intracellular Ca(2+) levels concomitant with increased activities of Ca(2+)-dependent protein kinase C (PKC), cAMP-dependent protein kinase and calcineurin as well as decreased activity of Ca(2+)-calmodulin-dependent protein kinase in hippocampal regions. Simultaneous ligand-binding studies revealed decreased binding to N-methyl-D-aspartic acid (NMDA) receptors. The combined results suggest that perturbed neuronal functions caused by ELF exposure may involve altered Ca(2+) signaling events contributing to aberrant NMDA receptor activities.

(E) Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya SR, Sashidhar RB, Subramanyam C. Extremely low frequency magnetic fields induce oxidative stress in rat brain. *Gen Physiol Biophys.* 2013 Dec 13. [Epub ahead of print] (AS, CE, OX, CC)

The present investigation was conducted to understand the influence of long-term exposure of rats to extremely low frequency magnetic fields (ELF-MF), focusing on oxidative stress (OS) on different regions of rat's brain. Male Wistar rats (21-day-old) were exposed to ELF-MF (50 Hz; 50 and 100 μT) for 90 days continuously; hippocampal, cerebellar and cortical regions from rats were analyzed for (i) reactive oxygen species (ROS), (ii) metabolites indicative of OS and (iii) antioxidant enzymes. In comparison to control group rats, the rats that were continuously exposed to ELF-MF caused OS and altered glutathione (GSH/GSSG) levels in dose-dependent manner in all the regions of the brain. Accumulation of ROS, lipid peroxidation end products and activity of superoxide dismutase in different regions was in the descending order of cerebellum < hippocampus < cortex. Decrement in GSH/GSSG levels and increment in glutathione peroxidase activity were in the descending order of hippocampus < cerebellum < cortex. The continuous exposure to ELF-MF caused OS in all the examined regions of brain more significantly at 100 μT than at 50 μT. Varied influences observed in different regions of the brain, as documented in this study, may contribute to altered metabolic patterns in its related regions of the central nervous system, leading to aberrant neuronal functions.

(E) Manjhi J, Kumar S, Behari J, Mathur R. Effect of extremely low frequency magnetic field in prevention of spinal cord injury-induced osteoporosis. J Rehabil Res Dev. 50(1):17-30, 2013. (AS, CE, MA)

The present study was designed to investigate the effect of extremely low frequency (ELF) magnetic field (MF) on spinal cord injury (SCI)-induced osteoporosis in rats. Adult male Wistar rats (n = 24) were equally divided into sham, SCI, and SCI+MF groups. Complete transection of spinal cord (thoracic 11 vertebra) was surgically performed under anesthesia, whereas in the sham group only laminectomy was done. Post-SCI day 1, rats were either exposed (2 h/d × 8 wk) to ELF-MF (17.96 micro-Tesla, 50 Hz; SCI+MF group) or sham exposed (SCI group). Basso, Beattie, and Bresnahan (BBB) score was recorded weekly. All the rats were sacrificed 8 wk post-SCI; tibia and femur bones were isolated for the analysis of bone mineral content (BMC; total calcium [Ca], phosphorus [P], carbon [C]), bone mineral density (BMD), and biochemical status (osteocalcin, collagen I, alkaline phosphatase). The BBB score decreased post-SCI, which partially recovered after ELF-MF. In SCI rats, there was a statistically significant decrease in BMC, Ca, P, C, BMD, and biochemical level in both the bones as compared with the sham group, which was attenuated in SCI+MF rats except the C content. Electron microscopic study revealed the enhancement of microstructural composition and compactness in cortical and trabecular parts of treated bones. The results suggest that the chronic (2 h/d × 8 wk) ELF-MF exposure (17.96 micro-Tesla, 50 Hz) to SCI rats is effective in attenuating SCI-induced osteoporosis.

(E) Martínez-Sámano J, Torres-Durán PV, Juárez-Oropeza MA, Verdugo-Díaz L. Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. Arch Med Res. 43(3):183-189, 2012. (AS, AE, CC, OX)

BACKGROUND AND AIMS: It is generally accepted that electromagnetic fields (EMF) can exert biological effects; however, the mechanisms by which EMF elicits responses are still unknown. The present study was designed to assess the immediate effects of acute EMF exposure, movement restriction, and the combination of both on the antioxidant systems and lipid content in the whole brain of rat. METHODS: Thirty two male Wistar rats were arranged in four groups: control, EMF exposed, movement restrained (MR), and EMF + MR for 2 h. Rats were then sacrificed and their brains analyzed for superoxide dismutase and catalase activities, reduced glutathione, nitric oxide, total cholesterol, and triacylglycerol levels, as well as plasma corticosterone concentrations. RESULTS: Acute exposure to EMF induces reduction in catalase and superoxide dismutase activities, whereas the combination of EMF + MR also decreases both reduced glutathione and nitric oxide levels. Our results show that the acute exposure to EMF does not induce elevation of stress-hormone corticosterone but impairs the antioxidant status in rat brain. CONCLUSIONS: Plasma corticosterone concentration and antioxidant data indicate that the acute exposure to EMF appears to be a mild stressor that leads to some adaptive responses due to the activation of systems controlling the brain oxidative balance.

(NE) Masuda H, de Gannes FP, Haro E, Billaudel B, Ruffié G, Lagroye I, Veyret B. Lack of effect of 50-Hz magnetic field exposure on the binding affinity of serotonin for the 5-HT 1B receptor subtype. Brain Res. 1368:44-51, 2011. (CS, AE, CC)

There is some concern that exposure to extremely low-frequency magnetic fields (MF) causes adverse health effects via signal transduction pathways. Two previous studies reported that exposure to 50-Hz MF decreased the binding affinity of the 1B receptor subtype of serotonin (5-HT) in rat brain membranes. The aim of this study was to investigate whether the exposure to MF affects binding to the 5-HT(1B) receptor and a physiological function associated with 5-HT(1B) receptor activation. Rat brain crude membrane fractions, including 5-HT(1B) receptor and C6-glia cells transfected with human 5-HT(1B) receptor gene, were exposed to 50-Hz MF at 1 mT using Merritt coils under temperature-regulated conditions. In the rat crude membrane, there was no significant difference in the affinity constant of [(3)H]-5-HT between exposed (K(d): 0.92 ± 0.38 nM) and sham-exposed (K(d): 1.00 ± 0.32 nM). The lack of affinity change after exposure was also confirmed using a chemical agonist of the 5-HT receptor, [(3)H]-5-carboxytryptamine (K(d): 0.59 ± 0.06 nM for exposed and 0.71 ± 0.08 nM for sham). Similar negative results in terms of affinity constant were obtained on the human 5-HT(1B) receptor in C6-glia cells. In addition, forskolin-stimulated cAMP production was inhibited by 5-HT administration in a dose-dependent manner in C6-glia cells, but exposure did not modify the inhibitory response. This study thus failed to confirm the previous results and findings suggest that exposure to MF below the current occupational limit does not affect the physiological function involved in 5-HT(1B) receptor subtypes.

(E) Partsvania B, Sulaberidze T, Modebadze Z, Shoshiashvili L. Extremely low-frequency magnetic fields effects on the snail single neurons. Electromagn Biol Med. 27(4):409-417, 2008. (CS, EE)

The aim of present work is to explore the influence of extremely low-frequency electromagnetic fields (8.34 and 217 Hz) utilized in cell phones on habituation of the mollusk single neuron to intracellular stimuli. The isolated nervous system of the mollusk *Helix Pomatia* was used in the experiments. Helmholtz coils were used to expose brain ganglia to the low-frequency electromagnetic fields. Peak values of the extremely low-frequency fields were between 1 and 6 mT. Neuron electrophysiology was investigated using a standard microelectrode technique. Exposure of the neuron to the low-frequency electromagnetic fields caused dehabituation to intracellular stimulus. The effect was proportional to the magnetic induction peak value. The observed dehabituation occurs by degradation of the signal to noise ratio and by alteration of the neuron's normal function.

(E) Perentos N, Croft RJ, McKenzie RJ, Cvetkovic D, Cosic I. The effect of GSM-like ELF radiation on the alpha band of the human resting EEG. Conf Proc IEEE Eng Med Biol Soc. 2008:5680-5683, 2008. (HU, EE)

Mobile phone handsets such as those operating in the GSM network emit extremely low frequency electromagnetic fields ranging from DC to at least 40 kHz. As a subpart of an extended protocol, the influence of these fields on the human resting EEG has been investigated in a fully counter balanced, double blind, cross-over design study that recruited 72 healthy volunteers. A decrease in the alpha frequency band was observed during the 20 minutes of ELF exposure in the exposed hemisphere only. This result suggests that ELF fields as emitted from GSM handsets during the DTX mode may have an effect on the resting alpha band of the human EEG.

(E) Piacentini R, Ripoli C, Mezzogori D, Azzena GB, Grassi C. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Ca(v)1-channel activity. J Cell Physiol. 215(1):129-139, 2008. (CS, AE, MC, MA)

We previously reported that exposure to extremely low-frequency electromagnetic fields (ELFEFs) increases the expression and function of voltage-gated Ca²⁺ channels and that Ca²⁺ influx through Ca(v)1 channels plays a key role in promoting the neuronal differentiation of neural stem/progenitor cells (NSCs). The present study was conducted to determine whether ELFEFs influence the neuronal differentiation of NSCs isolated from the brain cortices of newborn mice by modulating Ca(v)1-channel function. In cultures of differentiating NSCs exposed to ELFEFs (1 mT, 50 Hz), the percentage of cells displaying immunoreactivity for neuronal markers (beta-III-tubulin, MAP2) and for Ca(v)1.2 and Ca(v)1.3 channels was markedly increased. NSC-differentiated neurons in ELFEF-exposed cultures also exhibited significant increases in spontaneous firing, in the percentage of cells exhibiting Ca²⁺ transients in response to KCl stimulation, in the amplitude of these transients and of Ca²⁺ currents generated by the activation of Ca(v)1 channels. When the Ca(v)1-channel blocker nifedipine (5 microM) was added to the culture medium, the neuronal yield of NSC differentiation dropped significantly, and ELFEF exposure no longer produced significant increases in beta-III-tubulin- and MAP2-immunoreactivity rates. In contrast, the effects of ELFEFs were preserved when NSCs were cultured in the presence of either glutamate receptor antagonists or Ca(v)2.1- and Ca(v)2.2-channel blockers. ELFEF stimulation during the first 24 h of differentiation caused Ca(v)1-dependent increases in the number of cells displaying CREB phosphorylation. Our data suggest that ELFEF exposure promotes neuronal differentiation of NSCs by upregulating Ca(v)1-channel expression and function.

(E) Podda MV, Leone L, Barbati SA, Mastrodonato A, Li Puma DD, Piacentini R, Grassi C. Extremely low-frequency electromagnetic fields enhance the survival of newborn neurons in the mouse hippocampus. Eur J Neurosci. 2013 Dec 30. doi: 10.1111/ejn.12465. [Epub ahead of print] (AS, CS, CE, AE, BE, CC, MA)

In recent years, much effort has been devoted to identifying stimuli capable of enhancing adult neurogenesis, a process that generates new neurons throughout life, and that appears to be dysfunctional in the senescent brain and in several neuropsychiatric and neurodegenerative diseases. We previously reported that in vivo

exposure to extremely low-frequency electromagnetic fields (ELFEFs) promotes the proliferation and neuronal differentiation of hippocampal neural stem cells (NSCs) that functionally integrate in the dentate gyrus. Here, we extended our studies to specifically assess the influence of ELFEFs on hippocampal newborn cell survival, which is a very critical issue in adult neurogenesis regulation. Mice were injected with 5-bromo-2'-deoxyuridine (BrdU) to label newborn cells, and were exposed to ELFEFs 9 days later, when the most dramatic decrease in the number of newly generated neurons occurs. The results showed that ELFEF exposure (3.5 h/day for 6 days) enhanced newborn neuron survival as documented by double staining for BrdU and doublecortin, to identify immature neurons, or NeuN labeling of mature neurons. The effects of ELFEFs were associated with enhanced spatial learning and memory. In an in vitro model of hippocampal NSCs, ELFEFs exerted their pro-survival action by rescuing differentiating neurons from apoptotic cell death. Western immunoblot assay revealed reduced expression of the pro-apoptotic protein Bax, and increased levels of the anti-apoptotic protein Bcl-2, in the hippocampi of ELFEF-exposed mice as well as in ELFEF-exposed NSC cultures, as compared with their sham-exposed counterparts. Our results may have clinical implications for the treatment of impaired neurogenesis associated with brain aging and neurodegenerative diseases.

(E) Rauš S, Selaković V, Manojlović-Stojanoski M, Radenović L, Prolić Z, Janać B. Response of Hippocampal Neurons and Glial Cells to Alternating Magnetic Field in Gerbils Submitted to Global Cerebral Ischemia. Neurotox Res. 23(1):79-91, 2013. (AS, CE, MC, MA)

The purpose of this study was to determine whether exposure to an extremely low-frequency magnetic field (ELF-MF, 50 Hz) affects the outcome of postischemic damage in the hippocampus of Mongolian gerbils. After 10-min bilateral carotid occlusion, the gerbils were continuously exposed to ELF-MF (average magnetic induction at the center of the cage was 0.5 mT) for 7 days. The impact of ELF-MF was estimated immediately (the 7th day after reperfusion) and 7 days after cessation of exposure (the 14th day after reperfusion) compared with ischemic gerbils without ELF-MF exposure. Applying stereological methods, histological evaluation of changes in the hippocampus was done for determining its volume, volume densities of degenerating neurons and astrocytes, as well as the number of microglial cells per unit area. ELF-MF per se did not induce any morphological changes, while 10-min global cerebral ischemia led to neuronal death, especially in CA1 region of the hippocampus, as expected. Ischemic gerbils exposed to ELF-MF had significantly a lower degree of cell loss in the examined structure and greater responses of astrocytes and microglial cells than postischemic gerbils without exposure on the seventh day after reperfusion (immediate effect of ELF-MF). Similar response was observed on the 14th day after reperfusion (delayed effect of ELF-MF); however, differences in measured parameters were low and insignificant. Applied ELF-MF has possible neuroprotective function in the hippocampus, as the most sensitive brain structure in the model of global cerebral ischemia, through reduction of neuronal death and activation of astrocytes and microglial cells.

(E) Rauš S, Selaković V, Radenović L, Prolić Z, Janać B. Extremely low frequency magnetic field induced changes in motor behaviour of gerbils submitted to global cerebral ischemia. Behav Brain Res. 228(2):241-246, 2012. (AS, CE, BE, MA)

The purpose of this study was to evaluate behavioural effects of an extremely low frequency magnetic field (ELF-MF) in 3-month-old Mongolian gerbils submitted to global cerebral ischemia. After 10-min occlusion of both common carotid arteries, the gerbils were placed in the vicinity of an electromagnet and continuously exposed to ELF-MF (50Hz, 0.5mT) for 7 days. Their behaviour (locomotion, stereotypy, rotations, and immobility) was monitored on days 1, 2, 4, 7, and 14 after reperfusion for 60 min in the open field. It was shown that the 10-min global cerebral ischemia per se induced a significant motor activity increase (locomotion, stereotypy and rotations), and consequently immobility decrease until day 4 after reperfusion, compared to control gerbils. Exposure to ELF-MF inhibited development of ischemia-induced motor hyperactivity during the whole period of registration, but significantly in the first 2 days after reperfusion, when the postischemic hyperactivity was most evident. Motor activity of these gerbils was still significantly increased compared to control ones, but only on day 1 after reperfusion. Our results revealed that the applied ELF-MF (50Hz, 0.5mT) decreased motor hyperactivity induced by the 10-min global cerebral ischemia, via modulation of the processes that underlie this behavioural response.

(E) Rauš Balind S, Selaković V, Radenović L, Prolić Z, Janać B.Extremely Low Frequency Magnetic Field (50 Hz, 0.5 mT) Reduces Oxidative Stress in the Brain of Gerbils Submitted to Global Cerebral Ischemia. PLoS One. 2014 Feb 19;9(2):e88921. doi: 10.1371/journal.pone.0088921. eCollection 2014. (AS, CE, OX, CC, MA)

Magnetic field as ecological factor has influence on all living beings. The aim of this study was to determine if extremely low frequency magnetic field (ELF-MF, 50 Hz, 0.5 mT) affects oxidative stress in the brain of gerbils submitted to 10-min global cerebral ischemia. After occlusion of both carotid arteries, 3-month-old gerbils were continuously exposed to ELF-MF for 7 days. Nitric oxide and superoxide anion production, superoxide dismutase activity and index of lipid peroxidation were examined in the forebrain cortex, striatum and hippocampus on the 7(th) (immediate effect of ELF-MF) and 14(th) day after reperfusion (delayed effect of ELF-MF). Ischemia per se increased oxidative stress in the brain on the 7(th) and 14(th) day after reperfusion. ELF-MF also increased oxidative stress, but to a greater extent than ischemia, only immediately after cessation of exposure. Ischemic gerbils exposed to ELF-MF had increased oxidative stress parameters on the 7(th) day after reperfusion, but to a lesser extent than ischemic or ELF-MF-exposed animals. On the 14(th) day after reperfusion, oxidative stress parameters in the brain of these gerbils were mostly at the control levels. Applied ELF-MF decreases oxidative stress induced by global cerebral ischemia and thereby reduces possible negative consequences which free radical species could have in the brain. The results presented here indicate a beneficial effect of ELF-MF (50 Hz, 0.5 mT) in the model of global cerebral ischemia.

(E) Ravera S, Bianco B, Cugnoli C, Panfoli I, Calzia D, Morelli A, Pepe IM. Sinusoidal ELF magnetic fields affect acetylcholinesterase activity in cerebellum synaptosomal membranes. Bioelectromagnetics. 31(4):270-276, 2010. (CS, AE, CE)

The effects of extremely low frequency magnetic fields (ELF-MF) on acetylcholinesterase (AChE) activity of synaptosomal membranes were investigated. Sinusoidal fields with 50 Hz frequency and different amplitudes caused AChE activity to decrease about 27% with a threshold of about 0.74 mT. The decrease in enzymatic activity was independent of the time of permanence in the field and was completely reversible. Identical results were obtained with exposure to static MF of the same amplitudes. Moreover, the inhibitory effects on enzymatic activity are spread over frequency windows with different maximal values at 60, 200, 350, and 475 Hz. When synaptosomal membranes were solubilized with Triton, ELF-MF did not affect AChE activity, suggesting the crucial role of the membrane, as well as the lipid linkage of the enzyme, in determining the conditions for inactivation. The results are discussed in order to give an interpretation at molecular level of the macroscopic effects produced by ELF-MF on biological systems, in particular the alterations of embryo development in many organisms due to acetylcholine accumulation.

**** (E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012;2012:716023. (AS, CE, OX, DE)**

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic, cytotoxic hazards in brain and bone marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50 Hz, 0.5 mT for 30 days. The control group was treated as the exposed one with the sole difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-113, 2010. (AS, AE, CC)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ross ML, Koren SA, Persinger MA. Physiologically patterned weak magnetic fields applied over left frontal lobe increase acceptance of false statements as true. Electromagn Biol Med. 27(4):365-371, 2008. (HU, AE, BE)

Fifty men and women were exposed to only one of four experimentally generated magnetic fields over the left prefrontal region (above the eyebrow) or to a sham field immediately after the words "true" or "false" were presented following statements of definitions of words for a "foreign language". Three of the patterns (25 Hz, 50 Hz, or burst-firing) with intensities between 1 and 10 microT were presented for 1 s during the refutation process (immediately after the offset of "true" or "false") for specific statements from a total of 28 statements. The fourth pattern was a variable approximately 7-10 Hz (10 nT) field generated from the circuitry that was present continuously during the entire experiment. When the statements were presented again, the groups who had received the burst-firing ("limbic") or 25 Hz pulsed magnetic fields during the refutation process accepted about twice the number of false statements as true compared to those exposed to the 50 Hz field or sham-field conditions. The treatments did not significantly affect the numbers of true statements accepted as false. These results suggest that the appropriately pulsed magnetic field during the refutation process of what one has been told or has heard can increase the probability a person will accept a false statement as true.

(E) Salunke BP, Umathe SN, Chavan JG. Involvement of NMDA receptor in low-frequency magnetic field-induced anxiety in mice. Electromagn Biol Med. 2013 Oct 16. [Epub ahead of print] (AS, CE, CC, BE)

It had been reported that exposure to extremely low-frequency magnetic field (ELFMF) induces anxiety in human and rodents. Anxiety mediates via the activation of N-methyl-d-aspartate (NMDA) receptor, whereas activation of γ -aminobutyric acid (GABA)

receptor attenuates the same. Hence, the present study was carried out to understand the contribution of NMDA and/or GABA receptors modulation in ELFMF-induced anxiety for which Swiss albino mice were exposed to ELFMF (50 Hz, 10 G) by subjecting them to Helmholtz coils. The exposure was for 8 h/day for 7, 30, 60, 90 and 120 days. Anxiety level was assessed in elevated plus maze, open field test and social interaction test, on 7th, 30th, 60th, 90th and 120th exposure day, respectively. Moreover, the role of GABA and glutamate in ELFMF-induced anxiety was assessed by treating mice with muscimol [0.25 mg/kg intraperitoneally (i.p.)], bicuculline (1.0 mg/kg i.p.), NMDA (15 mg/kg i.p.) and MK-801 (0.03 mg/kg i.p.), as a GABA_A and NMDA receptor agonist and antagonist, respectively. Glutamate receptor agonist exacerbated while inhibitor attenuated the ELFMF-induced anxiety. In addition, levels of GABA and glutamate were determined in regions of the brain viz, cortex, striatum, hippocampus and hypothalamus. Experiments demonstrated significant elevation of GABA and glutamate levels in the hippocampus and hypothalamus. However, GABA receptor modulators did not produce significant effect on ELFMF-induced anxiety and elevated levels of GABA at tested dose. Together, these findings suggest that ELFMF significantly induced anxiety behavior, and indicated the involvement of NMDA receptor in its effect.

(E) Schmid MR, Murbach M, Lustenberger C, Maire M, Kuster N, Achermann P, Loughran SP. Sleep EEG alterations: effects of pulsed magnetic fields versus pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 2012 Jun 22. doi: 10.1111/j.1365-2869.2012.01025.x. [Epub ahead of print] (HU, AE, EE)

Studies have repeatedly shown that electroencephalographic power during sleep is enhanced in the spindle frequency range following radio frequency electromagnetic field exposures pulse-modulated with fundamental frequency components of 2, 8, 14 or 217 Hz and combinations of these. However, signals used in previous studies also had significant harmonic components above 20 Hz. The current study aimed: (i) to determine if modulation components above 20 Hz, in combination with radio frequency, are necessary to alter the electroencephalogram; and (ii) to test the demodulation hypothesis, if the same effects occur after magnetic field exposure with the same pulse sequence used in the pulse-modulated radio frequency exposure. In a randomized double-blind crossover design, 25 young healthy men were exposed at weekly intervals to three different conditions for 30 min before sleep. Cognitive tasks were also performed during exposure. The conditions were a 2-Hz pulse-modulated radio frequency field, a 2-Hz pulsed magnetic field, and sham. Radio frequency exposure increased electroencephalogram power in the spindle frequency range. Furthermore, delta and theta activity (non-rapid eye movement sleep), and alpha and delta activity (rapid eye movement sleep) were affected following both exposure conditions. No effect on sleep architecture and no clear impact of exposure on cognition was observed. These results demonstrate that both pulse-modulated radio frequency and pulsed magnetic fields affect brain physiology, and the presence of significant frequency components above 20 Hz are not fundamental for these effects to occur. Because responses were not identical for all exposures, the study does not support the

hypothesis that effects of radio frequency exposure are based on demodulation of the signal only.

(E) Selaković V, Rauš Balind S, Radenović L, Prolić Z, Janać B. Age-Dependent Effects of ELF-MF on Oxidative Stress in the Brain of Mongolian Gerbils. Cell Biochem Biophys. 66(3):513-521, 2013. (AS, CE, OX)

The aim of study was to investigate the effects of extremely low frequency magnetic field (ELF-MF; 50 Hz; 0.1, 0.25 and 0.5 mT) on oxidative stress in the brain of 3- (adult) and 10-month-old (middle-aged) gerbils. Nitric oxide (NO) level, superoxide (O(2) (-)) production, superoxide dismutase (SOD) activity, and index of lipid peroxidation (ILP) were measured in the forebrain cortex, striatum, hippocampus, and cerebellum immediately and 3 days after cessation of 7-day exposure. In all gerbils, ELF-MF significantly increased oxidative stress in all tested brain regions. This effect was correlated with the value of magnetic induction and was higher in middle-aged gerbils. Three days after cessation of exposure, the values of examined parameters were closer to control levels. In adult gerbils, the effect of ELF-MF of 0.1 mT on NO level, O(2) (-) production and SOD activity was almost fully disappeared, and ILP was at the control level regardless of the value of magnetic induction. In middle-aged gerbils, the effect of ELF-MF was still present but to a lesser degree than those observed immediately after cessation of exposure. These findings pointed out the ability of ELF-MF to induce age- and magnetic induction-dependent modification of oxidative stress in the brain.

(E) Shafiei SA, Firoozabadi SM, Rasoulzadeh Tabatabaie K, Ghabaee M. Study of the frequency parameters of EEG influenced by zone-dependent local ELF-MF exposure on the human head. Electromagn Biol Med. 31(2):112-12, 2012. (HU, AE, EE)

It has been reported that human subjects exposed to electromagnetic fields exhibit changes in human EEG signals at the frequency of stimulation. The aim of the present study was to expose different parts of the brain to extremely low-frequency magnetic fields locally and investigate EEG power spectrum alters at the frequency of stimulation. EEG relative power spectrum were evaluated at 3, 5, 10, 17, and 45 Hz frequencies at T4, T3, F3, Cz, and F4 points, respectively, when these points were exposed to magnetic fields with similar frequencies and 100 μ T intensity. The paired t-test results showed that power value of EEG did not alter significantly at the frequency of stimulation ($P < 0.05$). Further, significant changes in different EEG bands caused by locally exposing to ELF-MF in different points of brain were observed. The changes in the EEG bands were not limited necessarily to the exposure point.

(E) Shin EJ, Jeong JH, Kim HJ, Jang CG, Yamada K, Nabeshima T, Kim HC. Exposure to extremely low frequency magnetic fields enhances locomotor activity via activation of

dopamine D1-like receptors in mice. J Pharmacol Sci. 105(4):367-371, 2007. (AS, AE, CE, BE, CC)

We demonstrated that exposure to extremely low frequency magnetic fields (ELF-MF) enhanced dopamine levels in the rat striatum. To extend our understanding, we examined the role of dopaminergic receptors in ELF-MF-induced behavioral changes. Exposure to ELF-MF (2.4 mT, 1 h/day, for one or seven days) enhanced locomotor activity in a time-dependent manner. This hyperlocomotor activity paralleled an increase in c-Fos-like immunoreactivity (c-Fos-IR). Pretreatment with SCH23390, a dopaminergic D(1)-like receptor antagonist, but not with sulpiride, a dopaminergic D(2)-like receptor antagonist, inhibited ELF-MF-induced increased locomotor activity and c-Fos-IR. Thus, our results suggest that ELF-MF-induced behavioral responses are, at least in part, mediated by activation of dopamine D(1)-like receptors.

(E) Shin EJ, Nguyen XK, Nguyen TT, Pham DT, Kim HC. Exposure to extremely low frequency magnetic fields induces fos-related antigen-immunoreactivity via activation of dopaminergic D1 receptor. Exp Neurobiol. 20(3):130-6, 2011. (CE, BE, CC)

We previously demonstrated that repeated exposure to extremely low frequency magnetic fields (ELF-MF) increases locomotor activity via stimulation of dopaminergic D1 receptor (J. Pharmacol. Sci., 2007;105:367-371). Since it has been demonstrated that activator protein-1 (AP-1) transcription factors, especially 35-kDa fos-related antigen (FRA), play a key role in the neuronal and behavioral adaptation in response to various stimuli, we examined whether repeated ELF-MF exposure induces FRA-immunoreactivity (FRA-IR) in the striatum and nucleus accumbens (striatal complex) of the mice. Repeated exposure to ELF-MF (0.3 or 2.4 mT, 1 h/day, for consecutive fourteen days) significantly induced hyperlocomotor activity and FRA-IR in the striatal complex in a field intensity-dependent manner. ELF-MF-induced FRA-IR lasted for at least 1 year, while locomotor activity returned near control level 3 months after the final exposure to ELF-MF. Pretreatment with SCH23390, a dopaminergic D1 receptor antagonist, but not with sulpiride, a dopaminergic D2 receptor antagonist, significantly attenuated hyperlocomotor activity and FRA-IR induced by ELF-MF. Our results suggest that repeated exposure to ELF-MF leads to prolonged locomotor stimulation and long-term expression of FRA in the striatal complex of the mice via stimulation of dopaminergic D1 receptor.

(E) Stevens P. Affective response to 5 microT ELF magnetic field-induced physiological changes. Bioelectromagnetics. 28(2):109-114, 2007. (HU, AE, BE)

Research into effects of weak magnetic fields (MFs) at biologically relevant frequencies has produced ambiguous results. Although they do affect human physiology and behaviour, the direction of effects is inconsistent, with a range of complex and unrelated behaviours being susceptible. A possible explanation is that these effects, rather than being directly caused, are instead related to changes in affective state. A

previous study showed that MFs altered the affective content of concurrent perceptions, but it was unclear whether the emotional response was direct or indirect. Here it is shown that exposure to a 0-5 microT MF (DC-offset sinusoidal wave form) within EEG alpha-band frequencies (8-12 Hz), results in a reported change in emotional state. This relates to a decrease global field power but lacks the frontal alpha-asymmetry that would physiologically indicate a directly induced emotional state, suggesting that participant experiences are due to an interpretation of the effects of MF exposure.

(E) Strasák L, Bártoová E, Krejci J, Fojt L, Vetterl V. Effects of ELF-EMF on brain proteins in mice. Electromagn Biol Med. 28(1):96-104, 2009. (AS, AE, CC)

Effect of electromagnetic low frequency fields was studied on mice. We analyzed level of protein in brain of mouse. The levels of c-Jun and c-Fos in brains were measured using Western-blot techniques. Female and male laboratory mice were exposed for 4 days to magnetic field ($B_m = 2 \text{ mT}$, $f = 50 \text{ Hz}$). The exposure took place in cylindrical coil at laboratory temperature. After the experiment they were sacrificed and the level of protein c-Jun and c-Fos in different parts of brain were estimated. The expression of c-Fos was not affected by magnetic field on the other hand the expression of c-Jun decreased after magnetic field exposure. The results did not depend on sex of mice.

(E) Sun H, Che Y, Liu X, Zhou D, Miao Y, Ma Y. Effects of prenatal exposure to a 50-Hz magnetic field on one-trial passive avoidance learning in 1-day-old chicks. Bioelectromagnetics. 31(2):150-155, 2010. (AS, CE, BE, DE)

We investigated memory impairment in newly hatched chicks following in ovo exposure to a 50-Hz magnetic field (MF) of 2 mT (60 min/day) on embryonic days 12-18. Isolated and paired chicks were used to test the effect of stress during training, and memory retention was tested at 10, 30, and 120 min, following exposure to a bitter-tasting bead (100% methylantranilate). Results showed that memory was intact at 10 min in both isolated and paired chicks with or without MF exposure. However, while isolated chicks had good memory retention levels at 30 and 120 min, those exposed to MF did not. The results suggest a potential disruption of memory formation following in ovo exposure to MF, with this effect only evident in the more stressed, isolated chicks.

(E) Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull. 81(1):92-99, 2010. (AS, CE, BE, CC)

It is believed that different electromagnetic fields do have beneficial and harmful biological effects. The aim of the present work was to study the long-term consequences of 50 Hz electromagnetic field (ELF-EMF) exposure with special focus on the development of chronic stress and stress-induced psychopathology. Adult male Sprague-Dawley rats were exposed to ELF-EMF (50 Hz, 0.5 mT) for 5 days, 8h daily

(short) or for 4-6 weeks, 24h daily (long). Anxiety was studied in elevated plus maze test, whereas depression-like behavior of the long-treated group was examined in the forced swim test. Some days after behavioral examination, the animals were decapitated among resting conditions and organ weights, blood hormone levels as well as proopiomelanocortin mRNA level from the anterior lobe of the pituitary gland were measured. Both treatments were ineffective on somatic parameters, namely none of the changes characteristic to chronic stress (body weight reduction, thymus involution and adrenal gland hypertrophy) were present. An enhanced blood glucose level was found after prolonged ELF-EMF exposure ($p=0.013$). The hormonal stress reaction was similar in control and short-term exposed rats, but significant proopiomelanocortin elevation ($p<0.000$) and depressive-like behavior (enhanced floating time; $p=0.006$) were found following long-term ELF-EMF exposure. Taken together, long and continuous exposure to relatively high intensity electromagnetic field may count as a mild stress situation and could be a factor in the development of depressive state or metabolic disturbances. Although we should stress that the average intensity of the human exposure is normally much smaller than in the present experiment.

(E) Tasset I, Medina FJ, Jimena I, Agüera E, Gascón F, Feijóo M, Sánchez-López F, Luque E, Peña J, Drucker-Colín R, Túnez I. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. Neuroscience. 209:54-63, 2012a. (AS, CE, MC, CC, BE, OX, MA, ND)

There is evidence to suggest that the neuroprotective effect of exposure of extremely low-frequency electromagnetic fields (ELF-EMF) may be due, at least in part, to the effect of these fields on neurotrophic factors levels and cell survival, leading to an improvement in behavior. This study was undertaken to investigate the neuroprotective effects of ELFEF in a rat model of 3-nitropropionic acid (3NP)-induced Huntington's disease. Behavior patterns were evaluated, and changes in neurotrophic factor, cell damage, and oxidative stress biomarker levels were monitored in Wistar rats. Rats were given 3NP over four consecutive days (20 mg/kg body weight), whereas ELFEF (60 Hz and 0.7 mT) was applied over 21 days, starting after the last injection of 3NP. Rats treated with 3NP exhibited significantly different behavior in the open field test (OFT) and the forced swim test (FST), and displayed significant differences in neurotrophic factor levels and oxidative stress biomarkers levels, together with a neuronal damage and diminished neuronal density, with respect neuronal controls. ELFEF improved neurological scores, enhanced neurotrophic factor levels, and reduced both oxidative damage and neuronal loss in 3NP-treated rats. ELFEF alleviates 3NP-induced brain injury and prevents loss of neurons in rat striatum, thus showing considerable potential as a therapeutic tool.

(E) Tasset I, Pérez-Herrera A, Medina FJ, Arias-Carrión O, Drucker-Colín R, Túnez I.

Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. Brain Stimul. 2012b Apr 15. [Epub ahead of print] (AS, CE, CC, MA, ND)

Transcranial magnetic stimulation (TMS) is a non-invasive technique used recently to treat different neuropsychiatric and neurodegenerative disorders. Despite its proven value, the mechanisms through which TMS exerts its beneficial action on neuronal function remain unclear. Recent studies have shown that its beneficial effects may be at least partly due to a neuroprotective effect on oxidative and cell damage. This study shows that TMS can modulate the Nrf2 transcription factor in a Huntington's disease-like rat model induced by 3-nitropropionic acid (3-NP). Western blot analysis demonstrated that 3-NP caused a reduction in Nrf2 in both cytoplasm and nucleus, while TMS applied to 3-NP-treated rats triggered an increase in cytoplasm and nucleus Nrf2 levels. It was therefore concluded that TMS modulates Nrf2 expression and translocation and that these mechanisms may partly explain the neuroprotective effect of TMS, as well as its antioxidant and cell protection capacity.

(E) Todorović D, Marković T, Prolić Z, Mihajlović S, Rauš S, Nikolić L, Janać B. The influence of static magnetic field (50 mT) on development and motor behaviour of Tenebrio (Insecta, Coleoptera). Int J Radiat Biol. 2012 Aug 1. [Epub ahead of print] (AS, CE, DE, BE)

PURPOSE: There is considerable concern about potential effects associated with exposure to magnetic fields on organisms. Therefore, duration of pupa-adult development and motor behaviour of adults were analyzed in *Tenebrio obscurus* and *Tenebrio molitor* after exposure to static magnetic field (50 mT). MATERIAL AND METHODS: The experimental groups were: control (kept 5 m from the magnets), groups which pupae and adults were placed closer to the North pole, or closer to the South pole of magnetic dipole. The pupae were exposed to the magnetic field until the moment of adult eclosion. The pupa-adult development dynamics were recorded daily. Subsequently, behaviour (distance travelled, average speed and immobility) of adults exposed to the magnetic field was monitored in a circular open field arena. RESULTS: Static magnetic field did not affect pupa-adult developmental dynamic of examined *Tenebrio* species. Exposure to magnetic field did not significantly change motor behaviour of *T. obscurus* adults. The changes in the motor behaviour of *T. molitor* induced by static magnetic field were opposite in two experimental groups developed closer to the North pole or closer to the South pole of magnetic dipole. CONCLUSION: Static magnetic field (50 mT) did not affect on pupa-adult development dynamic of two examined *Tenebrio* species, but modulated their motor behaviour.

(NE) Türközer Z, Güler G, Seyhan N. Effects of exposure to 50 Hz electric field at different strengths on oxidative stress and antioxidant enzyme activities in the brain tissue of guinea pigs. Int J Radiat Biol. 84(7):581-590, 2008. (AS, CE, OX)

PURPOSE: The aim of this study was to evaluate the possible effects of varied exposure to 50 Hz extremely low frequency (ELF) electric field (EF) on the lipid peroxidation levels and antioxidant enzyme activities in the brain homogenates of guinea pigs. Subjects were exposed to 2 kV/m, 2.5 kV/m, 3 kV/m, 3.5 kV/m, 4 kV/m, 4.5 kV/m and 5 kV/m electric fields for three days, 8 h a day in both vertical and horizontal directions.

MATERIALS AND METHODS: Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were measured in order to identify possible alterations in lipid peroxidation levels and antioxidant status due to electric field exposure. Xanthine oxidase (XO), myeloperoxidase (MPO) and adenosine deaminase (ADA) activities were also evaluated in the same samples. **RESULTS:** Although the study showed several positive but non-significant findings ($p > 0.05$), we did not find significant differences among all of the exposed groups and sham groups in lipid peroxidation levels and enzyme activities ($p > 0.05$) at all strengths and in both directions. Furthermore, the result was the same when the comparison was made between the groups in vertical directions and horizontal directions ($p > 0.05$).

CONCLUSION: The present study observed effects of 50 Hz EF exposure on lipid peroxidation levels and antioxidant defense mechanisms but these were not statistically significant at the 95% confidence level. Further research on the effects ELF-EF exposure on lipid peroxidation levels and antioxidant defence mechanisms are warranted.

(E) van Nierop LE, Slottje P, van Zandvoort MJE, de Vocht F, Kromkout H. Effects of magnetic stray fields from a 7 Tesla MRI scanner on neurocognition: a double-blind randomised crossover study. Occup Environ Med doi:10.1136/oemed-2011-100468 (HU, BE)

Objective: This study characterises neurocognitive domains that are affected by movement-induced time-varying magnetic fields (TVMF) within a static magnetic stray field (SMF) of a 7 Tesla (T) MRI scanner. **Methods:** Using a double-blind randomised crossover design, 31 healthy volunteers were tested in a sham (0 T), low (0.5 T) and high (1.0 T) SMF exposure condition. Standardised head movements were made before every neurocognitive task to induce TVMF. **Results:** Of the six tested neurocognitive domains, we demonstrated that attention and concentration were negatively affected when exposed to TVMF within an SMF (varying from 5.0% to 21.1% per Tesla exposure, $p < 0.05$), particular in situations where high working memory performance was required. In addition, visuospatial orientation was affected after exposure (46.7% per Tesla exposure, $p = 0.05$). **Conclusion:** Neurocognitive functioning is modulated when exposed to movement-induced TVMF within an SMF of a 7 T MRI scanner. Domains that were affected include attention/concentration and visuospatial orientation. Further studies are needed to better understand the mechanisms and possible practical safety and health implications of these acute neurocognitive effects.

(E) Varró P, Szemerszky R, Bárdos G, Világi I. Changes in synaptic efficacy and seizure susceptibility in rat brain slices following extremely low-frequency electromagnetic field exposure. Bioelectromagnetics. 30(8):631-640, 2009. (AS, CS, FC)

The effects of electromagnetic fields (EMFs) on living organisms are recently a focus of scientific interest, as they may influence everyday life in several ways. Although the neural effects of EMFs have been subject to a considerable number of investigations, the results are difficult to compare since dissimilar exposure protocols have been applied on different preparations or animals. In the present series of experiments, whole rats or excised rat brain slices were exposed to a reference level-intensity (250-500 microT, 50 Hz) EMF in order to examine the effects on the synaptic efficacy in the central nervous system. Electrophysiological investigation was carried out *ex vivo*, on neocortical and hippocampal slices; basic synaptic functions, short- and long-term plasticity and seizure susceptibility were tested. The most pronounced effect was a decrease in basic synaptic activity in slices treated directly *ex vivo* observed as a diminution in amplitude of evoked potentials. On the other hand, following whole-body exposure an enhanced short- and long-term synaptic facilitation in hippocampal slices and increased seizure susceptibility in neocortical slices was also observed. However, these effects seem to be transient. We can conclude that ELF-EMF exposure exerts significant effects on synaptic activity, but the overall changes may strongly depend on the synaptic structure and neuronal network of the affected region together with the specific spatial parameters and constancy of EMF.

(E) Volkow ND, Tomasi D, Wang GJ, Fowler JS, Telang F, Wang R, Alexoff D, Logan J, Wong C, Pradhan K, Caparelli EC, Ma Y, Jayne M. Effects of low-field magnetic stimulation on brain glucose metabolism. Neuroimage. 51(2):623-628, 2010. (HU, AE, FC)

Echo planar imaging (EPI), the gold standard technique for functional MRI (fMRI), is based on fast magnetic field gradient switching. These time-varying magnetic fields induce electric (E) fields in the brain that could influence neuronal activity; but this has not been tested. Here we assessed the effects of EPI on brain glucose metabolism (marker of brain function) using PET and 18F 2-fluoro-2-deoxy-D-glucose ((18)FDG). Fifteen healthy subjects were in a 4 T magnet during the (18)FDG uptake period twice: with (ON) and without (OFF) EPI gradient pulses along the z-axis ($G(z)$: 23 mT/m; 250 μ s rise-time; 920 Hz). The E-field from these EPI pulses is non-homogeneous, increasing linearly from the gradient's isocenter (radial and z directions), which allowed us to assess the correlation between local strength of the E-field and the regional metabolic differences between ON and OFF sessions. Metabolic images were normalized to metabolic activity in the plane positioned at the gradient's isocenter where $E=0$ for both ON and OFF conditions. Statistical parametric analyses used to identify regions that differed between ON versus OFF ($p<0.05$, corrected) showed that the relative metabolism was lower in areas at the poles of the brain (inferior occipital and frontal and superior parietal cortices) for ON than for OFF, which was also documented with individual region of interest analysis. Moreover the magnitude of the metabolic decrements was significantly correlated with the estimated strength of E ($r=0.68$, $p<0.0001$); the stronger the E-field the larger the decreases. However, we did not detect differences between ON versus OFF conditions on mood ratings nor on

absolute whole brain metabolism. This data provides preliminary evidence that EPI sequences may affect neuronal activity and merits further investigation.

(E) Wang X, Liu Y, Lei Y, Zhou D, Fu Y, Che Y, Xu R, Yu H, Hu X, Ma Y. Extremely low-frequency electromagnetic field exposure during chronic morphine treatment strengthens downregulation of dopamine D2 receptors in rat dorsal hippocampus after morphine withdrawal. *Neurosci Lett.* 433(3):178-82, 2008. (AS, CE, CC)

The aim of this study was to investigate the effect of extremely low-frequency electromagnetic field (ELF-EMF) exposure during morphine treatment on dopamine D2 receptor (D2R) density in the rat dorsal hippocampus following withdrawal. Rats were exposed to ELF-EMF (20 Hz, 14 mT) or sham exposed for 1h per day before injection of morphine (10mg/kg, i.p.) once daily for 12 days. The saline control group was sham exposed for the same period. Immunohistochemistry was used to detect the density of D2Rs on the 1st, 3rd and 5th morphine withdrawal days. The results showed that the density of D2Rs in sham-exposed morphine-treated rats on the 1st and 3rd days of morphine withdrawal was significantly lower than that of the saline control group. The ELF-EMF-exposed morphine group also exhibited a significantly lower density of D2Rs on the 1st and 3rd withdrawal days relative to the sham-exposed morphine group. However, the D2R density in both groups tended to recover as morphine withdrawal days increased. The results suggest that dorsal hippocampal D2Rs are sensitive to morphine withdrawal and that this is potentiated by ELF-EMF pre-exposure during morphine treatment.

(E) Wang X, Zhao K, Wang D, Adams W, Fu Y, Sun H, Liu X, Yu H, Ma Y. Effects of exposure to a 50 Hz sinusoidal magnetic field during the early adolescent period on spatial memory in mice. *Bioelectromagnetics.* 34(4):275-284, 2013. (AS, CE, BE)

Adolescence is a critical developmental stage during which substantial remodeling occurs in brain areas involved in emotional and learning processes. Although a robust literature on the biological effects of extremely low frequency magnetic fields (ELF-MFs) has been documented, data on the effects of ELF-MF exposure during this period on cognitive functions remain scarce. In this study, early adolescent male mice were exposed from postnatal day (P) 23-35 to a 50 Hz MF at 2 mT for 60 min/day. On P36-45, the potential effects of the MF exposure on spatial memory performance were examined using the Y-maze and Morris water maze tasks. The results showed that the MF exposure did not affect Y-maze performance but improved spatial learning acquisition and memory retention in the water maze task under the present experimental conditions.

(E) Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. *PLoS One.* 5(11):e13883, 2010. (AE, CS, CC)

BACKGROUND: This study was inspired by coalescing evidence that magnetic therapy may be a viable treatment option for certain diseases. This premise is based on the ability of moderate strength fields (i.e., 0.1 to 1 Tesla) to alter the biophysical properties of lipid bilayers and in turn modulate cellular signaling pathways. In particular, previous results from our laboratory (Wang et al., BMC Genomics, 10, 356 (2009)) established that moderate strength static magnetic field (SMF) exposure altered cellular endpoints associated with neuronal function and differentiation. Building on this background, the current paper investigated SMF by focusing on the adenosine A(2A) receptor (A(2A)R) in the PC12 rat adrenal pheochromocytoma cell line that displays metabolic features of Parkinson's disease (PD). **METHODOLOGY AND PRINCIPAL FINDINGS:** SMF reproduced several responses elicited by ZM241385, a selective A(2A)R antagonist, in PC12 cells including altered calcium flux, increased ATP levels, reduced cAMP levels, reduced nitric oxide production, reduced p44/42 MAPK phosphorylation, inhibited proliferation, and reduced iron uptake. SMF also counteracted several PD-relevant endpoints exacerbated by A(2A)R agonist CGS21680 in a manner similar to ZM241385; these include reduction of increased expression of A(2A)R, reversal of altered calcium efflux, dampening of increased adenosine production, reduction of enhanced proliferation and associated p44/42 MAPK phosphorylation, and inhibition of neurite outgrowth. **CONCLUSIONS AND SIGNIFICANCE:** When measured against multiple endpoints, SMF elicited qualitatively similar responses as ZM241385, a PD drug candidate. Provided that the in vitro results presented in this paper apply in vivo, SMF holds promise as an intriguing non-invasive approach to treat PD and potentially other neurological disorders.

(E) Xiong J, He C, Li C, Tan G, Li J, Yu Z, Hu Z, Chen F. Changes of dendritic spine density and morphology in the superficial layers of the medial entorhinal cortex induced by extremely low-frequency magnetic field exposure. PLoS One. 2013 Dec 20; 8(12):e83561. doi: 10.1371/journal.pone.0083561. eCollection 2013. (AS, CE, MC)

In the present study, we investigated the effects of chronic exposure (14 and 28 days) to a 0.5 mT 50 Hz extremely low-frequency magnetic field (ELM) on the dendritic spine density and shape in the superficial layers of the medial entorhinal cortex (MEC). We performed Golgi staining to reveal the dendritic spines of the principal neurons in rats. The results showed that ELM exposure induced a decrease in the spine density in the dendrites of stellate neurons and the basal dendrites of pyramidal neurons at both 14 days and 28 days, which was largely due to the loss of the thin and branched spines. The alteration in the density of mushroom and stubby spines post ELM exposure was cell-type specific. For the stellate neurons, ELM exposure slightly increased the density of stubby spines at 28 days, while it did not affect the density of mushroom spines at the same time. In the basal dendrites of pyramidal neurons, we observed a significant decrease in the mushroom spine density only at the later time point post ELM exposure, while the stubby spine density was reduced at 14 days and partially restored at 28 days post ELM exposure. ELM exposure-induced reduction in the spine density in the apical dendrites of pyramidal neurons was only observed at 28 days, reflecting the distinct vulnerability of spines in the apical and basal dendrites. Considering the changes in

spine number and shape are involved in synaptic plasticity and the MEC is a part of neural network that is closely related to learning and memory, these findings may be helpful for explaining the ELM exposure-induced impairment in cognitive functions.

(E) Yi G, Wang J, Wei X, Deng B, Tsang KM, Chan WL, Han C. Effects of extremely low-frequency magnetic fields on the response of a conductance-based neuron model. Int J Neural Syst. 2014 Feb; 24(1):1450007. doi: 10.1142/S0129065714500075. Epub 2013 Dec 11. (CS, AE, EE)

To provide insights into the modulation of neuronal activity by extremely low-frequency (ELF) magnetic field (MF), we present a conductance-based neuron model and introduce ELF sinusoidal MF as an additive voltage input. By analyzing spike times and spiking frequency, it is observed that neuron with distinct spiking patterns exhibits different response properties in the presence of MF exposure. For tonic spiking neuron, the perturbations of MF exposure on spike times is maximized at the harmonics of neuronal intrinsic spiking frequency, while it is maximized at the harmonics of bursting frequency for burst spiking neuron. As MF intensity increases, the perturbations also increase. Compared with tonic spiking, bursting dynamics are less sensitive to the perturbations of ELF MF exposure. Further, ELF MF exposure is more prone to perturb neuronal spike times relative to spiking frequency. Our finding suggests that the resonance may be one of the neural mechanisms underlying the modulatory effects of the low-intensity ELF MFs on neuronal activities. The results highlight the impacts of ELF MFs exposure on neuronal activity from the single cell level, and demonstrate various factors including ELF MF properties and neuronal spiking characteristics could determine the outcome of exposure. These insights into the mechanism of MF exposure may be relevant for the design of multi-intensity magnetic stimulus protocols, and may even contribute to the interpretation of MF effects on the central nervous systems.

(NE) Zhang C, Li Y, Wang C, Lv R, Song T. Extremely low-frequency magnetic exposure appears to have no effect on pathogenesis of Alzheimer's disease in aluminum-overloaded rat. PLoS One. 2013 Aug 12;8(8):e71087. doi: 10.1371/journal.pone.0071087. eCollection 2013. (AS, CE, BE, MC, ND)

OBJECTIVE: Extremely low-frequency magnetic field (ELF-MF) has been reported to be of potential pathogenetic relevance to Alzheimer's disease (AD) for years. However, evidence confirming this function remains inconclusive. Chronic Al treatment has been identified as a contributing factor to cognitive function impairment in AD. This study aims to examine whether or not ELF-MF and Al have synergistic effects toward AD pathogenesis by investigating the effects of ELF-MF with or without chronic Al treatment on SD rats. **METHODS:** Sprague-Dawley (SD) rats were subjected one of the following treatments: sham (control group), oral Al (Al group), ELF-MF (100 μ T at 50 Hz) with oral Al (MF+Al group), or ELF-MF (100 μ T at 50 Hz) without oral Al (MF group).

RESULTS: After 12 wk of treatment, oral A β treatment groups (A β and MF+A β groups) showed learning and memory impairment as well as morphological hallmarks, including neuronal cell loss and high density of amyloid- β (A β) in the hippocampus and cerebral cortex. ELF-MF without A β treatment showed no significant effect on AD pathogenesis. ELF-MF+A β treatment induced no more damage than A β treatment did. CONCLUSIONS: Our results showed no evidence of any association between ELF-MF exposure (100 μ T at 50 Hz) and AD, and ELF-MF exposure does not influence the pathogenesis of AD induced by A β overload.

March, 2014

**Exhibit E: An Update on the Genetic Effects of Nonionizing
Electromagnetic Fields by Prof. Henry Lai, PhD,
University of Washington, Emeritus**

Introduction:

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect. With E representing a biological effect, and NE representing no biological effects, the recent literature finds RFR-genetic effects at: E=74 publications (65%); NE=40 publications (35%); and ELF-genetic effects at: E=49 (83%); NE=10 (17%).

Discussion:

1. The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman (Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. *Int. J. Radiat. Biol.* 87(4):409-415, 2011) in which they stated that ‘...the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.’ However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. *Bioelectromagnetics* 13:237-246, 1992; Lai, H. and Carino, M.A. Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. *Bioelectromagnetics* 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz

magnetic field affects rats' performance in the water maze. Bioelectromagnetics 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. Bioelectromagnetics 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency.

Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al., 2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonen et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, there are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF- Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

2. An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplatin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonen et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Jouni et al., 2012; Yoon et al., 2014); RFR- chemical mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

3. Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroui et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010; Panagopoulous et al., 2013; Udroui et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi.* 23(6):414-417, 2005; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. *Bioelectromagnetics.* 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. *Bioelectromagnetics.* 32(1):15-27, 2011.
4. Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (μ T) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. *Environ. Rev.* 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level.

- Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) (de Bruyn L and de Jager L. Effect of long-term exposure to a randomly varied 50 Hz power frequency magnetic field on the fertility of the mouse. *Electromag. Biol. Med.* 29(1-2):52-61, 2010).
5. Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found that neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et al. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in *E. coli* was *decreased* after exposure to a sinusoidal magnetic field and *increased* after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-

continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complicity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

6. Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease (Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. *J Alzheimers Dis.* 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. *PLoS One.* 5(11):e13883, 2010); bone regeneration (Lee HM, Kwon UH, Kim H, Kim HJ, Kim B, Park JO, Moon ES, Moon SH. Pulsed electromagnetic field stimulates cellular proliferation in human intervertebral disc cells. *Yonsei Med. J.* 51(6):954-959, 2010); cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. *Br. J. Cancer.* 105(5):640-648, 2011), and tissue regeneration (Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A, Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. *Cardiovasc. Res.* 82(3):411-420, 2009).

7. It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

References and abstracts

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2006 and serve as my comments to help the reader quickly identify the significance of each work. The summary sentences by each author are underlined. The list is divided into RF effects papers, and ELF effects papers.

(E- effect observed; NE- no effect observed) (LE- long term exposure; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; HU- human study; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IA- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; EH- compared with electro-hypersensitive subjects; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect).

An update on the research on genetic effects of radiofrequency/cell phone radiation

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril 92 1318-1325, 2009. (GT, RP, OX)

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may

lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices. J Pediatr Urol. 2012 Mar 30. [Epub ahead of print] (GT, OX, LE, RP)

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. **METHODS:** Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. **RESULTS:** We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$). **CONCLUSIONS:** These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) Atlı Şekeroğlu Z, Akar A, Sekeroğlu V. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900 MHz radio frequency electromagnetic fields. Int J Radiat Biol. 89(11):985-992, 2013. [Epub ahead of print] (GT, DE, LE)

Abstract Purpose: One of the most important issues regarding radio frequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radio frequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. **Materials and methods:** The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1 ± 4.8 V/m and 20.0 ± 3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. **Results:** Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats.

The recovery period did not reduce the damage to the same extent as the corresponding control groups. Conclusions: The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

(E) Avendaño C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. FertilSteril 97:39-45, 2012. (GT, RP)

OBJECTIVE: To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa. DESIGN: Prospective in vitro study. SETTING: Center for reproductive medicine. PATIENT(S): Semen samples from 29 healthy donors. INTERVENTION(S): Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop. MAIN OUTCOME MEASURE(S): Evaluation of sperm motility, viability, and DNA fragmentation. RESULT(S): Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups. CONCLUSION(S): To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

(E) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. Mutat Res 578:149-57, 2005. (GT, IA)

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at

0 and 21h incubation after exposure ($P>0.05$). There were significant difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment ($P<0.01$). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed ($P<0.05$ or $P<0.01$). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups ($P>0.05$). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H. Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. Toxicology. 232(3):311-316, 2007. (GT, IA)

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m⁻², respectively. The lymphocytes were irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant ($P>0.05$). The MTLs induced by UVC were 1.71+/-0.09, 2.02+/-0.08, 2.27+/-0.17, 2.27+/-0.06, 2.25+/-0.12, 2.24+/-0.11 microm, respectively, which were significantly higher than that (0.96+/-0.05 microm) of control ($P<0.01$). MTLs of some sub-groups in combinative exposure groups at 1.5-h incubation were significantly lower than those of corresponding UVC sub-groups ($P<0.01$ or $P<0.05$). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher than those of corresponding UVC sub-groups ($P<0.01$ or $P<0.05$). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power

frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 27:295-306, 2006. (GE)

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter

regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

(E) Belyaev IY, Markovà E, Hillert L, Malmgren LO, Persson BR. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/gamma-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-41, 2009. (GT, EH)

We have recently described frequency-dependent effects of mobile phone microwaves (MWs) of global system for mobile communication (GSM) on human lymphocytes from persons reporting hypersensitivity to electromagnetic fields and healthy persons. Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signal because of eventual "effective" frequencies within the wideband. Here, we report for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/gamma-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency. Remarkably, the effects of MWs on 53BP1/gamma-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM radiation emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM-915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($P < 0.02[53BP1]/0.01[\text{gamma-H2AX}]$) but not for control subjects ($P > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are needed to study the nature of these differences.

(NE) Bourthoumieu S, Joubert V, Marin B, Collin A, Leveque P, Terro F, Yardin C. Cytogenetic studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using R-banded karyotyping. Radiat Res 174:712-718, 2010. (GT)

It is important to determine the possible effects of exposure to radiofrequency (RF) radiation on the genetic material of cells since damage to the DNA of somatic cells may be linked to cancer development or cell death and damage to germ cells may lead to genetic damage in next and subsequent generations. The objective of this study was to investigate whether exposure to radiofrequency radiation similar to that emitted by mobile phones of second-generation standard Global System for Mobile Communication (GSM) induces genotoxic effects in cultured human cells. The cytogenetic effects of GSM-900 MHz (GSM-900) RF radiation were investigated using R-banded karyotyping after in vitro exposure of human cells (amniotic cells) for 24 h. The average specific absorption rate (SAR) was 0.25 W/kg. The exposures were carried out in wire-patch cells (WPCs) under strictly controlled conditions of temperature. The genotoxic effect was

assessed immediately or 24 h after exposure using four different samples. One hundred metaphase cells were analyzed per assay. Positive controls were provided by using bleomycin. We found no direct cytogenetic effects of GSM-900 either 0 h or 24 h after exposure. To the best of our knowledge, our work is the first to study genotoxicity using complete R-banded karyotyping, which allows visualizing all the chromosomal rearrangements, either numerical or structural.

(NE) Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH. Int J Radiat Biol 87:400-408, 2011. (GT)

PURPOSE: Since previous research found an increase in the rate of aneuploidies in human lymphocytes exposed to radiofrequencies, it seems important to perform further studies. The objective of this study was then to investigate whether the exposure to RF (radiofrequency) radiation similar to that emitted by mobile phones of a second generation standard, i.e., Global System for Mobile communication (GSM) may induce aneuploidy in cultured human cells. **MATERIALS AND METHODS:** The potential induction of genomic instability by GSM-900 MHz radiofrequency (GSM-900) was investigated after in vitro exposure of human amniotic cells for 24 h to average-specific absorption rates (SAR) of 0.25, 1, 2 and 4 W/kg in the temperature range of 36.3-39.7°C. The exposures were carried out in a wire-patch cell (WPC). The rate of aneuploidy of chromosomes 11 and 17 was determined by interphase FISH (Fluorescence In Situ Hybridisation) immediately after independent exposure of three different donors for 24 h. At least 100 interphase cells were analysed per assay. **RESULTS:** No significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h at average SAR up to 4 W/kg. **CONCLUSION:** Our study did not show any in vitro aneuploidogenic effect of GSM using FISH and is not in agreement with the results of previous research.

(NE) Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C. Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells. Bioelectromagnetics. 2012 Jul 5. doi: 10.1002/bem.21744. [Epub ahead of print] (GE)

The potential effects of radiofrequency (RF) exposure on the genetic material of cells are very important to determine since genome instability of somatic cells may be linked to cancer development. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through apoptosis. The objective of this study was to investigate whether the exposure to RF electromagnetic fields, similar to those emitted by mobile phones of the second generation standard, Global System for Mobile Communications (GSM), may induce expression of the p53 protein and its activation by post-translational modifications in cultured human cells. The potential induction of p53 expression and activation by GSM-900 was investigated after in vitro exposure of human amniotic cells for 24 h to average specific absorption rates (SARs) of 0.25, 1, 2, and 4 W/kg in the

temperature range of 36.3-39.7 °C. The exposures were carried out using a wire-patch cell (WPC) under strictly controlled conditions of temperature. Expression and activation of p53 by phosphorylation at serine 15 and 37 were studied using Western blot assay immediately after three independent exposures of cell cultures provided from three different donors. Bleomycin-exposed cells were used as a positive control. According to our results, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells.

(E) Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (GT, LE, DE, OX)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 $\mu\text{W}/\text{cm}^2$) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O_2^-), nitrogen oxide ($\text{NO}\cdot$), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. J Cell Physiol. 213(3):759-767, 2007. (GE)

Many environmental signals, including ionizing radiation and UV rays, induce activation of Egr-1 gene, thus affecting cell growth and apoptosis. The paucity and the controversial knowledge about the effect of electromagnetic fields (EMF) exposure of

nerve cells prompted us to investigate the bioeffects of radiofrequency (RF) radiation on SH-SY5Y neuroblastoma cells. The effect of a modulated RF field of 900 MHz, generated by a wire patch cell (WPC) antenna exposure system on Egr-1 gene expression, was studied as a function of time. Short-term exposures induced a transient increase in Egr-1 mRNA level paralleled with activation of the MAPK subtypes ERK1/2 and SAPK/JNK. The effects of RF radiations on cell growth rate and apoptosis were also studied. Exposure to RF radiation had an anti-proliferative activity in SH-SY5Y cells with a significant effect observed at 24 h. RF radiation impaired cell cycle progression, reaching a significant G2-M arrest. In addition, the appearance of the sub-G1 peak, a hallmark of apoptosis, was highlighted after a 24-h exposure, together with a significant decrease in mRNA levels of Bcl-2 and survivin genes, both interfering with signaling between G2-M arrest and apoptosis. Our results provide evidence that exposure to a 900 MHz-modulated RF radiation affect both Egr-1 gene expression and cell regulatory functions, involving apoptosis inhibitors like Bcl-2 and survivin, thus providing important insights into a potentially broad mechanism for controlling in vitro cell viability.

(E) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012 (GT, HU)

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased ($p < .05$) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 473:52-55. 2010. (GT, OX, WS)

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA

fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

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 Cervellati F, Valacchi G, Lunghi L, Fabbri E, Valbonesi P, Marci R, Biondi C, Vesce F. **17- β -estradiol counteracts the effects of high frequency electromagnetic fields on trophoblastic connexins and integrins. *Oxid Med Cell Longev.* 2013;2013:280850. doi: 10.1155/2013/280850. (GE)**

We investigated the effect of high-frequency electromagnetic fields (HF-EMFs) and 17- β -estradiol on connexins (Cxs), integrins (Ints), and estrogen receptor (ER) expression, as well as on ultrastructure of trophoblast-derived HTR-8/SVneo cells. HF-EMF, 17- β -estradiol, and their combination induced an increase of Cx40 and Cx43 mRNA expression. HF-EMF decreased Int alpha1 and β 1 mRNA levels but enhanced Int alpha5 mRNA expression. All the Ints mRNA expressions were increased by 17- β -estradiol and exposure to both stimuli. ER- β mRNA was reduced by HF-EMF but augmented by 17- β -estradiol alone or with HF-EMF. ER- β immunofluorescence showed a cytoplasmic localization in sham and HF-EMF exposed cells which became nuclear after treatment with hormone or both stimuli. Electron microscopy evidenced a loss of cellular contact in exposed cells which appeared counteracted by 17- β -estradiol. We demonstrate that 17- β -estradiol modulates Cxs and Ints as well as ER- β expression induced by HF-EMF, suggesting an influence of both stimuli on trophoblast differentiation and migration.

(NE) Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. **Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. *Eur J Cancer Prev* 14:175-179, 2005. (GT, IA)**
(Some interaction effects with chemicals are reported in this paper.)

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-

nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in Escherichia coli WP2 and TA102, respectively, while the contrary results in Salmonella typhimurium TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF did not change the rate of degradation observed using plasmid pBluescriptSK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

(NE) Chauhan V, Mariampillai A, Bellier PV, Qutob SS, Gajda GB, Lemay E, Thansandote A, McNamee JP. Gene expression analysis of a human lymphoblastoma cell line exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Radiat Res. 165(4):424-429, 2006. (GE)

This study was designed to determine whether radiofrequency (RF) fields of the type used for wireless communications could elicit a cellular stress response. As general indicators of a cellular stress response, we monitored changes in proto-oncogene and heat-shock protein expression. Exponentially growing human lymphoblastoma cells (TK6) were exposed to 1.9 GHz pulse-modulated RF fields at average specific absorption rates (SARs) of 1 and 10 W/kg. Perturbations in the expression levels of the proto-oncogenes FOS, JUN and MYC after exposure to sham and RF fields were assessed by real-time RT-PCR. In addition, the transcript levels of the cellular stress proteins HSP27 and inducible HSP70 were also monitored. We demonstrated that transcript levels of these genes in RF-field-exposed cells showed no significant difference in relation to the sham treatment group. However, concurrent positive (heat-shock) control samples displayed a significant elevation in the expression of HSP27, HSP70, FOS and JUN. Conversely, the levels of MYC mRNA were found to decline in the positive (heat-shock) control. In conclusion, our study found no evidence that the 1.9 GHz RF-field exposure caused a general stress response in TK6 cells under our experimental conditions.

(NE) Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int J Radiat Biol. 82(5):347-354, 2006. (GE)

Purpose: Several studies have reported that radiofrequency (RF) fields, as emitted by mobile phones, may cause changes in gene expression in cultured human cell-lines. The current study was undertaken to evaluate this possibility in two human-derived immune cell-lines. Materials and methods: HL-60 and Mono-Mac-6 (MM6) cells were individually exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields at a average specific absorption rate (SAR) of 1 and 10 W/kg at 37 +/- 0.5 degrees C for 6 h. Concurrent negative and positive (heat-shock for 1 h at 43 degrees C) controls were

conducted with each experiment. Immediately following RF field exposure (T = 6 h) and 18 h post-exposure (T = 24 h), cell pellets were collected from each of the culture dishes and analyzed for transcript levels of proto-oncogenes (c-jun, c-myc and c-fos) and the stress-related genes (heat shock proteins (HSP) HSP27 and HSP70B) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Results: No significant effects were observed in mRNA expression of HSP27, HSP70, c-jun, c-myc or c-fos between the sham and RF-exposed groups, in either of the two cell-lines. However, the positive (heat-shock) control group displayed a significant elevation in the expression of HSP27, HSP70, c-fos and c-jun in both cell-lines at T = 6 and 24 h, relative to the sham and negative control groups. Conclusion: This study found no evidence that exposure of cells to non-thermalizing levels of 1.9 GHz pulse-modulated RF fields can cause any detectable change in stress-related gene expression.

(NE) Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, Douglas GR, Williams A, McNamee JP. Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics. 7(21):3896-3905, 2007. (GE)

There is considerable controversy surrounding the biological effects of radiofrequency (RF) fields, as emitted by mobile phones. Previous work from our laboratory has shown no effect related to the exposure of 1.9 GHz pulse-modulated RF fields on the expression of 22,000 genes in a human glioblastoma-derived cell-line (U87MG) at 6 h following a 4 h RF field exposure period. As a follow-up to this study, we have now examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h postexposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg. In support of our previous results, we found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to nonirradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

(E) Chavdoula ED, Panagopoulos DJ, Margaritis LH. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Res 700:51-61, 2010. (RP, LE, GT)

In the present study we used a 6-min daily exposure of dipteran flies, *Drosophila melanogaster*, to GSM-900 MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous

and four different intermittent exposures of 6min total duration, and also to test whether intermittent exposure provides any cumulative effects on the insect's reproductive capacity as well as on the induction of apoptotic cell death. According to our previous experiments, a 6-min continuous exposure per day for five days to GSM-900 MHz and DCS-1800 MHz (Digital Cellular System) mobile phone radiation, brought about a large decrease in the insect's reproductive capacity, as defined by the number of F pupae. This decrease was found to be non thermal and correlated with an increased percentage of induced fragmented DNA in the egg chambers' cells at early- and mid-oogenesis. In the present experiments we show that intermittent exposure also decreases the reproductive capacity and alters the actin cytoskeleton network of the egg chambers, another known aspect of cell death that was not investigated in previous experiments, and that the effect is also due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

(E) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism *Saccharomyces cerevisiae* to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. *Bioelectromagnetics*. 33(7):550-560, 2012 . (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used *Saccharomyces cerevisiae* to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446, 2009. (GT, OX, RP)

BACKGROUND: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. PRINCIPAL FINDINGS: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P < 0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. CONCLUSIONS: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (GE, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern

of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats. Toxicol Int. 20(1):19-24, 2013. (GT, LE)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835×10^{-4} W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672×10^{-4} W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Engelmann JC, Deeken R, Müller T, Nimtz G, Roelfsema MR, Hedrich R. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv Appl Bioinform Chem. 1:71-83, 2008. (GE)

Mobile phone technology makes use of radio frequency (RF) electromagnetic fields transmitted through a dense network of base stations in Europe. Possible harmful effects of RF fields on humans and animals are discussed, but their effect on plants has received little attention. In search for physiological processes of plant cells sensitive to RF fields, cell suspension cultures of *Arabidopsis thaliana* were exposed for 24 h to a RF field protocol representing typical microwave exposition in an urban environment. mRNA of exposed cultures and controls was used to hybridize Affymetrix-ATH1 whole genome microarrays. Differential expression analysis revealed significant changes in

transcription of 10 genes, but they did not exceed a fold change of 2.5. Besides that 3 of them are dark-inducible, their functions do not point to any known responses of plants to environmental stimuli. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

(E) Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, Seyhan N. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Sci Total Environ. 410-411:59-64, 2011. (GT, OX)

The mutagenic and morphologic effects of 1.8GHz Global System for Mobile Communications (GSM) modulated RF (radiofrequency) radiation alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (hPBLs) were investigated in this study using Sister Chromatid Exchange (SCE) and electron microscopy. Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. The lymphocyte cultures were exposed to GSM modulated RF radiation at 1.8GHz for 6, 8, 24 and 48h with and without EGb 761. We observed morphological changes in pulse-modulated RF radiated lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8h and 24h and were more pronounced in cells exposed for 48h. Cytoplasmic lysis and destruction of membrane integrity of cells and nuclei were also seen in 48h RF exposed cells. There was a significant increase ($p < 0.05$) in SCE frequency in RF exposed lymphocytes compared to sham controls. EGb 761 pre-treatment significantly decreased SCE from RF radiation. RF radiation also inhibited cell viability in a time dependent manner. The inhibitory effects of RF radiation on the growth of lymphocytes were marked in longer exposure periods. EGb 761 pre-treatment significantly increased cell viability in RF+EGb 761 treated groups at 8 and 24h when compared to RF exposed groups alone. The results of our study showed that RF radiation affects cell morphology, increases SCE and inhibits cell proliferation. However, EGb 761 has a protective role against RF induced mutagenity. We concluded that RF radiation induces chromosomal damage in hPBLs but this damage may be reduced by EGb 761 pre-treatment.

(NE) Falzone N, Huyser C, Franken DR, Leszczynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiat Res 174:169-176, 2010. (GT, OX)

Abstract Recent reports suggest that mobile phone radiation may diminish male fertility. However, the effects of this radiation on human spermatozoa are largely unknown. The

present study examined effects of the radiation on induction of apoptosis-related properties in human spermatozoa. Ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at specific absorption rates (SARs) of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity, externalization of phosphatidylserine (PS), induction of DNA strand breaks, and generation of reactive oxygen species. Mobile phone radiation had no statistically significant effect on any of the parameters studied. This suggests that the impairment of fertility reported in some studies was not caused by the induction of apoptosis in spermatozoa.

(E) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci 80: 43-50, 2006. (GT, OX, LE, DE)

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(NE) Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 38(4):333-335, 2006. (GE, DE)

AIMS: To study immediate early gene, c-fos, expression as a marker of neural stress after whole of gestation exposure of the fetal mouse brain to mobile telephone-type radiofrequency fields. METHODS: Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Pregnant control mice were sham-exposed or freely mobile in a cage without further restraint. Immediately prior to parturition on gestational day 19, fetal heads were collected, fixed

in 4% paraformaldehyde and paraffin embedded. Any stress response in the brain was detected by c-fos immunohistochemistry in the cerebral cortex, basal ganglia, thalamus, hippocampus, midbrain, cerebellum and medulla. RESULTS: c-fos expression was of limited, but consistent, neuroanatomical distribution and there was no difference in immunoreactivity between exposed and control brains. CONCLUSION: In this animal model, no stress response was detected in the fetal brain using c-fos immunohistochemistry after whole of gestation exposure to mobile telephony.

(E) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz) in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010. (GT, WS)

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(E) Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 2013 Jul 25. [Epub ahead of print] (LE, GT, OX)

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of

unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (*Apis mellifera*) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009. (GT, OX)

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) Gandhi G, Anita, Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11:99-104, 2005. (GT, HU)

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to

2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. Int J Hum Genet 5:259-265, 2005. (GT, HU)

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concerns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any cytogenetic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

(E) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011. (GT, HU, OX)

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The

glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59-66, 2010. (GT, OX, LE, DE)

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012. (LE, GT, OX, DE)

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure

(-): Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(NE) Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radio frequency radiation (RFR)? Electromagn Biol Med. 29(3):98-104, 2010. (LE, GT)

People are exposed to many carcinogenic and mutagenic chemicals in their everyday lives. These include antineoplastic drugs, Polycyclic aromatic hydrocarbons (PAH)s, aromatic amines, nitrosamines, metals, and electromagnetic radiation. Based on the state of knowledge acquired during the last 50 years of research on possible biological effects of electromagnetic fields (EMF), the majority of the scientific community is convinced that exposure to EMF below the existing security limits does not cause a risk to the health of the general public. However, this position is questioned by others, who are of the opinion that the available research data are contradictory or inconsistent and, therefore, unreliable. In this study, we aimed to investigate if there is any effect of 1800 MHz GSM modulated radio frequency radiation (RFR) on the number of micronucleus in exfoliated bladder cells of rat which will be informative about the genotoxic damage. Exposure period was 20 min/day, 5 days/week during a month. Six female Wistar rats were used for two groups: Group I (n=6): controls; Group II (n=6): 1.8 GHz exposed animals. 1800 MHz RFR did not showed a significant MN frequencies in rat bladder cells when compared with the control group ($p>0.05$). 1800 MHz RFR-exposed animals did not produce any genotoxic effect when compared with the control group ($p>0.05$). Kinetic studies are important for any biomarker, especially those in which tissue differentiation and maturation processes will heavily influence the time between induction of damage and collection of damaged cells for micronucleus analysis.

(NE) Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100-MHz radio frequency radiation. Electromagn Biol Med. 2013 Nov 27. [Epub ahead of print] (GT, LE)

Abstract In this study, we aimed to investigate the effects of 1800 and 2100 MHz Radio Frequency (RF) radiation on the number of micronucleus (MN) in exfoliated bladder cells of rat which shows the genotoxic damage. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. Thirty male wistar albino rats were used for five groups: Group I (n = 6): 1800 MHz RF exposed animals for one month, Group II (n = 6): 2100 MHz RF exposed animals for one month, Group III (n = 6): 2100 MHz RF exposed for two months, Group IV (n = 6): control group for one month, Group V (n = 6): control group for two months. Rats of the control groups were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups ($p > 0.05$). There was no statistically significant difference between 2100 MHz RF exposed groups, either. Further studies are needed to demonstrate if there is any genotoxic effect, micronucleus formation in other tissues of rats.

(NE) Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI, Kure EH. Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes in vitro. Anticancer Res 29:2885-2892, 2009. (GT, IA, WS)

BACKGROUND: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. MATERIALS AND METHODS: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m continuous-wave radiation at 18.0 GHz or 10 W/m pulsed-wave at 16.5 GHz, alone or in combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. RESULTS: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. CONCLUSION: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.

(NE) Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, Skiaker R, Hauger E, Lågeide L, Vistnes AI, Kure EH. Cytogenetic effects of exposure to 2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Res 29:4323-4330, 2009. (GT, IA)

BACKGROUND: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. MATERIALS AND METHODS:

Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m continuous waves, or 2.3 GHz, 10 W/m pulsed waves (200 Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. RESULTS: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. CONCLUSION: Exposure during the whole cell cycle in inhibited cultures did not result in significant differences in chromosomal aberrations as compared to controls.

(E) Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940MHz) on the structure of calf thymus DNA. Ecotoxicol Environ Saf. 2012 Nov 16. pii: S0147-6513(12)00368-5. doi: 10.1016/j.ecoenv.2012.10.016. [Epub ahead of print] (GT)

Currently, the biological effects of nonionizing electromagnetic fields (EMFs) including radiofrequency (RF) radiation have been the subject of numerous experimental and theoretical studies. The aim of this study is to evaluate the possible biological effects of mobile phone RF (940MHz, 15V/m and SAR=40mW/kg) on the structure of calf thymus DNA (ct DNA) immediately after exposure and 2h after 45min exposure via diverse range of spectroscopic instruments. The UV-vis and circular dichroism (CD) experiments depict that mobile phone EMFs can remarkably cause disturbance on ct DNA structure. In addition, the DNA samples, immediately after exposure and 2h after 45min exposure, are relatively thermally unstable compared to the DNA solution, which was placed in a small shielded box (unexposed ct DNA). Furthermore, the exposed DNA samples (the DNA samples that were exposed to 940MHz EMF) have more fluorescence emission when compared with the unexposed DNA, which may have occurred attributable to expansion of the exposed DNA structure. The results of dynamic light scattering (DLS) and zeta potential experiments demonstrate that RF-EMFs lead to increment in the surface charge and size of DNA. The structure of DNA immediately after exposure is not significantly different from the DNA sample 2h after 45min exposure. In other words, the EMF-induced conformational changes are irreversible. Collectively, our results reveal that 940MHz can alter the structure of DNA. The displacement of electrons in DNA by EMFs may lead to conformational changes of DNA and DNA disaggregation. Results from this study could have an important implication on the health effects of RF-EMFs exposure. In addition, this finding could proffer a novel strategy for the development of next generation of mobile phone.

(NE) Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users. Toxicol Lett. 193(1):124-130, 2010. (GT, HU)

Mobile phones are being used extensively throughout the world, with more than four billion accounts existing in 2009. This technology applies electromagnetic radiation in the microwave range. Health effects of this radiation have been subject of debate for a

long time, both within the scientific community and within the general public. This study investigated the effect of mobile phone use on genomic instability of the human oral cavity's mucosa cells. 131 Individuals donated buccal mucosa cells extracted by slightly scraping the oral cavity with a cotton swab. Every participant filled out a questionnaire about mobile phone use including duration of weekly use, overall period of exposure and headset usage. 13 Individuals did not use mobile phones at all, 85 reported using the mobile phone for three hours per week or less, and 33 reported use of more than three hours per week. Additionally, information on age, gender, body weight, smoking status, medication and nutrition was retrieved. For staining of the cells a procedure using alpha-tubulin-antibody and chromomycin A(3) was applied. Micronuclei and other markers were evaluated in 1000 cells per individual at the microscope. A second scorer counted another 1000 cells, resulting in 2000 analyzed cells per individual. Mobile phone use did not lead to a significantly increased frequency of micronuclei.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Schrader T, Stopper H. 900 MHz radiation does not induce micronucleus formation in different cell types. *Mutagenesis*. 27(4):477-483, 2012 . (GT)

The exposure of the population to non-ionising electromagnetic radiation is still increasing, mainly due to mobile communication. Whether low-intensity electromagnetic fields can cause other effects apart from heating has been a subject of debate. One of the effects, which were proposed to be caused by mobile phone radiation, is the occurrence of mitotic disturbances. The aim of this study was to investigate possible consequences of these mitotic disturbances as manifest genomic damage, i.e. micronucleus induction. Cells were irradiated at a frequency of 900 MHz, which is located in one of the main frequency bands applied for mobile communication. Two cell types were used, HaCaT cells as human cells and A(L) cells (human-hamster hybrid cells), in which mitotic disturbances had been reported to occur. After different post-exposure incubation periods, cells were fixed and micronucleus frequencies were evaluated. Both cell types did not show any genomic damage after exposure. To adapt the protocol for the micronucleus test into the direction of the protocol for mitotic disturbances, the post-exposure incubation period was reduced and exposure time was extended to one cell cycle length. This did not result in any increase of the genomic damage. In conclusion, micronucleus induction was not observed as a consequence of exposure to non-ionising radiation, even though this agent was reported to cause mitotic disturbances under similar experimental conditions.

(NE) Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. *Bioelectromagnetics* 27:494-504, 2006. (GT)

A large-scale in vitro study focusing on low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields

induce apoptosis or other cellular stress response that activate p53 or the p53-signaling pathway. First, we evaluated the response of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and wideband code division multiple access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced apoptosis or any signs of stress. Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg, and CW radiation at 80 mW/kg for 24 or 48 h. Human IMR-90 fibroblasts from fetal lungs were exposed to both W-CDMA and CW radiation at a SAR of 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the percentage of apoptotic cells were observed between the test groups exposed to RF signals and the sham-exposed negative controls, as evaluated by the Annexin V affinity assay. No significant differences in expression levels of phosphorylated p53 at serine 15 or total p53 were observed between the test groups and the negative controls by the bead-based multiplex assay. Moreover, microarray hybridization and real-time RT-PCR analysis showed no noticeable differences in gene expression of the subsequent downstream targets of p53 signaling involved in apoptosis between the test groups and the negative controls. Our results confirm that exposure to low-level RF signals up to 800 mW/kg does not induce p53-dependent apoptosis, DNA damage, or other stress response in human cells.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics 28:99-108, 2007. (GE)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-

exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsp were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY. Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation. Int J Radiat Biol 84:734-741, 2008. (GT, GE)

PURPOSE: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells. **MATERIALS AND METHODS:** Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. **RESULTS:** RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 approximately 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses. **CONCLUSION:** These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

(NE) Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park WY. Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells. Int J Radiat Biol 84:909-915, 2008. (GT, GE)

Purpose: Radiofrequency (RF) exposure at the frequency of mobile phones has been reported not to induce cellular damage in in vitro and in vivo models. We chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells could be exposed to mobile phone frequencies. **Materials and methods:** Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. **Results:** Neither of cell cycle changes nor DNA damage was detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. We tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, we found that only

29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. Conclusion: From these results, we could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg in HEI-OC1 auditory hair cells.

(E) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012. (LE, GT, IA)

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 $\mu\text{W}/\text{cm}^2$ power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ -radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ -radiation was similar and not significantly different from those exposed to γ -radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ -radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

(NE) Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. Int J Radiat Biol. 83(4):213-220, 2007. (LE, GT)

PURPOSE: The aim of the study was to investigate genotoxicity of long-term exposure to radiofrequency (RF) electromagnetic fields by measuring micronuclei in erythrocytes. The blood samples were collected in two animal studies evaluating possible cocarcinogenic effects of RF fields. **METHODS:** In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz signal similar to that emitted by analog NMT (Nordic Mobile Telephone) phones at a whole-body specific absorption rate (SAR) of 1.5 W/kg, or to a pulsed 902.4 MHz signal similar to that of digital GSM (Global System for Mobile Communications) phones at 0.35 W/kg. A third group was sham-exposed, and a fourth group served as cage controls. All but the cage control animals were exposed to 4 Gy of x-rays during three first weeks of the experiment. In study B, female transgenic mice (line K2) and their nontransgenic littermates were exposed for 52 weeks (1.5 h/d, 5 d/week). Two digital mobile phone signals, GSM and DAMPS (Digital Advanced Mobile Phone System), were used at 0.5 W/kg. All but the cage-control animals were exposed 3 times per week to an ultraviolet

radiation dose of 1.2 MED (minimum erythema dose). RESULTS AND CONCLUSIONS: The results did not show any effects of RF fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The results were consistent in two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals.

(E) Karaca E, Durmaz B, Altug H, Yildiz T, Guducu C, Irgi M, Koksal MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol 106:53-58, 2012. (GT, GE)

Erratum: J Neurooncol 2012 May;107:665.

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorption rate (SAR) 0.725 W/kg signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Behari J. Fifty-gigahertz Microwave exposure effect of radiations on rat brain. Appl Biochem Biotechnol 158:126-139, 2009. (GT, OX, LE)

The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups-group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 $\mu\text{W}/\text{cm}^2$ with nominal specific absorption rate 8.0×10^{-4} w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a significant decrease in GPx and SOD activity ($p = <0.05$) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control ($p = <0.001$). In addition to these, PKC decreased significantly in whole brain and hippocampus ($p < 0.05$). All data are expressed as mean

+/- standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

(E) Kesari KK, Behari J, Kumar S. Mutagenic response of 2.45 GHz radiation exposure on rat brain. Int J Radiat Biol 86:334-343, 2010. (GT, OX, LE)

Purpose: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. Material and methods: Male rats of wistar strain (35 days old with 130 +/- 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. Results: A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance (ANOVA) method was adopted for statistical analysis. Conclusion: The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

(E) Khalil AM, Gagaa M, Alshamali A. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum Exp Toxicol 31(7):734-740, 2012. (GT, OX)

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across

time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(E) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M. In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008. (GT, IA)

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

(E) Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian J Exp Biol 48:586-592, 2010. (GT, OX)

Wistar rats (70 days old) were exposed for 2 h a day for 45 days continuously at 10 GHz [power density 0.214 mW/cm², specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm², SAR 8.0 x10⁽⁻⁴⁾ W/kg). Micronuclei (MN), reactive oxygen species (ROS), and antioxidant enzymes activity were estimated in the blood cells and serum. These radiations induce micronuclei formation and significant increase in ROS production. Significant changes in the level of serum glutathione peroxidase, superoxide dismutase and catalase were observed in exposed group as compared with control group. It is concluded that microwave exposure can be affective at genetic level. This may be an indication of tumor promotion, which comes through the overproduction of reactive oxygen species.

(E) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen MolBiol 33, 154-158, 2010. (GT, HU, LE)

The potential effect of electromagnetic fields (EMFs) emitted from video display terminals (VDTs) to elicit biological response is a major concern for the public. The software professionals are subjected to cumulative EMFs in their occupational environments. This study was undertaken to evaluate DNA damage and incidences of micronuclei in such professionals. To the best of our knowledge, the present study is the first attempt to carry out cytogenetic investigations on assessing bioeffects in personal computer users. The study subjects (n = 138) included software professionals using VDTs for more than 2 years with age, gender, socioeconomic status matched controls (n = 151). DNA damage and frequency of micronuclei were evaluated using alkaline comet assay and cytochalasin blocked micronucleus assay respectively. Overall DNA damage and incidence of micronuclei showed no significant differences between the exposed and control subjects. With exposure characteristics, such as total duration (years) and frequency of use (minutes/day) sub-groups were assessed for such parameters. Although cumulative frequency of use showed no significant changes in the DNA integrity of the classified sub-groups, the long-term users (> 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells.

(E) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a. (GT, OX, RP)

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α -tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived

cell line: a protective role of melatonin. Int J Radiat Biol. 2013b Aug 19. [Epub ahead of print] (GT, OX, RP)

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby, listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res 602(1-2):135-42, 2006. (GT, GE)

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly

increased Hsp70 protein expression ($P < 0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P > 0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P > 0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, GE, WS, IA)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009. (GT, OX, WS)

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without

menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p < 0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure ($p < 0.05$ and $p < 0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. *Bioelectromagnetics* 31:417-424, 2010. (GT, OX)

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(NE) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. *Mutagenesis* 21:139-42, 2006. (GT, IA)

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and

chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(E) Manti L, Braselmann H, Calabrese ML, Massa R, Pugliese M, Scampoli P, Sicignano G, Grossi G. Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro. Radiat Res 169:575-583, 2008. (GT, IA)

The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed in vitro to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence in situ hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

(E) Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E, Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat Res. 169(1):28-37, 2008. (GT)

We investigated the effects of 72 h in vitro exposure of 10 human lymphocyte samples to radiofrequency electromagnetic fields (800 MHz, continuous wave) on genomic instability. The lymphocytes were exposed in a specially designed waveguide resonator at specific absorption rates (SARs) of 2.9 and 4.1 W/kg in a temperature range of 36-37 degrees C. The induced aneuploidy of chromosomes 1, 10, 11 and 17 was determined by interphase FISH using semi-automated image analysis. We observed increased levels of aneuploidy depending on the chromosome studied as well as on the level of exposure.

In chromosomes 1 and 10, there was increased aneuploidy at the higher SAR, while for chromosomes 11 and 17, the increases were observed only for the lower SAR. Multisomy (chromosomal gains) appeared to be the primary contributor to the increased aneuploidy. The effect of temperature on the level of aneuploidy was examined over the range of 33.5-40 degrees C for 72 h with no statistically significant difference in the level of aneuploidy compared to 37 degrees C. These findings suggest the possible existence of an athermal effect of RF radiation that causes increased levels of aneuploidy. These results contribute to the assessment of potential health risks after continuous chronic exposure to RF radiation at SARs close to the current levels set by ICNIRP guidelines.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. ASEB J 19(12):1686-1688, 2005. (GT, GE)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (GE)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to

non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nylund R, Leszczynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. *Proteomics* 6:4769-4780, 2006. (GE, CS)

We have examined in vitro cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

(E) Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. *Mutat Res* 626:69-78, 2007. (GT, RP)

In the present study, the TUNEL (Terminal deoxynucleotidetransferaseUTP Nick End Labeling) assay - a well known technique widely used for detecting fragmented DNA in

various types of cells - was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect *Drosophila melanogaster*. The flies were exposed *in vivo* to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J Panagopoulos, A. Karabarbounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of *D. melanogaster*, *Electromagn. Biol Med* 23 (2004) 29-43; D.J Panagopoulos, N. Messini, A. Karabarbounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within "safety levels" alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), *Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields*, Heraklion, Crete, Greece, October 17-20, 2000, pp. 169-175, ISBN: 960-86733-0-5; D.J Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of *D. melanogaster*, in: P. Stavroulakis (Ed.), *Biological Effects of Electromagnetic Fields*, Springer, 2003, pp. 545-578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7-8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7-8.

(NE) Papparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. *Bioelectromagnetics*. 29(4):312-323, 2008. (GE)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. *Mutat Res* 596:76-80, 2006. (GT, LE)

This investigation concerns with the effect of low intensity microwave (2.45 and 16.5GHz, SAR 1.0 and 2.01W/kg, respectively) radiation on developing rat brain. Wistar rats (35 days old, male, six rats in each group) were selected for this study. These animals were exposed for 35 days at the above mentioned frequencies separately in two different exposure systems. After the exposure period, the rats were sacrificed and the whole brain tissue was dissected and used for study of single strand DNA breaks by micro gel electrophoresis (comet assay). Single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. One-way ANOVA method was adopted for statistical analysis. This study shows that the chronic exposure to these radiations cause statistically significant (p<0.001) increase in DNA single strand breaks in brain cells of rat.

(E) Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test. *Mutat Res*. 2012 Oct 8. pii: S1383-5718(12)00291-4. doi: 10.1016/j.mrgentox.2012.08.010. [Epub ahead of print] (GT)

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone radiation in the *Allium cepa* test. Three groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9hours. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 hours of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the *Allium cepa* test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

(NE) Qutob SS, Chauhan V, Bellier PV, Yauk CL, Douglas GR, Berndt L, Williams A, Gajda GB, Lemay E, Thansandote A, McNamee JP. Microarray gene expression profiling of a human glioblastoma cell line exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. *Radiat Res* 165:636-644, 2006. (GE)

The widespread use of mobile phones has led to public concerns about the health effects associated with exposure to radiofrequency (RF) fields. The paramount concern

of most persons relates to the potential of these fields to cause cancer. Unlike ionizing radiation, RF fields used for mobile telecommunications (800-1900 MHz) do not possess sufficient energy to directly damage DNA. Most rodent bioassay and in vitro genotoxicity/mutation studies have reported that RF fields at non-thermal levels have no direct mutagenic, genotoxic or carcinogenic effects. However, some evidence has suggested that RF fields may cause detectable postexposure changes in gene expression. Therefore, the purpose of this study was to assess the ability of exposure to a 1.9 GHz pulse-modulated RF field for 4 h at specific absorption rates (SARs) of 0.1, 1.0 and 10.0 W/kg to affect global gene expression in U87MG glioblastoma cells. We found no evidence that non-thermal RF fields can affect gene expression in cultured U87MG cells relative to the nonirradiated control groups, whereas exposure to heat shock at 43 degrees C for 1 h up-regulated a number of typical stress-responsive genes in the positive control group. Future studies will assess the effect of RF fields on other cell lines and on gene expression in the mouse brain after in vivo exposure.

(E) Remondini D, Nylund R, Reivinen J, Poullietier de Gannes F, Veyret B, Lagroye I, Haro E, Trillo MA, Capri M, Franceschi C, Schlatterer K, Gminski R, Fitzner R, Tauber R, Schuderer J, Kuster N, Leszczynski D, Bersani F, Maercker C. Gene expression changes in human cells after exposure to mobile phone microwaves. *Proteomics* 6:4745-4754, 2006. (GE, CS)

Possible biological effects of mobile phone microwaves were investigated in vitro. In this study, which was part of the 5FP EU project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods), six human cell types, immortalized cell lines and primary cells, were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labeled for transcriptome analysis on whole-genome cDNA arrays. The results were evaluated statistically using bioinformatics techniques and examined for biological relevance with the help of different databases. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells, and HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes. Analysis of the affected gene families does not point towards a stress response. However, following microwave exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism.

(NE) Ros-Llor I, Sanchez-Siles M, Camacho-Alonso F, Lopez-Jornet P. Effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells. *Oral Dis.* 18:786-792, 2012. (GT)

Objective: In the last two decades, the use of mobile phones has increased enormously all over the world. The controversy regarding whether radiofrequency (RF) fields exert effects upon biological systems is a concern for the general population. An evaluation is made of DNA damage and cytokinetic defects, proliferative potential, and cell death

because of RF radiation emitted by mobile phones in healthy young users. Study design: This cohort study was carried out in 50 Caucasian mobile phone users. We collected two cell samples from each subject (a total of 100 cell samples), corresponding to the right and left cheek mucosa, respectively. Case histories and personal information were assessed, including age, gender, body height and weight, history of cancer, smoking and alcohol consumption, exposure to chemical carcinogens or radiation, and dietary habits. Sampling comprised cell collection from both cheeks with a cytobrush, centrifugation, slide preparation, fixation, and staining, followed by fluorescent microscopic analysis. A total of 2000 exfoliated cells were screened for nuclear abnormalities, especially micronucleus. Results: No statistically significant changes were recorded in relation to age, gender, body mass index, or smoking status. A comparison of the results vs the control area according to the side of the face on which the mobile phone was placed, and in relation to the duration of exposure (years) to mobile phone radiation in the total 100 samples, yielded no significant differences. Conclusions: No genotoxic effects because of RF exposure were observed in relation to any of the study parameters.

(NE) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006. (CT)

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (GE)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(NE) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfi MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009. (NT, IA)

Abstract Sannino, A., Di Costanzo, G., Brescia, F., Sarti, M., Zeni, O., Juutilainen, J and Scarfi, M. R. Human Fibroblasts and 900 MHz Radiofrequency Radiation: Evaluation of DNA Damage after Exposure and Co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171, 743-751 (2009). The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human

**fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008.
(GT, CS)**

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is disconcerting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. **METHODS:** Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. **RESULTS:** UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN ($P = 0.02$). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation ($P = 0.02$), the number of MN after 12 h ($P = 0.02$). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. **CONCLUSION:** UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012. (LE, GT, DE)

We investigated the cytogenotoxic effects of high frequency electromagnetic fields (HF-EMF) for 45 day and the effect of a recovery period of 15 day after exposure to EMF on bone marrow cells of immature and mature rats. The animals in treatment groups were exposed to 1800 MHz EMF at SAR of 0.37 W/kg and 0.49 W/kg for 2h/day for 45 day. Two recovery groups were kept for a recovery period of 15 day without EMF after exposure to HF-EMF. Two control groups for both immature and mature rats were also included. Significant differences were also observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) in all treatment groups. The cytogenotoxic damage was more remarkable in immature rats and, the recovery period did not improve this damage in immature rats. Because much higher and irreversible cytogenotoxic damage was observed in immature rats than in mature rats, further studies are needed to understand effects of EMF on DNA damage and DNA repair, and to determine safe limits for environment and human, especially for children.

(NE) Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. J Radiat Res. 51(3):277-284, 2010. (GE)

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

(E) Souza LD, Cerqueira ED, Meireles JR. Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users. Electromagn Biol Med. 2013 May 28. [Epub ahead of print] (GE, HU)

Abstract Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in hours per week (t) spent using mobile phones: group I, $t > 5$ h; group II, $t > 1$ h and ≤ 5 h; and group III, $t \leq 1$ h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p < 0.05$).

(NE) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. *Mutat Res.* 626(1-2):42-47, 2007. (GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline (pH>13) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. *Int J Radiat Biol* 82:339-346, 2006. (GT, IA)

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did

not modify any measured effects of the x-radiation. Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(E) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi*. 24:465-467, 2006. [Article in Chinese] **(GT)**

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. METHODS: Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). RESULTS: The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. CONCLUSION: No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR \leq 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. *Electromagn Biol Med* 27:418-425, 2008. **(GT, IA)**

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different

among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900MHz electromagnetic fields in the earthworm *Eisenia fetida*. *Ecotoxicol Environ Saf.* 90:7-12, 2013. (GT, OX, WS)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms *Eisenia fetida* exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of *E. fetida* exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. *Cell Biochem Biophys* 56:39-47, 2010. (GT, OX, DE, LE)

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group IV). 1800 MHz GSM-like RF radiation whole-body exposure (15

min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I ($P < 0.05$, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10 dG and MDA levels between Group VI and Group V ($P > 0.05$, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V ($P < 0.05$, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C. Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. Electromagn Biol Med. 31(1):1-18, 2012. (GE)

Electric, magnetic, and electromagnetic fields are ubiquitous in our society, and concerns have been expressed regarding possible adverse effects of these exposures. Research on Extremely Low-Frequency (ELF) magnetic fields has been performed for more than two decades, and the methodology and quality of studies have improved over time. Studies have consistently shown increased risk for childhood leukemia associated with ELF magnetic fields. There are still inadequate data for other outcomes. More recently, focus has shifted toward Radio Frequencies (RF) exposures from mobile telephony. There are no persuasive data suggesting a health risk, but this research field is still immature with regard to the quantity and quality of available data. This technology is constantly changing and there is a need for continued research on this issue. To investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times. We evaluated the effect of high-frequency EMF on gene expression and we identified functional pathways influenced by 900 MHz MW-EMF exposure.

(E) Trošić I, Pavčić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011. (GT)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) ($N = 9$) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m², whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week

during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Valbonesi P, Franzellitti S, Piano A, Contin A, Biondi C, Fabbri E. Evaluation of HSP70 Expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat Res 169:270-279, 2008. (GT, GE)

The aim of this study was to determine whether high-frequency electromagnetic fields (EMFs) could induce cellular effects. The human trophoblast cell line HTR-8/SVneo was used as a model to evaluate the expression of proteins (HSP70 and HSC70) and genes (HSP70A, B, C and HSC70) of the HSP70 family and the primary DNA damage response after nonthermal exposure to pulse-modulated 1817 MHz sinusoidal waves (GSM-217 Hz; 1 h; SAR of 2 W/kg). HSP70 expression was significantly enhanced by heat, which was applied as the prototypical stimulus. The HSP70A, B and C transcripts were differentially expressed under basal conditions, and they were all significantly induced above basal levels by thermal stress. Conversely, HSC70 protein and gene expression was not influenced by heat. Exposing HTR-8/SVneo cells to high-frequency EMFs did not change either HSP70 or HSC70 protein or gene expression. A significant increase in DNA strand breaks was caused by exposure to HO, which was used as a positive stimulus; however, no effect was observed after exposure of cells to high-frequency EMFs. Overall, no evidence was found that a 1-h exposure to GSM-217 Hz induced a HSP70-mediated stress response or primary DNA damage in HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations are needed.

(E) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Effects of the exposure to intermittent 1.8 GHz radio frequency electromagnetic fields on HSP70 expression and MAPK signaling pathways in PC12 cells. Int J Radiat Biol. 2014 Feb 11. [Epub ahead of print] (GE, WS)

Purpose: We previously reported effects on heat shock protein 70 (HSP70) mRNA expression, a cytoprotective protein induced under stressful condition, in human trophoblast cells exposed to amplitude-modulated Global System for Mobile

Communication (GSM) signals. In the present work the same experimental conditions were applied to the rat PC12 cells, in order to assess the stress responses mediated by HSP70 and by the Mitogen Activated Protein Kinases (MAPK) in neuronal-like cells, an interesting model to study possible effects of mobile phone frequencies exposure. Materials and methods: HSP70 gene expression level was evaluated by reverse transcriptase polymerase chain reaction, HSP70 protein expression and MAPK phosphorylation were assessed by Western blotting. PC12 cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave signal (CW, carrier frequency without modulation) or to two different GSM modulation schemes, GSM-217Hz and GSM-Talk (which generates temporal changes between two different GSM signals, active during talking or listening phases respectively, thus simulating a typical conversation). Specific adsorption rate (SAR) was 2 W/kg. Results: After PC12 cells exposure to the GSM-217Hz signal for 16 or 24 h, HSP70 transcription significantly increased, whereas no effect was observed in cells exposed to the CW or GSM-Talk signals. HSP70 protein expression and three different MAPK signaling pathways were not affected by the exposure to any of the three different 1.8 GHz signals. Conclusion: The positive effect on HSP70 mRNA expression, observed only in cells exposed to the GSM-217Hz signal, is a repeatable response previously reported in human trophoblast cells and now confirmed in PC12 cells. Further investigations towards a possible role of 1.8 GHz signal modulation are therefore advisable.

(NE) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006. (GT, LE, IA)

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 µg/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

(NE) Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res 166, 532–538, 2006. (GT)

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm² or 10 mW/cm², 2.13 W/kg or 20.71 W/kg, and 36.9 ± 0.1°C or 37.5 ± 0.2°C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy γ radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to γ irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

(NE) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicity. Radiat Res. 2013 Jan 14. [Epub ahead of print] (GT)

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for

outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.]Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese] (GT, IA, OX)

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly ($P < 0.05$). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced ($P > 0.05$) as compared to sham exposure group. Conclusion: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhong M, Zhang L, Zhou Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Yu Z. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189-196. 2010. (GT, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in

the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. PLoS One. 8(1):e54906, 2013. (GT, CS)

BACKGROUND: Although IARC clarifies radiofrequency electromagnetic fields (RF-EMF) as possible human carcinogen, the debate on its health impact continues due to the inconsistent results. Genotoxic effect has been considered as a golden standard to determine if an environmental factor is a carcinogen, but the currently available data for RF-EMF remain controversial. As an environmental stimulus, the effect of RF-EMF on cellular DNA may be subtle. Therefore, more sensitive method and systematic research strategy are warranted to evaluate its genotoxicity. **OBJECTIVES:** To determine whether RF-EMF does induce DNA damage and if the effect is cell-type dependent by adopting a more sensitive method γ H2AX foci formation; and to investigate the biological consequences if RF-EMF does increase γ H2AX foci formation. **METHODS:** Six different types of cells were intermittently exposed to GSM 1800 MHz RF-EMF at a specific absorption rate of 3.0 W/kg for 1 h or 24 h, then subjected to immunostaining with anti- γ H2AX antibody. The biological consequences in γ H2AX-elevated cell type were further explored with comet and TUNEL assays, flow cytometry, and cell growth assay. **RESULTS:** Exposure to RF-EMF for 24 h significantly induced γ H2AX foci formation in Chinese hamster lung cells and Human skin fibroblasts (HSFs), but not the other cells. However, RF-EMF-elevated γ H2AX foci formation in HSF cells did not result in detectable DNA fragmentation, sustainable cell cycle arrest, cell proliferation or viability change. RF-EMF exposure slightly but not significantly increased the cellular ROS level. **CONCLUSIONS:** RF-EMF induces DNA damage in a cell type-dependent manner, but the elevated γ H2AX foci formation in HSF cells does not result in significant cellular dysfunctions.

(NE) Yadav AS, Sharma MK. Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations. Mutat Res.650(2):175-180, 2008. (LE, GT, HU)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any in vivo effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex-age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including

micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35 years in exposed subjects along with the 9.84 \pm 0.745 micronucleated cells (MNCs) and 10.72 \pm 0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75 \pm 0.774 MNC and 4.00 \pm 0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17 \pm 2.750 and in exposed subjects is 13.06 \pm 1.793. The value of means of KH in exposed subjects (1.84 \pm 0.432) is slightly higher than in controls (1.42 \pm 0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65 \pm 0.276) as compared to controls (0.50 \pm 0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72 \pm 0.374) in comparison to controls (0.67 \pm 0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0-1, 1-2, 2-3 and 3-4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (LE, GE)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008. (GT, IA, OX)

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the

phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(NE) Yildirim MS, Yildirim A, Zamani AG, Okudan N. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns. 21(2):243-251, 2010. (HU, LE, GT)

The use of mobile telephones has rapidly increased worldwide as well as the number of mobile phone base stations that lead to rise low level radiofrequency emissions which may in turn have possible harm for human health. The national radiation protection board has published the known effects of radio waves exposure on humans living close to mobile phone base stations. However, several studies have claimed that the base station has detrimental effects on different tissues. In this study, we aimed to evaluate the effects of mobile phone base stations on the micronucleus (MN) frequency and chromosomal aberrations on blood in people who were living around mobile phone base stations and healthy controls. Frequency of MN and chromosomal aberrations in study and control groups was 8.96 +/- 3.51 and 6.97 +/- 1.52 (p: 0.16); 0.36 +/- 0.31 and 0.75 +/- 0.61 (p: 0.07), respectively. Our results show that there was not a significant difference of MN frequency and chromosomal aberrations between the two study groups. The results claim that cellular phones and their base stations do not produce important carcinogenic changes.

(E) Zalata, A., A. Z. El-Samanoudy, D. Shaalan, Y. El-Baiomy, and T. Mostafa. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression of sperm. Int J Fertil Steril, In Press. Published online ahead of print. (GT, GE, RP)

Background: Use of cellular phones that emits radiofrequency electromagnetic field (RF-EMF) has been increased exponentially and became a part of everyday life. This study aimed to investigate the effects of RF-EMF radiation emitted from cellular phones on sperm motility variables, sperm DNA fragmentation and clusterin (CLU) gene expression. Materials and Methods: 124 semen samples were grouped into; normozoospermia (N, n=26), asthenozoospermia (A, n=32), asthenoteratozoospermia (AT, n=31) and

oligoasthenoteratozoospermia (OAT, n=35). Semen samples were divided into two aliquots; samples not exposed to cell phone and samples exposed to cell phone radiation (850 MHz, maximum power < 1 watt; SAR 1.46 W/kg at 10 cm distance) for 1 hr. Before and immediately after exposure both aliquots were subjected to assessment of sperm motility, acrosin activity, sperm DNA fragmentation and CLU gene expression. Statistical differences were analyzed using paired t-student test for comparisons where $P < 0.05$ was set as significant. Results: There was significant decrease in sperm motility, sperm linear velocity, sperm linearity index, sperm acrosin activity and significant increase in sperm DNA fragmentation percent, CLU gene expression and CLU protein levels in the exposed semen samples to RF-EMF compared with non-exposed samples in OAT > AT > A > N groups ($P < 0.05$).

Conclusions: Cell phone emissions have a negative impact on exposed sperm motility indices, sperm acrosin activity, sperm DNA fragmentation and CLU gene expression especially in OAT cases.

(NE) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008. (GT)

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi XueZaZhi 40:149-152, 2006. [Article in Chinese] (GT)

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. **METHODS:** The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. **RESULTS:** The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. **CONCLUSION:** 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (GE, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. **METHODS:** Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative reverse transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). **RESULTS:** Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h

continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). **CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).**

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175, 2007. (GE)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (GE, CS)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons,

but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

(E) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. *Mutat Res.* 695(1-2):16-21, 2010.

(GT, IA)

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. *Mutat Res.* 677(1-2):100-104, 2009.

(GT, IA)

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA)

served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi, Prihoda TJ, Kuster N, Tillmann T, Dasenbrock C. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. Int J Radiat Biol. 85(5):454-464, 2009. (GT, LE)

PURPOSE: The aim of the present investigation was to determine the incidence of micronuclei in peripheral blood erythrocytes of B6C3F1 mice that had been chronically exposed to radiofrequencies (RF) used for mobile communication. **MATERIALS AND METHODS:** 'Ferris wheels' were used to expose tube-restrained male and female mice to simulated environmental RF signals of the Global System for Mobile Communications (GSM, 902 MHz) or Digital Cellular System (DCS, 1747 MHz). RF signals were applied to the mice for 2 hours/day on 5 days/week for two years, at maximal whole-body-averaged specific absorption rates of 0.4, 1.3, and 4.0 W/kg body weight. Concurrent sham-exposed mice, cage controls, and positive controls injected with mitomycin C were included in this investigation. At necropsy, peripheral blood smears were prepared, and coded slides were stained using May-Grunwald-Giemsa or acridine orange. The incidence of micronuclei was recorded for each mouse in 2000 polychromatic and 2000 normochromatic erythrocytes. **RESULTS:** There were no significant differences in the frequency of micronuclei between RF-exposed, sham-exposed, and cage control mice, irrespective of the staining/counting method used. Micronuclei were, however, significantly increased in polychromatic erythrocytes of the positive control mice. **CONCLUSIONS:** In conclusion, the data did not indicate RF-induced genotoxicity in mice after two years of exposure.

Update on genetic effects of extremely-low frequency electromagnetic fields

(NE) Albert GC, McNamee JP, Marro L, Bellier PV, Prato FS, Thomas AW. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 μ T, 60 Hz magnetic field. *Int J Radiat Biol.* 85(2):144-152, 2009. (GT, IA)

AIM: To investigate the extent of damage in nucleated cells in peripheral blood of healthy human volunteers exposed to a whole-body 60 Hz, 200 microT magnetic field. **MATERIALS AND METHODS:** In this study, 10 male and 10 female healthy human volunteers received a 4 h whole-body exposure to a 200 microT, 60 Hz magnetic field. In addition, five males and five females were treated in a similar fashion, but were exposed to sham conditions. For each subject, a blood sample was obtained prior to the exposure period and aliquots were used as negative- (pre-exposure) and positive- [1.5 Gray (Gy) (60)Cobalt ((60)Co) gamma-irradiation] controls. At the end of the 4 h exposure period, a second blood sample was obtained. The extent of DNA damage was assessed in peripheral human blood leukocytes from all samples using the alkaline comet assay. To detect possible clastogenic effects, the incidence of micronuclei was assessed in phytohemagglutinin (PHA)-stimulated lymphocytes using the cytokinesis-block micronucleus assay. **RESULTS:** There was no evidence of either increased DNA damage, as indicated by the alkaline comet assay, or increased incidence of micronuclei (MN) in the magnetic field exposed group. However, an in vitro exposure of 1.5 Gy gamma-irradiation caused a significant increase in both DNA damage and MN induction. **CONCLUSIONS:** This study found no evidence that an acute, whole-body exposure to a 200 microT, 60 Hz magnetic field for 4 hours could cause DNA damage in human blood.

(E) Alcaraz M, Olmos E, Alcaraz-Saura M, Achel DG, Castillo J. Effect of long-term 50 Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice. *Electromagn Biol Med.* 2013 Jun 19. [Epub ahead of print] (GT)

Abstract In recent years extremely low-frequency magnetic fields (ELF-EMF) have become widely used in human activities, leading to an increased chance of exposure to ELF-EMF. There are few reports on in vivo mammalian genotoxic effects using micronucleus (MN) assays, which generally have been used as a short-term screening system. We analyzed the possible genotoxic effect induced by long-term exposure (7, 14, 21, 28 d) of a 50 Hz ELM-MF to mice by measuring the increase in frequency of micronucleated polychromatic erythrocyte in their bone marrow (MNPCEs) and we compared it with that induced by 50 cGy of X-rays. Subsequently, we tried to reduce this chromosomal damage by administering four antioxidants substances with radioprotective capacities: dimethyl sulfoxide (DMSO), 6-n-propyl-2-thiouracil (PTU),

grape-procyanidins (P) and citrus flavonoids extract (CE). The increase in micronucleated cells was higher in both physical treatments (Control < ELF-EMF ($p < 0.01$) < X-rays ($p > 0.001$)); however, the antioxidant substances only showed a genoprotective capacity against the damage induced by ionizing radiation (Ci > PTU = DMSO ($p < 0.001$) > P = CE ($p < 0.001$). The 50 Hz ELM-MF increased MNPCEs in mouse bone marrow, expressing a genotoxic capacity. Administration of antioxidant substances with radioprotective capacities known to act through the elimination of free radicals did not diminish the genotoxic effect induced by ELM-MF.

(E) Balamuralikrishnan B, Balachandar V, Kumar SS, Stalin N, Varsha P, Devi SM, Arun M, Manikantan P, Venkatesan C, Sasikala K, Dharwadkar SN. Evaluation of Chromosomal Alteration in Electrical Workers Occupationally Exposed to Low Frequency of Electro Magnetic Field (EMFs) in Coimbatore Population, India. Asian Pac J Cancer Prev. 13(6):2961-2966, 2012. (HU, LE, GT)

Extremely low frequency electromagnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of nonionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure ($P < 0.05$). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power

frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Borhani N, Rajaei F, Salehi Z, Javadi A. Analysis of DNA fragmentation in mouse embryos exposed to an extremely low-frequency electromagnetic field. *Electromagn Biol Med.* 30(4):246-252, 2011. (GT, DE, LE)

Effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on DNA damage in biological systems are still a matter of dispute. The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in cells (blastomers) of mouse blastocysts. Eighty female NMRI mice were randomly divided into 2 groups of 40 animals each. The control group was left unexposed whereas the animals in the EMF-group were exposed to a 50-Hz EMF at 0.5 mT 4 h per day, 6 days a week for a duration of 2 weeks. After the 8(th) day of exposure, the female mice in both groups were superovulated (with injections of pregnant mare serum gonadotropin and human chorionic gonadotropin) and then mated overnight. At approximately 4 days after mating (102 h after the human chorionic gonadotropin treatment), blastocysts were obtained by flushing the uterus horns. The mean numbers of pregnant mice, blastocysts after flushing, blastomers within the blastocysts, and the DNA fragmentation index following staining in both groups were compared using statistical methods (SPSS, the Chi-square test, the Student's t-test and the Mann-Whitney U-test, $P < 0.05$). The results showed that the mean number of blastocysts after flushing was significantly decreased in the EMF-group compared to that of the control group ($P < 0.03$). The DNA fragmentation index was significantly increased in the EMF-group compared to control (10.53% vs. 7.14%; $P < 0.001$). However, there was no

significant difference in the mean numbers of blastomers and numbers of pregnant mice between the EMF-exposed and control group. Our findings indicate that the EMF exposure in preimplantation stage could have detrimental effects on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

(E) Bułdak RJ, Polaniak R, Bułdak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Bułdak A, Birkner E. Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics. 2012 Apr 25. doi: 10.1002/bem.21732. [Epub ahead of print] (GT, IA, OX)

The aim of this study was to assess the influence of cisplatin and an extremely low frequency electromagnetic field (ELF-EMF) on antioxidant enzyme activity and the lipid peroxidation ratio, as well as the level of DNA damage and reactive oxygen species (ROS) production in AT478 carcinoma cells. Cells were cultured for 24 and 72 h in culture medium with cisplatin. Additionally, the cells were irradiated with 50 Hz/1 mT ELF-EMF for 16 min using a solenoid as a source of the ELF-EMF. The amount of ROS, superoxide dismutase (SOD) isoenzyme activity, glutathione peroxidase (GSH-Px) activity, DNA damage, and malondialdehyde (MDA) levels were assessed. Cells that were exposed to cisplatin exhibited a significant increase in ROS and antioxidant enzyme activity. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity. A significant reduction in MDA concentrations was observed in all of the study groups, with the greatest decrease associated with treatment by both cisplatin and ELF-EMF. Cisplatin induced the most severe DNA damage; however, when cells were also irradiated with ELF-EMF, less DNA damage occurred. Exposure to ELF-EMF alone resulted in an increase in DNA damage compared to control cells. ELF-EMF lessened the effects of oxidative stress and DNA damage that were induced by cisplatin; however, ELF-EMF alone was a mild oxidative stressor and DNA damage inducer. We speculate that ELF-EMF exerts differential effects depending on the exogenous conditions. This information may be of value for appraising the pathophysiologic consequences of exposure to ELF-EMF.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (GT)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH₂ methylene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO₂- stretching phosphate bands

decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β -sheet contents in amide I, and around the 1740 cm^{-1} band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β -sheet contents as to α -helix components of amide I region, as well.

(E) Celikler S, Aydemir N, Vatan O, Kurtuldu S, Bilaloglu R. A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations. Int J Environ Health Res. 19(6):421-430, 2009. (GT, HU)

A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05 , respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

(E) Chen GD, Lu DQ, Jiang H, Xu ZP.[Effects of 50 Hz magnetic fields on gene expression in MCF-7 cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):15-22, 2008. [Article in Chinese] (GT, GE)

OBJECTIVE: To investigate whether 50 Hz magnetic fields (MF) can change the gene expression profile in MCF-7 cells and to screen MF responsive genes. **METHODS:** In vitro cultured MCF-7 cells were continuously exposed or sham-exposed to 0.4 mT of 50 Hz MF for 24 hours. Affymetrix Human Genome Genechips (U133A) were applied to analyze gene expression profiles in MF exposed and sham-exposed MCF-7 cells and the data were processed with Genechip data analysis software MAS 5.0 and DMT 3.0. Real-time RT-PCR assay was employed to examine the differentially expressed genes. **RESULT:** Thirty differentially expressed genes were screened with 100 % consistency change calls in the MF exposed MCF-7 cells. Six independent real-time RT-PCR analyses showed that SCNN1A, METTL3 and GPR137B were slightly but statistically significantly changed in MCF-7 cells after exposure to 50 Hz MF ($P < 0.05$), while other analyzed genes exhibited slight up-and down-fluctuations in expressions and no increase or decrease in each gene expression reached statistical significance ($P > 0.05$). **CONCLUSION:** The present study identified three 50 Hz MF responsive genes in MCF-7 cells and the biological consequences of expression changes in these MF responsive genes need to be

further investigated. 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(NE) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism *Saccharomyces cerevisiae* to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. *Bioelectromagnetics*. 33(7):550-560, 2012. (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used *Saccharomyces cerevisiae* to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Cho S, Lee Y, Lee S, Choi YJ, Chung HW. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. *Drug Chem Toxicol*. 2014 Jan 30. [Epub ahead of print] (GT, IA)

Gadolinium (Gd) and its chelated derivatives are widely utilized for various industrial and medical purposes, particularly as a contrast agent for magnetic resonance imaging (MRI). There are many studies of Gd nephrotoxicity and neurotoxicity, whereas research on cyto- and genotoxicity in normal human lymphocytes is scarce. It is important to investigate the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on Gd toxicity, as patients are co-exposed to Gd and ELF-EMF generated by MRI scanners. We investigated the cytotoxicity and genotoxicity of Gd and the possible enhancing effect of ELF-EMF on Gd toxicity in cultured human lymphocytes by performing a micronuclei (MN) assay, trypan blue dye exclusion, single cell gel electrophoresis, and apoptosis analyses using flow cytometry. Isolated lymphocytes were exposed to 0.2-1.2 mM of Gd only or in combination with a 60-Hz ELF-EMF of 0.8-mT field strength. Exposing human lymphocytes to Gd resulted in a concentration- and time-dependent decrease in cell viability and an increase in MN frequency, single strand DNA breakage,

apoptotic cell death, and ROS production. ELF-EMF (0.8 mT) exposure also increased cell death, MN frequency, olive tail moment, and apoptosis induced by Gd treatment alone. These results suggest that Gd induces DNA damage and apoptotic cell death in human lymphocytes and that ELF-EMF enhances the cytotoxicity and genotoxicity of

Gd.

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(E) Cho YH, Jeon HK, Chung HW. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. J Toxicol Environ Health A. 70(15-16):1252-1258, 2007. (GT, IA)

This study was carried out to examine the interaction of extremely low-frequency electromagnetic fields (ELF-EMF) on delayed chromosomal instability by bleomycin (BLM) in human fibroblast cells. A micronucleus-centromere assay using DNA probes for chromosomes 1 and 4 was performed and a 60-Hz ELF-EMF of 0.8 mT field strength was applied either alone or with BLM throughout the culture period. The frequencies of micronuclei (MN) and aneuploidy were analyzed at 28, 88, and 240 h after treatment with BLM. The coexposure of cells to BLM and ELF-EMF led to a significant increase in the frequencies of MN and aneuploidy compared to the cells treated with BLM alone. No difference was observed between field-exposed and sham-exposed control cells. The frequency of MN induced by BLM was increased at 28 h, and further analysis showed a persistent increase up to 240 h, but the new levels were not significantly different from the level at 28 h. BLM increased the frequencies of aneuploidy at 28, 88, and 240 h, and significantly higher frequency of aneuploidy was observed in the cells analyzed at 240 h compared to the cells examined at 28 h. No interaction of ELF-EMF on delayed chromosomal instability by BLM was observed. Our results suggest that ELF-EMF enhances the cytotoxicity of BLM. BLM might induce delayed chromosomal instability, but no effect of ELF-EMF was observed on the BLM-induced delayed chromosomal instability in fibroblast cells.

(E) Collard JF, Lazar C, Nowé A, Hinsenkamp M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol. 111(1):37-45, 2013. (GE)

An acceleration of differentiation at the expense of proliferation is observed in our previous publications and in the literature after exposure of various biological models to low frequency and low-amplitude electric and electromagnetic fields. This observation is related with a significant modification of genes expression. We observed and compared over time this modification. This study use microarray data obtained on epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields. This protocol is repeated with samples collected on three different healthy patients. The sampling over time allows comparison of the effect of the stimulus at a given time with the evolution of control group. After 4 days, we observed a significant difference of the

genes expression between control (D4C) and stimulated (D4S) ($p < 0.05$). On the control between day 4 and 7, we observed another group of genes with significant difference ($p < 0.05$) in their expression. We identify the common genes between these two groups and we select from them those expressing no difference between stimulate at 4 days (D4S) and control after 7 days (D7C). The same analysis was performed with D4S-D4C-D12C and D7S-D7C-D12C. The lists of genes which follow this pattern show acceleration in their expressions under stimulation appearing on control at a later time. In this list, genes such as DKK1, SPRR3, NDRG4, and CHEK1 are involved in cell proliferation or differentiation. Numerous other genes are also playing a function in mitosis, cell cycle or in the DNA replication transcription and

translation.

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(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C.

Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Exp Neurol.* 226(1):173-182, 2010. (LE, GE, DE)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel $\alpha(1C)$ subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Di Campi E, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. Curr Microbiol. 60(6):412-418, 2010. (GE)

The aim of this work was to investigate the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of *Helicobacter pylori*. Bacterial cultures and 2-day-old biofilm of *H. pylori* ATCC 43629 were exposed to ELF-EMF (50 Hz frequency-1 mT intensity) for 2 days to assess their effect on the cell adhesion and on the mature biofilm detachment, respectively. All the exposed cultures and the respective sham exposed controls were studied for: the cell viability status, the cell morphological analysis, the biofilm mass measurement, the genotypic profile, and the *luxS* and *amiA* gene expression. The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability, as well as, in morphotypes measured by the prevalence of spiral forms (58.41%) in respect to the controls (33.14%), whereas, on mature biofilm, no significant differences were found when compared to the controls. The measurement of biofilm cell mass was significantly reduced in exposed cultures in both examined experimental conditions. No changes in DNA patterns were recorded, whereas a modulation in *amiA* gene expression was detected. An exposure to ELF-EMF of *H. pylori* biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the *H. pylori* capability to protect itself.

(E) Dominici L, Villarini M, Fatigoni C, Monarca S, Moretti M. Genotoxic hazard evaluation in welders occupationally exposed to extremely low-frequency magnetic fields (ELF-MF). Int J Hyg Environ Health. 215(1):68-75, 2011. (GT, HU)

Electric arc welding is known to involve considerable exposure to extremely low-frequency magnetic fields (ELF-MF). A cytogenetic monitoring study was carried out in a group of welders to investigate the genotoxic risk of occupational exposure to ELF-MF. This study assessed individual occupational exposure to ELF-MF using a personal magnetic-field dosimeter, and the cytogenetic effects were examined by comparing micronuclei (MN) and sister chromatid exchange (SCE) frequencies in the lymphocytes of the exposed workers with those of non-exposed control subjects (blood donors) matched for age and smoking habit. Cytogenetic analyses were carried out on 21 workers enrolled from two different welding companies in Central Italy and compared to 21 controls. Some differences between the groups were observed on analysis of SCE and MN, whereas replication indices in the exposed were found not to differ from the controls. In particular, the exposed group showed a significantly higher frequency of MN (group mean \pm SEM: 6.10 \pm 0.39) compared to the control group (4.45 \pm 0.30). Moreover, the increase in MN is associated with a proportional increase in ELF-MF exposure levels with a dose-response relationship. A significant decrease in SCE frequency was observed in exposed subjects (3.73 \pm 0.21) compared to controls (4.89 \pm 0.12). The hypothesis of a correlation between genotoxic assays and ELF-MF exposure value was partially

supported, especially as regards MN assay. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(E) Du XG, Xu SS, Chen Q, Lu DQ, Xu ZP, Zeng QL. [Effects of 50 Hz magnetic fields on DNA double-strand breaks in human lens epithelial cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):9-14, 2008. [Article in Chinese] (GT)

OBJECTIVE: To investigate the effects of 50 Hz magnetic fields (MF) on DNA double-strand breaks in human lens epithelial cells (hLECs). **METHODS:** The cultured human lens epithelial cells were exposed to 0.4 mT 50 Hz MF for 2 h, 6 h, 12 h, 24 h and 48 h. Cells exposed to 4-nitroquinoline-1-oxide, a DNA damage agent, at a final concentration of 0.1 micromol/L for 1 h were used as positive controls. After exposure, cells were fixed with 4 % paraformaldehyde and for H2AX (gamma H2AX) immunofluorescence measurement. gamma H2AX foci were detected at least 200 cells for each sample. Cells were classified as positive when more than three foci per cell were observed. Mean values of foci per cell and percentage of foci positive cells were adopted as indexes of DNA double-strand breaks. **RESULT:** The mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 24 h were (2.93 +/- 0.43) and (27.88 +/- 2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77 +/- 0.37) and (19.38 +/- 2.70)%, P < 0.05], and the mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 48 h were (3.14 +/- 0.35) and (31.00 +/- 3.44)%, which were significantly higher than those of sham-exposure group (P < 0.01). However there was no significant difference between 50 Hz MF exposure groups for 2 h, 6 h, 12 h and sham-exposure group for above two indexes (P > 0.05). **CONCLUSION:** 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(E) El-Bialy NS, Rageh MM. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. Biomed Res Int. 2013;2013:189352. doi: 10.1155/2013/189352. Epub 2013 Jan 14. (GT, IA)

The present study examines the therapeutic efficacy of the administration of low-dose cisplatin (cis) followed by exposure to extremely low-frequency magnetic field (ELF-MF), with an average intensity of 10 mT, on Ehrlich carcinoma in vivo. The cytotoxic and genotoxic actions of this combination were studied using comet assay, mitotic index (MI), and the induction of micronucleus (MN). Moreover, the inhibition of tumor growth was also measured. Treatment with cisplatin and ELF-MF (group A) increased the number of damaged cells by 54% compared with 41% for mice treated with cisplatin alone (group B), 20% for mice treated by exposure to ELF-MF (group C), and 9% for the control group (group D). Also the mitotic index decreased significantly for all treated groups (P < 0.001). The decrement percent for the treated groups (A, B, and C) were 70%, 65%, and 22%, respectively, compared with the control group (D). Additionally, the

rate of tumor growth at day 12 was suppressed significantly ($P < 0.001$) for groups A, B, and C with respect to group (D). These results suggest that ELF-MF enhanced the cytotoxic activity of cisplatin and potentiate the benefit of using a combination of low-dose cisplatin and ELF-MF in the treatment of Ehrlich carcinoma.

(E) Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. *Mutat Res.* 630(1-2):69-77, 2007. (GT, LE)

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4h) and long-term (4h/day for 45 days) to a horizontal 50Hz, 1mT uniform magnetic field generated by a Helmholtz coil system. Mitomycin C (MMC, 2mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p < 0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p < 0.001$ and $p < 0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p < 0.001$ and $p < 0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-MF was significantly lower than negative control ($p < 0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p -values of all each groups < 0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p -values of all groups < 0.01). These observations indicate the in vivo susceptibility of mammals to the genotoxicity potential of ELF-MF.

(E) Fedrowitz M, Löscher W. Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 μ T) for 2 weeks. *Int J Radiat Biol.* 88(5):425-429, 2012. (GE, LE) See also: Fedrowitz M, Hass R, Löscher W. Effects of 50 Hz magnetic field exposure on the stress marker α -amylase in the rat mammary gland. *Int J Radiat Biol.* 88(7):556-564, 2012.

PURPOSE: The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously,

we observed rat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. **MATERIALS AND METHODS:** Female F344 and Lewis rats were exposed to MF (50 Hz, 100 μ T) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. **RESULTS:** A remarkably decreased α -amylase gene expression, decreases in carbonic anhydrase 6 and lactoperoxidase, both relevant for pH regulation, and an increased gene expression of cystatin E/M, a tumor suppressor, were observed in MF-exposed F344, but not in Lewis rats. **CONCLUSION:** The MF-exposed F344 breast tissue showed alterations in gene expression, which were absent in Lewis and may therefore be involved in the MF-susceptibility of F344. Notably α -amylase might serve as a promising target to study MF effects, because first experiments indicate that MF exposure alters the functionality of this enzyme in breast tissue.

(E) Focke F, Schuermann D, Kuster N, Schär P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res. 683(1-2):74-83, 2010. (GT)

Extremely low frequency electromagnetic fields (ELF-EMFs) were reported to affect DNA integrity in human cells with evidence based on the Comet assay. These findings were heavily debated for two main reasons; the lack of reproducibility, and the absence of a plausible scientific rationale for how EMFs could damage DNA. Starting out from a replication of the relevant experiments, we performed this study to clarify the existence and explore origin and nature of ELF-EMF induced DNA effects. Our data confirm that intermittent (but not continuous) exposure of human primary fibroblasts to a 50 Hz EMF at a flux density of 1 mT induces a slight but significant increase of DNA fragmentation in the Comet assay, and we provide first evidence for this to be caused by the magnetic rather than the electric field. Moreover, we show that EMF-induced responses in the Comet assay are dependent on cell proliferation, suggesting that processes of DNA replication rather than the DNA itself may be affected. Consistently, the Comet effects correlated with a reduction of actively replicating cells and a concomitant increase of apoptotic cells in exposed cultures, whereas a combined Fpg-Comet test failed to produce evidence for a notable contribution of oxidative DNA base damage. Hence, ELF-EMF induced effects in the Comet assay are reproducible under specific conditions and can be explained by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

(E) Frisch P, Li GC, McLeod K, Laramée CB. Induction of heat shock gene expression in RAT1 primary fibroblast cells by ELF electric fields. Bioelectromagnetics. 34(5):405-413, 2013. (GE)

Recent studies have demonstrated that the Ku70 gene fragment can be placed in the anti-sense orientation under the control of a heat-inducible heat shock protein 70

(HSP70) promoter and activated through heat shock exposure. This results in attenuation of the Ku70 protein expression, inhibiting cellular repair processes, and sensitizing the transfected cells to exposures such as the ionizing radiation exposures used clinically. However, achieving the tissue temperatures necessary to thermally induce the HSP70 response presents significant limitations to the clinical application of this strategy. Previous findings suggest an alternative approach to inducing a heat shock response, specifically through the use of extremely low frequency (ELF) electrical field stimulation. To further pursue this approach, we investigated HSP70 responses in transfected rat primary fibroblast (RAT1) cells exposed to 10 Hz electric fields at intensities of 20-500 V/m. We confirmed that low frequency electric fields can induce HSP70 heat shock expression, with peak responses obtained at 8 h following a 2 h field exposure. However, the approximate threefold increase in expression is substantially lower than that obtained using thermal stimulation, raising questions of the clinical utility of the response.

(E) Giorgi G, Marcantonio P, Bersani F, Gavoçi E, Del Re B. Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time. Int J Radiat Biol. 87(6):601-608, 2011. (GT, WS)

PURPOSE: To examine the effect of extremely low frequency magnetic field (ELF-MF) exposure on transposon (Tn) mobility in relation to the exposure time, the frequency and the wave shape of the field applied. **MATERIALS AND METHODS:** Two Escherichia coli model systems were used: (1) Cells unable to express β -galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express β -galactosidase (LacZ(+)) upon its transposition; therefore in these cells transposition activity can be evaluated by analysing LacZ(+) clones; (2) cells carrying Fertility plasmid (F(+)), and a Tn5 element located on the chromosome; therefore in these cells transposition activity can be estimated by a bacterial conjugation assay. Cells were exposed to sinusoidal (SiMF) or pulsed-square wave (PMF) magnetic fields of various frequencies (20, 50, 75 Hz) and for different exposure times (15 and 90 min). **RESULTS:** Both mini-Tn10 and Tn5 transposition decreased under SiMF and increased under PMF, as compared to sham exposure control. No significant difference was found between frequencies and between exposure times. **CONCLUSIONS:** ELF-MF exposure affects transposition activity and the effects critically depend on the wave shape of the field, but not on the frequency and the exposure time, at least in the range observed.

(E) Heredia-Rojas JA, Rodríguez de la Fuente AO, Alcocer González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. In Vitro Cell Dev Biol Anim. 46(9):758-63, 2010. (GE)

It has been reported that 50-60 Hz magnetic fields (MF) with flux densities ranging from microtesla to millitesla are able to induce heat shock factor or heat shock proteins in

various cells. In this study, we investigated the effect of 60 Hz sinusoidal MF at 8 and 80 μ T on the expression of the luciferase gene contained in a plasmid labeled as electromagnetic field-plasmid (pEMF). This gene construct contains the specific sequences previously described for the induction of hsp70 expression by MF, as well as the reporter for the luciferase gene. The pEMF vector was transfected into INER-37 and RMA E7 cell lines that were later exposed to either MF or thermal shock (TS). Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system for evaluate luciferase activity. An increased luciferase gene expression was observed in INER-37 cells exposed to MF and TS compared with controls ($p < 0.05$), but MF exposure had no effect on the RMA E7 cell line.

(NE) Huwiler SG, Beyer C, Fröhlich J, Hennecke H, Egli T, Schürmann D, Rehrauer H, Fischer HM. Genome-wide transcription analysis of Escherichia coli in response to extremely low-frequency magnetic fields. Bioelectromagnetics. 2012 Feb 13. doi: 10.1002/bem.21709. [Epub ahead of print] (GE)

The widespread use of electricity raises the question of whether or not 50 Hz (power line frequency in Europe) magnetic fields (MFs) affect organisms. We investigated the transcription of Escherichia coli K-12 MG1655 in response to extremely low-frequency (ELF) MFs. Fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent; all at 50 Hz, 1 mT) were applied and gene expression was monitored at the transcript level using an Affymetrix whole-genome microarray. Bacterial cells were grown continuously in a chemostat (dilution rate $D = 0.4 \text{ h}^{-1}$) fed with glucose-limited minimal medium and exposed to 50 Hz MFs with a homogenous flux density of 1 mT. For all three types of MFs investigated, neither bacterial growth (determined using optical density) nor culturable counts were affected. Likewise, no statistically significant change (fold-change > 2 , $P \leq 0.01$) in the expression of 4,358 genes and 714 intergenic regions represented on the gene chip was detected after MF exposure for 2.5 h (1.4 generations) or 15 h (8.7 generations). Moreover, short-term exposure (8 min) to the sinusoidal continuous and power line intermittent signal neither affected bacterial growth nor showed evidence for reliable changes in transcription. In conclusion, our experiments did not indicate that the different tested MFs (50 Hz, 1 mT) affected the transcription of E. coli.

(NE) Jin YB, Kang GY, Lee JS, Choi JI, Lee JW, Hong SC, Myung SH, Lee YS. Effects on micronuclei formation of 60-Hz electromagnetic field exposure with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Int J Radiat Biol. 88(4):374-380, 2012. (GT, IA)

PURPOSE: Epidemiological studies have demonstrated a possible correlation between exposure to extremely low-frequency magnetic fields (ELF-MF) and cancer. However, this correlation has yet to be definitively confirmed by epidemiological studies. The principal objective of this study was to assess the effects of 60 Hz magnetic fields in a

normal cell line system, and particularly in combination with various external factors, via micronucleus (MN) assays. **MATERIALS AND METHODS:** Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells were exposed for 4 h to a 60 Hz, 1 mT uniform magnetic field with or without ionizing radiation (IR, 2 Gy), H₂O₂ (100 µM) and cellular myelocytomatosis oncogene (c-Myc) activation. **RESULTS:** The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic effects were observed when ELF-MF was combined with IR, H₂O₂, and c-Myc activation. **CONCLUSIONS:** Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H₂O₂ and c-Myc activation.

(NE) Jin YB, Choi SH, Lee JS, Kim JK, Lee JW, Hong SC, Myung SH, Lee YS. Absence of DNA damage after 60-Hz electromagnetic field exposure combined with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Radiat Environ Biophys. 2013 Dec 5. [Epub ahead of print] (GT, IA)

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H₂O₂ (50 µM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H₂O₂, or c-Myc activation.

(E) Jouni FJ, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutat Res. 741(1-2):116-121, 2012. (LE, GT, OX, IA)

The investigation was performed to evaluate the influence of the static magnetic field on oxidative stress in Vicia faba cultivated in soil from high background natural radioactivity in Iran. Soil samples were collected from Ramsar, Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year. The soil samples were then divided into 2 separate groups including high and low natural radioactivity. The plants were continuously exposed to static magnetic field of 15 mT for 8 days, each 8h/day. The results showed that in the plants cultivated in soils with high background natural radioactivity and low background natural radioactivity the activity of antioxidant enzymes as well as flavonoid content were lower than those of the control. Treatment of plants with static magnetic field showed similar results in terms of lowering of antioxidant defense system and increase of peroxidation of membrane lipids. Accumulation of ROS also resulted in chromosomal aberration and

DNA damage. This phenomenon was more pronounced when a combination of natural radiation and treatment with static magnetic field was applied. The results suggest that exposure to static magnetic field causes accumulation of reactive oxygen species in V. faba and natural radioactivity of soil exaggerates oxidative stress.

(E) Kim J, Ha CS, Lee HJ, Song K. Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Biochem Biophys Res Commun. 400(4):739-744, 2010. (GT)

We investigated the effects of extremely low frequency time-varying magnetic fields (MFs) on human normal and cancer cells. Whereas a single exposure to a 60-Hz time-varying MF of 6 mT for 30min showed no effect, repetitive exposure decreased cell viability. This decrease was accompanied by phosphorylation of γ -H2AX, a common DNA double-strand break (DSB) marker, and checkpoint kinase 2 (Chk2), which is critical to the DNA damage checkpoint pathway. In addition, repetitive exposure to a time-varying MF of 6 mT for 30 min every 24 h for 3 days led to p38 activation and induction of apoptosis in cancer and normal cells. Therefore, these results demonstrate that repetitive exposure to MF with extremely low frequency can induce DNA DSBs and apoptosis through p38 activation. These results also suggest the need for further evaluation of the effects of repetitive exposure to environmental time-varying MFs on human health.

(E) Kim J, Yoon Y, Yun S, Park GS, Lee HJ, Song K. Time-varying magnetic fields of 60 Hz at 7 mT induce DNA double-strand breaks and activate DNA damage checkpoints without apoptosis. Bioelectromagnetics. 33(5):383-393, 2012. (GT, WS)

The potential genotoxic effect of a time-varying magnetic field (MF) on human cells was investigated. Upon continuous exposure of human primary fibroblast and cervical cancer cells to a 60 Hz MF at 7 mT for 10-60 min, no significant change in cell viability was observed. However, deoxyribonucleic acid (DNA) double-strand breaks (DSBs) were detected, and the DNA damage checkpoint pathway was activated in these cells without programmed cell death (called apoptosis). The exposure of human cells to a 60 Hz MF did not induce intracellular reactive oxygen species (ROS) production, suggesting that the observed DNA DSBs are not directly caused by ROS. We also compared the position and time dependency of DNA DSBs with numerical simulation of MFs. The Lorentz force and eddy currents in these experiments were numerically calculated to investigate the influence of each factor on DNA DSBs. The DNA DSBs mainly occurred at the central region, where the MF was strongest, after a 30-min exposure. After 90 min, however, the amount of DNA DSBs increased rapidly in the outer regions, where the eddy current and Lorentz force were strong.

(NE) Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene Expression Profiles in White Blood Cells of Volunteers Exposed to a 50 Hz Electromagnetic Field. Radiat Res. 178(3): 138-149, 2012. (GE, HU)

Consistent and independently replicated laboratory evidence to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia has not been obtained. In particular, although gene expression responses have been reported in a wide variety of cells, none has emerged as robust, widely replicated effects. DNA microarrays facilitate comprehensive searches for changes in gene expression without a requirement to select candidate responsive genes. To determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF, each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of $62.0 \pm 7.1 \mu\text{T}$ for 2 h or to a sham exposure ($0.21 \pm 0.05 \mu\text{T}$) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure ($0.085 \pm 0.01 \mu\text{T}$) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/aprimidinic (AP) sites in A172 cells. Int J Radiat Biol. 84(1):53-59, 2008. (GT, IA)

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/aprimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. **MATERIALS AND METHODS:** The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (H₂O₂)) alone, or to an ELF magnetic field with the genotoxic agents. After exposure, DNA was extracted, and the number of AP sites was measured. **RESULTS:** There was no difference in the number of AP sites between cells exposed to an ELF magnetic field and sham controls. With MMS or H₂O₂ alone, the number of AP sites increased with longer treatment times. Exposure to an ELF magnetic field in combination with the genotoxic agents increased AP-site levels compared with the genotoxic agents alone. **CONCLUSIONS:** Our results suggest that the number of AP sites induced by MMS or H₂O₂ is enhanced by exposure to ELF magnetic fields at 5

millitesla (mT). This may occur because such exposure can enhance the activity or lengthen the lifetime of radical pairs.

(E) Lee JW, Kim MS, Kim YJ, Choi YJ, Lee Y, Chung HW. Genotoxic effects of 3 T magnetic resonance imaging in cultured human lymphocytes. Bioelectromagnetics. 32(7):535-542, 2011. (GT)

The clinical and preclinical use of high-field intensity (HF, 3 T and above) magnetic resonance imaging (MRI) scanners have significantly increased in the past few years. However, potential health risks are implied in the MRI and especially HF MRI environment due to high-static magnetic fields, fast gradient magnetic fields, and strong radiofrequency electromagnetic fields. In this study, the genotoxic potential of 3 T clinical MRI scans in cultured human lymphocytes in vitro was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis. Human lymphocytes were exposed to electromagnetic fields generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min. We observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. The frequencies of MN in lymphocytes exposed to complex electromagnetic fields for 0, 22, 45, 67, and 89 min were 9.67, 11.67, 14.67, 18.00, and 20.33 per 1000 cells, respectively. Similarly, the frequencies of CAs in lymphocytes exposed for 0, 45, 67, and 89 min were 1.33, 2.33, 3.67, and 4.67 per 200 cells, respectively. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (GE)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events

were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(E) Li SS, Zhang ZY, Yang CJ, Lian HY, Cai P. Gene expression and reproductive abilities of male *Drosophila melanogaster* subjected to ELF-EMF exposure. *Mutat Res.* 758(1-2):95-103, 2013. (GE, LE, RP)

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male *Drosophila melanogaster* were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-regulated following long-term exposures (expression >2- or <0.5-fold, respectively). In addition, there are 238 up-regulated genes and 598 down-regulated genes in the intersection of short-term and long-term exposure (expression >2- or <0.5-fold). The DEGs (differentially expressed genes) in *D. melanogaster* following short-term exposures were involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. Long-term exposure let to changes in expression of genes involved in metabolic processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration. In the intersection of short-term and long-term exposure, a series of DEGs were related to apoptosis, aging, immunological stress and reproduction. To check the ELF-EMF effects on reproduction, some experiments on male reproduction ability were performed. Their results indicated that short-term ELF-EMF exposure may decrease the reproductive ability of males, but long-term exposures had no effect on reproductive ability. Down-regulation of ark gene in the exposed males suggests that the decrease in reproductive capacity may be induced by the effects of ELF-EMF exposure on spermatogenesis through the caspase pathway. QRT-PCR analysis confirmed that jra, ark and decay genes were down regulated in males exposed for 1 Generation (1G) and 72 h, which suggests that apoptosis may be inhibited in vivo. ELF-EMF exposure may have accelerated cell senescence, as suggested by the down-regulation of both cat and jra genes and the up-regulation of hsp22 gene. Up-regulation of totA and hsp22 genes during exposure suggests that exposed flies might induce an in vivo immune response to counter the adverse effects encountered during ELF-EMF exposure. Down-regulation of cat genes suggests that the partial oxidative protection system might be restrained, especially during short-term exposures. This study

demonstrates the bioeffects of ELF-EMF exposure and provides evidence for understanding the in vivo mechanisms of ELF-EMF exposure on male *D. melanogaster*.

(E) Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. *Biochim Biophys Acta*. 1763(4):402-12, 2006.

(GE)

This study focused on the cell activating capacity of extremely low frequency magnetic fields (ELF-MF) on human umbilical cord blood-derived monocytes. Our results confirm the previous findings of cell activating capacity of ELF-MF (1.0 mT) in human monocytes, which was detected as an increased ROS release. Furthermore, gene expression profiling (whole-genome cDNA array Human Unigene RZPD-2) was performed to achieve a comprehensive view of involved genes during the cell activation process after 45 min ELF-MF exposure. Our results indicate the alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response. Significant regulations could be analyzed for 5 genes (expression >2- or <0.5-fold): IL15RA (Interleukin 15 receptor, alpha chain), EPS15R (Epidermal growth factor receptor pathway substrate 15 - like 1), DNMT3A (Hypothetical protein MGC16121), DNMT3A (DNA (cytosine-5) methyltransferase 3 alpha), and one gene with no match to known genes, DKFZP586J1624. Real-time RT-PCR analysis of the kinetic of the expression of IL15RA, and IL10RA during 45 min ELF-MF exposure indicates the regulation of cell activation via the alternative pathway, whereas the delayed gene expression of FOS, IL2RA and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes. Accordingly, we suggest that ELF-MF activates human monocytes via the alternative pathway.

(E) Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. *PLoS One*. 2011 Mar 23;6(3):e18021. (GT, IA)

BACKGROUND: Extremely low frequency (ELF) magnetic fields (MF) are generated by power lines and various electric appliances. They have been classified as possibly carcinogenic by the International Agency for Research on Cancer, but a mechanistic explanation for carcinogenic effects is lacking. A previous study in our laboratory showed that pre-exposure to ELF MF altered cancer-relevant cellular responses (cell cycle arrest, apoptosis) to menadione-induced DNA damage, but it did not include endpoints measuring actual genetic damage. In the present study, we examined whether pre-exposure to ELF MF affects chemically induced DNA damage level, DNA repair rate, or micronucleus frequency in human SH-SY5Y neuroblastoma cells.

METHODOLOGY/PRINCIPAL FINDINGS: Exposure to 50 Hz MF was conducted at 100 μ T for 24 hours, followed by chemical exposure for 3 hours. The chemicals used for inducing DNA damage and subsequent micronucleus formation were menadione and methyl methanesulphonate (MMS). Pre-treatment with MF enhanced menadione-

induced DNA damage, DNA repair rate, and micronucleus formation in human SH-SY5Y neuroblastoma cells. Although the results with MMS indicated similar effects, the differences were not statistically significant. No effects were observed after MF exposure alone. **CONCLUSIONS:** The results confirm our previous findings showing that pre-exposure to MFs as low as 100 μ T alters cellular responses to menadione, and show that increased genotoxicity results from such interaction. The present findings also indicate that complementary data at several chronological points may be critical for understanding the MF effects on DNA damage, repair, and post-repair integrity of the genome.

(E) Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. *Mutat Res.* 760:33-41, 2014. (GT, OX, IA)

Epidemiological studies have suggested that exposure to 50Hz magnetic fields (MF) increases the risk of childhood leukemia, but there is no mechanistic explanation for carcinogenic effects. In two previous studies we have observed that a 24-h pre-exposure to MF alters cellular responses to menadione-induced DNA damage. The aim of this study was to investigate the cellular changes that must occur already during the first 24h of exposure to MF, and to explore whether the MF-induced changes in DNA damage response can lead to genomic instability in the progeny of the exposed cells. In order to answer these questions, human SH-SY5Y neuroblastoma cells were exposed to a 50-Hz, 100- μ T MF for 24h, followed by 3-h exposure to menadione. The main finding was that MF exposure was associated with increased level of micronuclei, used as an indicator of induced genomic instability, at 8 and 15d after the exposures. Other delayed effects in MF-exposed cells included increased mitochondrial activity at 8d, and increased reactive oxygen species (ROS) production and lipid peroxidation at 15d after the exposures. Oxidative processes (ROS production, reduced glutathione level, and mitochondrial superoxide level) were affected by MF immediately after the exposure. In conclusion, the present results suggest that MF exposure disturbs oxidative balance immediately after the exposure, which might explain our previous findings on MF altered cellular responses to menadione-induced DNA damage. Persistently elevated levels of micronuclei were found in the progeny of MF-exposed cells, indicating induction of genomic instability.

(E) Ma Q, Deng P, Zhu G, Liu C, Zhang L, Zhou Z, Luo X, Li M, Zhong M, Yu Z, Chen C, Zhang Y. Extremely low-frequency electromagnetic fields affect transcript levels of neuronal differentiation-related genes in embryonic neural stem cells. *PLoS One.* 2014 Mar 3;9(3):e90041. doi: 10.1371/journal.pone.0090041. eCollection 2014. (GE)

Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) can affect the processes of brain development, but the underlying mechanism is largely unknown. The proliferation and differentiation of embryonic

neural stem cells (eNSCs) is essential for brain development during the gestation period. To date, there is no report about the effects of ELF-EMF on eNSCs. In this paper, we studied the effects of ELF-EMF on the proliferation and differentiation of eNSCs. Primary cultured eNSCs were treated with 50 Hz ELF-EMF; various magnetic intensities and exposure times were applied. Our data showed that there was no significant change in cell proliferation, which was evaluated by cell viability (CCK-8 assay), DNA synthesis (Edu incorporation), average diameter of neurospheres, cell cycle distribution (flow cytometry) and transcript levels of cell cycle related genes (P53, P21 and GADD45 detected by real-time PCR). When eNSCs were induced to differentiation, real-time PCR results showed a down-regulation of Sox2 and up-regulation of Math1, Math3, Ngn1 and Tuj1 mRNA levels after 50 Hz ELF-EMF exposure (2 mT for 3 days), but the percentages of neurons (Tuj1 positive cells) and astrocytes (GFAP positive cells) were not altered when detected by immunofluorescence assay. Although cell proliferation and the percentages of neurons and astrocytes differentiated from eNSCs were not affected by 50 Hz ELF-EMF, the expression of genes regulating neuronal differentiation was altered. In conclusion, our results support that 50 Hz ELF-EMF induce molecular changes during eNSCs differentiation, which might be compensated by post-transcriptional mechanisms to support cellular homeostasis.

(E) Mairs RJ, Hughes K, Fitzsimmons S, Prise KM, Livingstone A, Wilson L, Baig N, Clark AM, Timpson A, Patel G, Folkard M, Angerson WJ, Boyd M. Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro. *Mutat Res.* 626(1-2):34-41, 2007. (GT, IA)

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations. Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy gamma-irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation. Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hypermutability at low dose.

(E) Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, Aristei C, Ambrosini MV.

Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. *Int J Radiat Biol.* 86(8):701-710, 2010. (GT, LE)

PURPOSE: The question of whether exposure to extremely low frequency magnetic fields (ELF-MF), may contribute to cerebral cancer and neurodegeneration is of current interest. In this study we investigated whether exposure to ELF-MF (50 Hz-1 mT) harms cerebral DNA and induces expression of 70-kDa heat shock protein (hsp70). **MATERIALS AND METHODS:** CD1 mice were exposed to a MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h. Unexposed and sham-exposed mice were used as controls. Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. Food intake, weight gain, and motor activity were also evaluated. **RESULTS:** An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure, as compared to controls. DNA damage, as can be evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. Neither a short (15 h) nor long (7 days) MF-exposure induced hsp70 expression, metabolic and behavioural changes. **CONCLUSIONS:** These results indicate that in vivo ELF-MF induce reversible brain DNA damage while they do not elicit the stress response.

(E) Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. Int J Radiat Biol. 84(9):742-751, 2008. (IA)

PURPOSE: Effects on DNA damage response were investigated in murine L929 cells exposed to 50 Hz magnetic fields (MF) with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ). **MATERIALS AND METHODS:** Cells were exposed to MF at 100 or 300 microT combined with MQ (150 microM, 1 hour) or UVB radiation (160 J/m²) using various exposure schedules. The samples were stained with propidium iodide (PI) and analysed by flow cytometer for cell cycle stages. Apoptotic cells were defined as sub G(1) events. **RESULTS:** In cells first exposed to 100 microT MF for 24 h, the response to subsequent MQ treatment was significantly altered so that the proportion of sub G(1) cells was decreased and the proportion of cells in the G(2)/M phase was increased. When a 300 microT MF was used, also the proportion of cells in the G(1) phase was decreased. MF exposures after MQ treatment did not alter responses to MQ. No effects were found from MF exposure alone or from MF combined with UVB radiation. **CONCLUSIONS:** The results strengthen previous findings suggesting that pre-exposure to MF can alter cellular responses to other agents, and indicate that MF as low as 100 microT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.

(NE) Mizuno K, Narita E, Yamada M, Shinohara N, Miyakoshi J. ELF magnetic fields do not affect cell survival and DNA damage induced by ultraviolet B. Bioelectromagnetics. 35(2):108-115, 2014. (GT, IA)

We investigated whether extremely low frequency (ELF) magnetic field exposure has modification effects on cell survival after ultraviolet B (UV-B) irradiation and on repair

process of DNA damage induced by UV-B irradiation in WI38VA13 subcloned 2RA and XP2OS(SV) cells. The ELF magnetic field exposure was conducted using a Helmholtz coil-based system that was designed to generate a sinusoidal magnetic field at 5 mT and 60 Hz. Cell survival was assessed by WST assay after UV-B irradiation at 20-80 J/m², ELF magnetic field exposure for 24 h, followed by incubation for 48 h. DNA damage was assessed by quantification of cyclobutane pyrimidine dimer formation and 6-4 photoproduct formation using ELISA after UV-B irradiation at 20-80 J/m² followed by ELF magnetic field exposure for 24 h. No significant changes were observed in cell survival between ELF magnetic field and sham exposures. Similarly, DNA damage induced by UV-B irradiation did not change significantly following ELF magnetic field exposure. Our results suggest that ELF magnetic field exposure at 5 mT does not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. ASEB J 19(12):1686-1688, 2005. (GT, GE)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(NE) Okudan N, Celik I, Salbacak A, Cicekcibasi AE, Buyukmumcu M, Gökbel H. Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice. Neuro Endocrinol Lett. 31(2):208-214, 2010. (GT)

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs). **METHODS:** One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 microT 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined. **RESULTS:** ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 microT group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 microT-exposure group, and the level was quite similar to that of the 5 microT-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 microT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 microT-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner. **CONCLUSIONS:** The results of the present study suggest that ≤ 5 microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

(E) Panagopoulos DJ, Karabarbounis A, Lioliosis C. ELF alternating magnetic field decreases reproduction by DNA damage induction. Cell Biochem Biophys. 67(2):703-16, 2013. (LE, GT, RP)

In the present experiments, the effect of 50-Hz alternating magnetic field on *Drosophila melanogaster* reproduction was studied. Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The reproductive capacity was assessed by the number of F1 pupae according to a well-defined protocol of ours. The magnetic field was found to decrease reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis)-in contrast to exposure to microwave radiation of earlier work of ours in which the DNA fragmentation was induced at all developmental stages of early and mid-oogenesis. Moreover, the TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle

cells and also in the oocyte, in agreement with the microwave exposure of our earlier works. According to previous reports, cell death induction in the oocyte was observed only in the case of microwave exposure and not after exposure to other stress factors as toxic chemicals or food deprivation. Now it is also observed for the first time after ELF magnetic field exposure. Finally, in contrast to microwave exposure of previous experiments of ours in which the germarium checkpoint was found to be more sensitive than stage 7-8, in the magnetic field exposure of the present experiments the mid-oogenesis checkpoint was found to be more sensitive than the germarium.

(E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012;2012:716023. (LE, GT, DE, OX)

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic, cytotoxic hazards in brain and bone marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50 Hz, 0.5 mT for 30 days. The control group was treated as the exposed one with the sole difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-13, 2010. (GE)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed

animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ruiz-Gómez MJ, Sendra-Portero F, Martínez-Morillo M. Effect of 2.45 mT sinusoidal 50 Hz magnetic field on *Saccharomyces cerevisiae* strains deficient in DNA strand breaks repair. *Int J Radiat Biol.* 86(7):602-611, 2010. (GT)

PURPOSE: To investigate whether extremely-low frequency magnetic field (MF) exposure produce alterations in the growth, cell cycle, survival and DNA damage of wild type (wt) and mutant yeast strains. **MATERIALS AND METHODS:** wt and high affinity DNA binding factor 1 (hdf1), radiation sensitive 52 (rad52), rad52 hdf1 mutant *Saccharomyces cerevisiae* strains were exposed to 2.45 mT, sinusoidal 50 Hz MF for 96 h. MF was generated by a pair of Helmholtz coils. During this time the growth was monitored by measuring the optical density at 600 nm and cell cycle evolution were analysed by microscopic morphological analysis. Then, yeast survival was assayed by the drop test and DNA was extracted and electrophoresed. **RESULTS:** A significant increase in the growth was observed for rad52 strain ($P = 0.005$, Analysis of Variance [ANOVA]) and close to significance for rad52 hdf1 strain ($P = 0.069$, ANOVA). In addition, the surviving fraction values obtained for MF-exposed samples were in all cases less than for the controls, being the P value obtained for the whole set of MF-treated strains close to significance ($P = 0.066$, Student's t-test). In contrast, the cell cycle evolution and the DNA pattern obtained for wt and the mutant strains were not altered after exposure to MF. **CONCLUSIONS:** The data presented in the current report show that the applied MF (2.45 mT, sinusoidal 50 Hz, 96 h) induces alterations in the growth and survival of *S. cerevisiae* strains deficient in DNA strand breaks repair. In contrast, the MF treatment does not induce alterations in the cell cycle and does not cause DNA damage.

(E) Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. *Bioelectromagnetics.* 32(7):570-579, 2011. (GT)

Effects of magnetic field (MF) at 50 Hz on chromatin conformation were studied by the method of anomalous viscosity time dependence (AVTD) in human lymphocytes from two healthy donors. MF within the peak amplitude range of 5-20 μT affected chromatin conformation. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure.

Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study we show that individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 μ T may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

(E) Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med. 2014 Jan 24. [Epub ahead of print] (LE, GT, HU, OX)

There is apprehension about widespread use of electrical and electromagnetic gadgets which are supposed to emit electromagnetic radiations. Reports are controversy. These electromagnetic fields (EMFs) have considerable effect on endocrine system of exposed subjects. This study was focused to assess the possible bioeffects of extremely low-frequency (ELF)-EMFs on epinephrine level, DNA damage and oxidative stress in subjects occupationally exposed to 132 kV high-voltage substations. The blood sample of 142 exposed subjects and 151 non-exposed individuals was analyzed. Plasma epinephrine was measured by enzyme-linked immunosorbent assay, DNA damage was studied by alkaline comet assay along with oxidative stress. Epinephrine levels of sub-groups showed mean concentration of 75.22 ± 1.46 , 64.43 ± 8.26 and 48.47 ± 4.97 for high, medium and low exposed groups, respectively. DNA damage ranged between 1.69 μ m and 9.91 μ m. The oxidative stress levels showed significant increase. The individuals employed in the live-line procedures were found to be vulnerable for EM stress with altered epinephrine concentrations, DNA damage and increased oxidative stress.

(E) Udrouiu I, Cristaldi M, Ieradi LA, Bedini A, Giuliani L, Tanzarella C. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol. 82(8):561-567, 2006. (GT, DE, LE)

PURPOSE: To detect possible clastogenic and aneugenic properties of a 50 Hz, 650 μ T magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dysmotility, Sclerodactyly, Telangiectasia) antibody staining was performed on liver and peripheral blood sampled from newborn mice exposed to an ELF (Extremely Low Frequency) magnetic field during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood sampled from adult mice exposed to the same magnetic field for the same period.

RESULTS: Data obtained in newborn mice show a significant increase in micronuclei frequencies. In absolute terms, most of the induced micronuclei were CREST-negative (i.e., formed by a chromosome fragment). However, in relative terms, ELF exposure caused a two-fold increase in CREST-negative micronuclei and a four-fold increase in CREST-positive micronuclei (i.e., formed by a whole chromosome). No significant effect was recorded on exposed adults. **CONCLUSIONS:** These findings suggest the need for

investigation of aneugenic properties of ELF magnetic fields in order to establish a possible relationship to carcinogenesis.

(NE) Verschaeve L, Anthonissen R, Grudniewska M, Wudarski J, Gevaert L, Maes A. Genotoxicity investigation of ELF-magnetic fields in Salmonella typhimurium with the sensitive SOS-based VITOTOX test. Bioelectromagnetics. 32(7):580-584, 2011. (GT, IA)

We performed a genotoxicity investigation of extremely low-frequency (ELF) magnetic fields (MFs, 50 Hz, 100 and 500 μ T, 1 and 2 h exposure) alone and in combination with known chemical mutagens using the VITOTOX test. This test is a very sensitive reporter assay of Salmonella typhimurium bacteria based on the SOS response. Our study showed that ELF-MFs do not induce SOS-based mutagenicity in S. typhimurium bacteria and do not show any synergetic effect when combined with chemical mutagens.

(E) Villarini M, Ambrosini MV, Moretti M, Dominici L, Taha E, Piobbico D, Gambelunghe C, Mariucci G. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study. Int J Radiat Biol. 89(7):562-570, 2013. (LE, GT)

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

(E) Wahab MA, Podd JV, Rapley BI, Rowland RE. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 50 Hz magnetic fields. Bioelectromagnetics. 28(4):281-288, 2007. (GT, WS)

The in vitro cytomolecular technique, sister chromatid exchange (SCE), was applied to test the clastogenic potentiality of extremely low frequency (ELF) electromagnetic fields (EMFs) on human peripheral blood lymphocytes (HPBLs). SCE frequencies were scored in dividing peripheral blood lymphocytes (PBLs) from six healthy male blood donors in

two rounds of experiments, R1 and R2, to determine reproducibility. Lymphocyte cultures in the eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1 microT or 1 mT for 72 h. A significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls was observed in both rounds. The highest SCE frequency in R1 was 10.03 for a square continuous field, and 10.39 for a square continuous field was the second highest frequency in R2. DNA crosslinking at the replication fork is proposed as a model which could explain the mechanistic link between ELF EMF exposure and increased SCE frequency.

(E) Wang Z, Sarje A, Che PL, Yarema KJ. Moderate strength (0.23-0.28 T) static magnetic fields (SMF) modulate signaling and differentiation in human embryonic cells. BMC Genomics. 10:356, 2009. (GE)

BACKGROUND: Compelling evidence exists that magnetic fields modulate living systems. To date, however rigorous studies have focused on identifying the molecular-level biosensor (e.g., radical ion pairs or membranes) or on the behavior of whole animals leaving a gap in understanding how molecular effects are translated into tissue-wide and organism-level responses. This study begins to bridge this gulf by investigating static magnetic fields (SMF) through global mRNA profiling in human embryonic cells coupled with software analysis to identify the affected signaling pathways. **RESULTS:** Software analysis of gene expression in cells exposed to 0.23-0.28 T SMF showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine IL-6. We found the short-term (<24 h) activation of IL-6 involved the coordinate up-regulation of toll-like receptor-4 (TLR4) with complementary changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 in a manner that augmented the activation of TLR4 and IL-6. Loss of GM3 also provided a plausible mechanism for the attenuation of cellular responses to SMF that occurred over longer exposure periods. Finally, SMF-mediated responses were manifest at the cellular level as morphological changes and biochemical markers indicative of pre-oligodendrocyte differentiation. **CONCLUSION:** This study provides a framework describing how magnetic exposure is transduced from a plausible molecular biosensor (lipid membranes) to cell-level responses that include differentiation toward neural lineages. In addition, SMF provided a stimulus that uncovered new relationships - that exist even in the absence of magnetic fields - between gangliosides, the time-dependent regulation of IL-6 signaling by these glycosphingolipids, and the fate of embryonic cells.

(NE) Williams PA, Ingebretsen RJ, Dawson RJ. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (GT)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress.

Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (*recA*). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

(E) Yokus B, Akdag MZ, Dasdag S, Cakir DU, Kizil M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int J Radiat Biol. 84(10):789-795, 2008. (GT)

PURPOSE: To detect the genotoxic effects of extremely low frequency (ELF) -magnetic fields (MF) on oxidative DNA base modifications [8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] in rat leucocytes, measured following exposure to ELF-MF. **MATERIALS AND METHODS:** After exposure to ELF-MF (50 Hz, 100 and 500 microT, for 2 hours/day during 10 months), DNA was extracted, and measurement of DNA lesions was achieved by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). **RESULTS:** Levels of FapyAde, FapyGua and 8OHdG in DNA were increased by both 100 microT and 500 microT ELF-MF as compared to a cage-control and a sham group; however, statistical significance was observed only in the group exposed to 100 microT. **CONCLUSION:** This is the first study to report that ELF-MF exposure generates oxidatively induced DNA base modifications which are mutagenic in mammalian cells, such as FapyGua, FapyAde and 8-OH-Gua, in vivo. This may explain previous studies showing DNA damage and genomic instability. These findings support the hypothesis that chronic exposure to 50-Hz MF may be potentially genotoxic. However, the intensity of ELF-MF has an important influence on the extent of DNA damage.

(E) Yoon HE, Lee JS, Myung SH, Lee YS. Increased γ -H2AX by exposure to a 60-Hz magnetic fields combined with ionizing radiation, but not hydrogen peroxide, in non-tumorigenic human cell lines. Int J Radiat Biol. 2014 Jan 28. [Epub ahead of print] (GT, IA)

Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate

genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H₂O₂ on the DNA damage response of expression of phosphorylated H2AX (γ -H2AX) and production of γ -H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased γ -H2AX expression, as well as γ -H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of γ -H2AX and γ -H2AX foci production when combined with IR, but not when combined with H₂O₂. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on γ -H2AX.

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Exhibit F: An Update on Physical and Biological Variables, Cancer and Safety Standards by Igor Belyaev, Dr.Sc., Cancer Research Institute Slovak Academy of Sciences, Slovak Republic

This review is divided into comments on two separate sections, one for extremely-low frequency (ELF) and the other for radiofrequency (RFR) studies. Comments are presented to address deficiencies in the Preliminary Opinion on EMF issued by the SCENIHR Committee. The comments are relevant to sections of the BioInitiative Working Group letter including brain tumors, oxidative damage, genomic instability, mitochondrial damage, carcinogenic classifications, biological plausibility and methodological deficiencies.

Comments on ELF Sections

ELF Carcinogenicity

Page 131 of the SCENIHR provides misleading and flawed conclusions on ELF and neoplastic diseases. As a matter of fact, the increased risk of childhood leukemia with daily average exposure above 0.3 to 0.4 μT is as strong as never before. All available studies from Europe, America and Asia consistently show such correlation. It has been further supported by recent meta-analysis by Zhao et al. (Zhao, Liu et al. 2014). The statement of lack of mechanisms for ELF effects is wrong. Recent studies provided more evidence for such mechanisms even if they have not been comprehensively studied, see below. Considerations of ELF carcinogenicity in the SCENIHR report did not use standard methods such as the Bradford Hill criteria which do not require complete knowledge of mechanisms in case when epidemiological evidence is overwhelming as in case of childhood leukemia (Zhao, Liu et al. 2014).

Similar to effects of MW, the ELF effects depend on variety of parameters that should be taken into account and have not been considered by the SCENIHR report when comparing data from different studies.

Baldi et al analyzed the relationship between residential and occupational exposure to electromagnetic field and brain tumors in adults (Baldi, Coureau et al. 2010). A case-control study was carried out in southwestern France between May 1999 and April 2001. A total of 221 central nervous system tumors (105 gliomas, 67 meningiomas, 33 neurinomas and 16 others) and 442 individually age- and sex-matched controls selected from general population were included. Electromagnetic field exposure to ELF and radiofrequency separately was assessed in occupational settings through expert judgment based on complete job calendar, and at home by assessing the distance to power lines. Confounders such as education, use of home pesticide, residency in a rural area and occupational exposure to chemicals were taken into account. Separate analyses were performed for gliomas, meningiomas and acoustic neurinomas. A nonsignificant increase in risk was found for occupational exposure to electromagnetic fields [odds ratio (OR = 1.52, 0.92-2.51)]. This increase became significant for meningiomas, especially when considering ELF separately [OR = 3.02; 95 percent confidence interval (95% CI) = 1.10-8.25]. The risk of meningioma was also higher in subjects living in the vicinity of power

lines (<100 m), even if not significant (OR = 2.99, 95% CI 0.86-10.40). These data suggest that occupational or residential exposure to ELF may play a role in the occurrence of meningioma. The insignificance of data obtained in group RF+ELF is well explained by majority of RF data showing no significant relationship of RF exposure with increased risks of meningioma (Carlberg, Soderqvist et al. 2013).

ELF affects cell proliferation

In line with many previous studies, new studies unmentioned in the SCENIHR report provide further evidence that ELF can affect cell proliferation under specific conditions of exposure (Segatore, Setacci et al. 2012; Bae, Do et al. 2013; Jadidi, Safari et al. 2013). Bai et al. investigated ELF effects on proliferation of epidermal stem cells (ESC) (Bai, Zhang et al. 2012). The ESC obtained from human foreskin were grafted into type-I three-dimensional collagen sponge scaffolds, and then were exposed with EMF (frequency 50 Hz, intensity 5 mT) for 14 days, 30 min daily. The effects of EMF on growth and proliferation of ESC were analyzed with staining of hematoxylin and eosin (H&E) and 4',6-diamidino-2-phenylindole (DAPI) under microscope or scanning electron microscope. The data of DAPI staining for 2 d, 7 d, 10 d and 14 d were collected respectively to investigate the cells proliferation. EMF promoted ESC proliferation compared with controls.

Belyaev analyzed the effect of ELF-MF on chromatin conformation in *E. coli* GE499 cells using anomalous viscosity time dependence technique (AVTD) (Belyaev 2011). Possible genotoxic effects of the specific combination of static and ELF-MF, which has been proven to affect chromatin conformation, were investigated by a clonogenic assay, by assessing cell-growth kinetics, and by analysis of the SOS-response by means of inducible *recA-lacZ* fusion-gene products and the beta-galactosidase assay. The genotoxic agent nalidixic acid (NAL) was used as a positive control and in combination with ELF-MF. Nalidixic acid decreased AVTD and induced a cytotoxic effect. In contrast to NAL, ELF-MF fields increased AVTD, stimulated cell growth, and increased cloning efficiency. In line with many previous studies, these effects depended on the frequency within the range of 7-11Hz. While NAL induced an SOS-response, exposure to ELF-MF did not induce the *recA-lacZ* fusion-gene product. Exposure to ELF-MF did not modify the genotoxic effects of NAL either. All together, the data show that ELF-MF, under specific conditions of exposure, acted as a non-toxic but cell-growth stimulating agent.

Cid et al verified hypothesis that ELF MF effect on cancer progression could be mediated by MF-induced effects on the cellular response to melatonin (MEL), a potentially oncostatic neurohormone (Cid, Ubeda et al. 2012). HepG2 cells were exposed to intermittent 50 Hz, 10 microT MF, in the presence or absence of MEL at physiological (10 nM) or pharmacological doses (1 microM). The results indicated that the MF exerts significant cytoproliferative and dedifferentiating effects that can be prevented by 10 nM MEL. Conversely, MEL exerts cytostatic and differentiating effects on HepG2 that are abolished by simultaneous exposure to MF.

Dependence of ELF effects on number of physical and biological parameters

The SCENIHR report did not take into account dependence of ELF effects on number of physical and biological parameters when comparing the data from different studies. This is in significant contrast with generally accepted methodology which requires considering a number of such parameters which include cell type, frequency, intensity (Belyaev, Alipov et al. 1999; Belyaev and Alipov 2001; Shcheglov, Alipov et al. 2002) and which are similarly important for the MW effects (IARC 2013). Due to this fundamental flaw, incorrect comparisons of studies, which used completely different parameters were performed in the SCENIHR report. For example, negative study by (Buldak et al., 2012) was opposed to positive study (Luukkonen et al. 2011) on Page 164-165. Significant and decisive differences between these studies include exposure time (24 h in (Luukkonen et al. 2011) versus 16 min in (Buldak et al., 2012)), cell type (human neuroblastoma SHSY5Y cells (Luukkonen et al. 2011) versus AT478 murine carcinoma cells (Buldak et al., 2012)). Recent study by the same authors confirmed and further extended evidence that prolonged exposure to ELF of human neuroblastoma SHSY5Y cells induce reactive oxygen species (ROS) and genomic instability (Luukkonen, Liimatainen et al. 2014).

Fijałkowski et al. analyzed effects of the rotating magnetic field (RMF, $f = 1-50$ Hz, RMF magnetic induction $B = 22-34$ mT, ptime of exposure $t = 60$ min, temperature of incubation 37 °C) on the growth rate, cell metabolic activity and ability to form biofilms by *E. coli* and *S. aureus* (Fijałkowski, Nawrotek et al. 2013). RMP exposure increased the growth dynamics, cell metabolic activities and percentage of biofilm-forming bacteria in both *S. aureus* and *E. coli* cultures. In line with many other studies, it was found that the RMF effects depended on frequencies and magnetic induction.

Sarimov et al have reported that magnetic field (MF) at 50 Hz within the peak amplitude range of 5-20 microT affected chromatin conformation in human lymphocytes from two healthy donors. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure. Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 microT were observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

ELF induced ROS and genomic instability

Induction ROS and is generally considered as a candidate mechanism for carcinogenicity for EMF (IARC 2013). Several recent studies unmentioned in the SCENIHR report provided further evidence for this mechanism in case of ELF exposure (Duan, Wang et al. 2013; Khaki, Khaki et al. 2013).

Duan et al. exposed mice to ELF-EMF at 50 Hz, 8 mT, 28 days (Duan, Wang et al. 2013). A water maze test indicated that ELF-EMF exposure deteriorated significantly learning and memory abilities as compared with the control group. Administration of

lotus seedpod procyanidins (LSPCs) had remarkably improved learning and memory abilities in exposed animals compared with the ELF-EMF group. ELF-EMF exposure significantly increased malondialdehyde (MDA), reactive oxygen species (ROS), nitric oxide (NO) and nitric oxide synthase (NOS), while the activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) were decreased significantly. Along with improved learning and memory abilities in exposed animals, LSPCs administration effectively prevented oxidative damage caused by the ELF-EMF, most likely through the ability of LSPCs to scavenge oxygen free radicals and to stimulate antioxidant enzyme activity. The majority of experimental studies (9 out of 10 animal studies) show oxidative stress induced by ELF in brain (Consales, Merla et al. 2012).

Mechanisms for effects of weak ELF

While all mechanisms of ELF effects are not known with certainty, new important data emerged about these mechanisms which were neglected by the SCENIHR report. For ELF fields, these mechanisms involve magnetoreception of fields in the μT -range which is observed in many studied animals including lizards (Nishimura, Okano et al. 2010). It should be stressed that the lack of precise knowledge for this mechanism (radical pairs and magnetite are mainly considered) does not preclude general acceptance of these phenomena. In analogy, and in accordance to the Bradford Hill criteria, lack of precise knowledge on mechanism for leukemogenesis of weak ELF $\geq 0.3 \mu\text{T}$, which was consistently shown in children in multiple studies (Zhao, Liu et al. 2014) should not preclude classification of μT -range ELF as an IARC carcinogen group 1.

The SCENIHR report completely neglects variety of mechanisms based on ELF effects on ions (Halgamuge and Abeyrathne 2011; Foletti, Grimaldi et al. 2013). Despite physical differences in and incompleteness of these mechanisms all of them relate ELF effects with ion cyclotron resonance frequencies and their harmonics/subharmonics (Belyaev and Alipov 2001; Sarimov, Markova et al. 2005). Poniedzialek et al. analyzed ELF effects on reactive oxygen species (ROS) production in human neutrophils in peripheral blood in vitro (Poniedzialek, Rzymiski et al. 2013). Two fluorescent dyes were used: 2',7'-dichlorofluorescein-diacetate and dihydrorhodamine. Phorbol 12-myristate 13-acetate (PMA), known as strong stimulator of the respiratory burst, was also used. Three different levels of magnetic induction have been analyzed: 10, 40 and 60 μT . The experiments demonstrated that only EMF tuned to the calcium ion cyclotron resonance frequency was able to affect ROS production in neutrophils. Statistical analysis showed that this effect depended on magnetic induction value of applied EMF.

ELF section omits significant number of ELF positive studies

Except for aforementioned studies, ELF section of the SCENIHR report omits significant number of other ELF positive studies. These include but not limited to (Mariucci, Villarini et al. 2010; Nishimura, Okano et al. 2010; Ravera, Bianco et al. 2010; Severini, Bosco et al. 2010; Ulku, Akdag et al. 2011; Bai, Zhang et al. 2012; Ince, Akdag et al. 2012; Martirosyan 2012; Portelli, Madapatha et al. 2012; Balassa, Varro et al. 2013; Gang, Parker et al. 2013; Iorio, Bennato et al. 2013; Kang, Hong et al. 2013; Khaki, Khaki et al. 2013; Li, Zhang et al. 2013; Martirosyan, Baghdasaryan et al. 2013;

Panagopoulos, Karabarbounis et al. 2013; Shams Lahijani, Tehrani et al. 2013; Villarini, Ambrosini et al. 2013).

Mariucci et al exposed CD1 mice to ELF MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h (Mariucci, Villarini et al. 2010). Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure. This damage, evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. The results indicate that in vivo ELF-MF exposure induces transient brain DNA damage did not induce hsp70. Importantly, these results were further replicated by the same research group (Villarini, Ambrosini et al. 2013).

Ulku et al. investigated a set of elements in costa of rats chronically exposed to ELF-MF, 100 and 500 μ T, 2 h/day during 10 months (Ulku, Akdag et al. 2011). The levels of elements were measured by using atomic absorption spectrophotometry (AAS) and ultraviolet (UV) spectrophotometry. Ca levels decreased in the ELF-500 exposure group in comparison to sham group ($p < 0.05$). Statistically significant decrease was found in Mg levels in the ELF-500 exposure group in comparison to sham and ELF-100 exposure groups ($p < 0.05$). Zn levels were found to be lower in the ELF-500 exposure group than those in the sham and ELF-100 exposure groups ($p < 0.05$). No significant differences were determined between groups in terms of the levels of P, Cu and Fe. Thus, long-term ELF-MF exposure could change the levels of some important elements such as Ca, Zn and Mg in rat bones.

Balassa et al. analyzed effects of a long-term ELF-MF (0.5 and 3 mT, 50 Hz) exposure on synaptic functions in the developing brain (Balassa, Varro et al. 2013). Rats were chronically exposed to MF during two critical periods of brain development, i.e. in utero during the second gestation week or as newborns for 7 days starting 3 days after birth, respectively. Excitability and plasticity of neocortical and hippocampal areas were tested on brain slices by analyzing extracellular evoked field potentials. The basic excitability of hippocampal slices (measured as amplitude of population spikes) was increased by both types of treatment (fetal 0.5 mT, newborn 3 mT). Neocortical slices seemed to be responsive mostly to the newborn treatment, the amplitude of excitatory postsynaptic potentials was increased. Fetal ELF-MF exposure significantly inhibited the paired-pulse depression (PPD) and there was a significant decrease in the efficacy of LTP (long-term potentiation induction) in neocortex, but not in hippocampus. On the other hand, neonatal treatment had no significant effect on plasticity phenomena. Results demonstrated that ELF-MF has significant effects on basic neuronal functions and synaptic plasticity in brain slice preparations originating from rats exposed either in fetal or in newborn period.

Gang et al. exposed planarian to either 140 or 400 nT peak amplitude-modulated 7 Hz magnetic fields for 6 min once per hour, 8 h per night for 5 days (Gang, Parker et al. 2013). The planarian exposed to either intensity magnetic field exhibited faster

regeneration of photoreceptors and auricles compared to sham field and reference groups. The magnetic field exposure accommodated 50% of the variance during the faster growth days. Authors concluded that naturally-patterned, intermittently-presented weaker electromagnetic fields may produce enhanced regeneration rates in flat worms similar to those observed for 60 Hz, higher intensity fields.

Severini et al. exposed cohorts of *Xenopus laevis laevis* (Daudin) tadpoles during their immature period (approximately 60 days) to a 50 Hz magnetic field of $63.9 \leq B \leq 76.4$ microT rms (root mean square, average values) magnetic flux density (Severini, Bosco et al. 2010). Mean developmental rate of ELF-exposed cohorts was reduced with respect to controls (0.43 vs. 0.48 stages/day, $p < 0.001$) starting from early larval stages. Exposure increased the mean metamorphosis period of tadpoles by 2.4 days compared with the controls ($p < 0.001$). Maturation rates of exposed and control tadpoles changed during maturation period. Important mortality, deformations or teratogenic effects were not observed in exposed matured tadpoles. Authors concluded that a long-term exposure of *X. laevis* tadpoles to a relatively weak 50 Hz magnetic field causes a sub-lethal effect that slows down their larval developmental rate and delays their metamorphosis.

Panagopoulos et al studied the effect of 50-Hz alternating magnetic field on *Drosophila melanogaster* reproduction (Panagopoulos, Karabarbounis et al. 2013). Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The magnetic field decreased reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis). The TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle cells and also in the oocyte.

Kang et al. analyzed specific electromagnetic field conditions (frequency and magnetic flux density) which significantly regulate osteogenic differentiation of adipose-derived stem cells (ASCs) (Kang, Hong et al. 2013). Before inducing osteogenic differentiation, ASC stemness was determined and uniform electromagnetic field was created using the solenoid coil. Then, authors selected positive (30/45 Hz, 1mT) and negative (7.5 Hz, 1mT) osteogenic differentiation conditions by quantifying alkaline phosphatase (ALP) mRNA expression. Osteogenic marker (runt-related transcription factor 2) expression was higher in the 30/45Hz condition and lower in the 7.5 Hz condition as compared with the nonexposed group. Both positive and negative regulation of ALP activity and mineralized nodule formation supported these responses. The data indicated that the ELF effects on osteogenic differentiation differ depending on the electromagnetic field

conditions and thus provided evidence that ELF can control stem cell differentiation depending on frequency and intensity.

Iorio et al. investigated whether ELF-MF could affect myoblast migration (Iorio, Bennato et al. 2013). ELF-MF (1 mT; 50 Hz) resulted in a transient but significant increase of myoblast migration. This effect was associated with a marked increase of μ - and m-calpain activity followed by the concomitant variation in their subcellular localization. No significant changes in intracellular distribution and protein levels of calpastatin were detected. A significant decrease of myristoylated alanine-rich C-kinase substrate (MARCKS) expression and modifications of actin dynamics were reported. This study provided evidence for involvement of calpains in ELF-MF-mediated myoblast migration.

Page 129, line 26-27. This statement misleads the reader who is not expert in effects of weak EMF to judge results as nonreplicable. In fact, ELF effects similar to MW effects depend on cell type (Belyaev 2010) and this study just provides further support for this dependence. In addition, reference to (Focke, Schuermann et al. 2010) is missing in Reference list.

Page 130. Study of Girgert et al (Girgert, Hanf et al. 2010) is erroneously marked as Girgert et al 2009 and reference if not provided in the Reference list.

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Exhibit F: An Update on Physical and Biological Variables, Cancer and Safety Standards by Igor Belyaev, Dr.Sc., Cancer Research Institute Slovak Academy of Sciences, Slovak Republic

Comments on RFR Sections

Main conclusions on health effects from RFR fields

1. The positive and negative studies were selected by unclear criteria, which (i) are different from those generally accepted and used by IARC and (ii) resulted in omission of majority of positive findings and almost all laboratory studies which were performed using conditions of EMF exposure similar as general public is exposed (see text below and reference list).
2. The report shows fundamental flaw in assessment of mechanisms for non-thermal EMF effects.
3. Analysis of data seem to be biased in favor of negative studies and negative interpretations.

Flawed assessment of negative studies

The main fundamental flaw of the report is neglecting the mechanistic data on non-thermal (NT) effects of microwaves (MW). As reported in multiple studies, these effects depend on variety of biological and physical parameters including polarization, frequency, modulation and environmental EMF (see (Belyaev 2010) and (IARC 2013)). The *in vitro* and *in vivo* studies included in the preliminary Opinion are largely negative studies only. Moreover, negative studies cannot be directly compared to positive studies if the exposure was performed under different conditions as it almost always done. Thus, obtained so far data of negative studies cannot be extrapolated to all real cell phone signals. The negative studies cannot neither dismiss positive studies, which were performed under other conditions, nor provide evidence for safety of majority of signals used for mobile communication. The reported "inconsistency" of *in vitro* and *in vivo* data (see for example page 120) and "conflicting results" (see for example page 121) has at least one simple explanation because the studies were performed under different conditions. Thus, results of most studies cannot be directly compared and conclusion by the SCENIHR report on inconsistency. Conflicting results instead reflect the level of superficial analysis. Another fundamental flaw deals with neglecting many studies showing dependence of the NT MW effects on exposure duration or dose (defined in radiation physics as multiplication of SAR on exposure duration), see (Belyaev 2010). In addition to laboratory studies, when brain cancer risk was epidemiologically examined as a function of dose received in different time windows before diagnosis, increasing trend was observed with increasing RFR dose, for exposures 7 years or more in the past (Cardis, Armstrong et al. 2011). This study provided straightforward evidence for one of most important Bradford Hill criteria - dependence on dose.

Another important parameter is intermittence of exposure which involves interaction with adaptation mechanisms and accumulative effects of NT MW. Chavdoula et al. used a 6 min daily exposure of dipteran flies, *Drosophila melanogaster*, to GSM-900 MHz mobile phone electromagnetic radiation (EMR), to compare the effects between continuous and four different intermittent exposures of 6 min total duration on the insect's reproductive capacity as well as on the induction of apoptosis (Chavdoula, Panagopoulos et al. 2010). It was found that intermittent exposure, similar to continuous exposure, decreases the reproductive capacity and alters the actin-cytoskeleton network of the egg chambers, another known aspect of cell death, and that this effect is due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be nearly equally effective as continuous exposure of the same total duration, however, longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the described effects of the GSM exposure.

The preliminary Opinion bases its conclusions mostly on SAR value, which is a main parameter for thermal MW effects but has much less value for NT MW to which general public is exposed to (Belyaev 2010; Panagopoulos, Johansson et al. 2013).

RFR epidemiologic evidence for carcinogenicity

The SCENIHR preliminary Opinion has conclusions on brain cancer that are heavily based on the Danish subscriber cohort study of mobile phone subscribers. However this study has not assessed exposure, has been heavily criticized and thus far is inconclusive. This study is not informative even according to the requirement of this SCENIHR reports : "*The minimum requirement for exposure assessment for an epidemiological study to be informative is to include reasonably accurate individual exposure characterization over a relevant period of time capturing all major sources of exposure for the pertinent part of the body*" (page 10). The preliminary Opinion is internally inconsistent with this requirement as the authors have based their review largely on epidemiological studies, where individual exposure was not accurately assessed. These studies include those coauthored by Dr Schüz who is one of the authors for this SCENIHR report. For example, the UK Million women study (Benson et al 2013) included only two simple questions regarding usage of mobile phone which cannot estimate individual exposure in any reasonable degree. Following the general bias of this report in favor of negative finding, the authors forgot to state that this study found statistically significant increase of acoustic neuroma for long term users vs never users (10+ years: RR = 2.46, 95% CI = 1.07–5.64, $P = 0.03$), the risk increasing with duration of use (trend among users, $P = 0.03$).

Another example is the underestimation of importance of the positive findings of de Vocht et al (2013) on global link of mobile phone usage and brain cancer. "*The study is not informative for causal inference, as popular use of mobile phones can also reflect standard of living, which is also associated with, for example, availability of diagnostic services*". The SCENIHR's preliminary Opinion did not mention that this statement is relevant to most negative studies and especially to the Danish subscriber cohort study upon which this preliminary Opinion heavily relies. In contrast, the meta-analyses of studies which included only data on ipsilateral tumors in subjects using mobile phones

for at least 10 years, show large and statistically significant increases in risk of ipsilateral brain gliomas and acoustic neuromas (Levis, Minicuci et al. 2011). The risk of head tumors was nearly doubled and was induced by long-term mobile phone use.

Consideration of the data on childhood cancers in relation to base stations is also biased in favor of weighting negative studies. While limitation of positive study by (Li et al. 2012) is provided, no limitations of negative study by (Elliott et al. 2010) is considered in contrast to about one-page description of such limitations provided by the authors (Elliott et al. 2010). In addition, the report did not provide the main positive result of the (Li et al. 2012) study which has shown increased (brain+leukemia) incidence related to base stations.

Brain cancer time trend analysis

The SCENIHR report provides biased consideration of available information. It should be noted that histology analysis and localization of tumors in respect to irradiation from mobile phone is of key importance for this analysis.

At the time of IARC meeting in 2011 the following data were available and included into the IARC monograph (IARC 2013):

USA

According to data collected by the Surveillance, Epidemiology, and End Results (SEER) Program, age- and sex-specific trends and overall temporal trends in rates of incidence of brain cancer in the USA were flat or downward between 1992 and 2006, with the exception of women aged 20–29 years (Inskip *et al.*, 2010). In this age group, a statistically significant increasing trend was driven by the rising incidence in tumors of the frontal lobe. [It is the temporal lobe that is most heavily exposed to radiation when using a mobile phone at the ear (Cardis *et al.*, 2008).] Incidence of brain cancer in USA "could be consistent with the modest excess risks in the Interphone study" (Little, Rajaraman et al. 2012).

UK

Overall rates of incidence of cancer of the brain in males or females, or in any specific age group were not increased in England between 1998 and 2007 (de Vocht, Burstyn et al. 2011). For men and women, the incidence of tumors (primarily glioma) was increased ($p < 0.01$) in the temporal lobe that is most heavily exposed to radiation when using a mobile phone at the ear (Cardis, Deltour et al. 2008). The incidence increased also in frontal lobe for men ($p < 0.01$) and in the frontal lobe for women, although not statistically significant ($p = 0.07$). The incidence decreased in other parts of the brain. In a subsequent paper, the same authors reported separate time trends for cancers of the temporal lobe in the periods 1979–99 and 2000–08 (de Vocht, Burstyn et al. 2011). For men, a linear regression of age-adjusted rates showed an overall annual increase in 2000–2008 of 3.3% (95% CI, 1.1–5.4), whereas it was lower 2.0% (95% CI, 1.4–2.6) for 1979–1999. For women, a linear regression of age-adjusted rates showed an overall annual increase in 2000–2008 of 2.8% (95% CI, 0.9–4.8), whereas it was lower 1.4% (95% CI, 0.7–2.2) for 1979–1999. This change may be suggestive of increased rates for

brain cancers of the temporal lobe in the recent years. [The linear regression used for this analysis was not an appropriate method and therefore the 95% confidence intervals reported may not be reliable.] p.190

After the IARC meeting in 2011 the following data were available

USA

Zada et al. studied incidence trends of primary malignant brain tumors in the Los Angeles area during 1992-2006 (Zada, Bond et al. 2012). Incidence data for histologically-confirmed brain tumors were obtained from the Los Angeles County Cancer Surveillance Program (LAC), the California Cancer Registry (CCR), and the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program for 1992 to 2006. Annual percentage change (APC) was calculated for microscopically confirmed histological subtypes and anatomic sub sites. The overall incidence of primary malignant brain tumors decreased over the time period with the exception of glioblastoma multiforme (GBM) (astrocytoma grade IV). The annual age adjusted incidence rate of that tumor type increased statistically significant in the frontal lobe with APC +2.4 % to +3.0 % ($p < 0.001$) and temporal lobe APC +1.3 % to +2.3 % ($p < 0.027$) across all registries. In the California Cancer Registry the incidence of glioblastoma multiforme increased also in cerebellum, APC +11.9 % ($p < 0.001$). In the parietal and occipital lobes or in overlapping lobes no statistically significant changes in incidence were seen. For lower grade astrocytoma decreases of annual age adjusted incidence rates were observed. The authors concluded that despite decreased incidences in other brain regions there was an increase in the incidence of glioblastoma multiforme in frontal and temporal lobes and cerebellum. These parts of the brain are characterized by highest absorbed dose of radiation from mobile phones (Cardis, Deltour et al. 2008; Deltour, Wiart et al. 2011).

China

Ding et al. (Ding and Wang 2011) investigated time trends in the incidence of brain and nervous tumor in urban Shanghai, from 1983 to 2007, applying joinpoint regression models to analyze the annual incidence rates. From 1983 to 2007, the age-adjusted incidence rate of brain and nervous tumors increased gradually by 1.2% per year (95% confidence interval [CI] = 0.4% to 1.9%) among men and 2.8% per year (95% CI = 2.1 to 3.4) among women. While the authors concluded that this study did not support an association between cellular telephone use and increased risk of brain and nervous tumors, the conclusion was made on assumption about *latency periods shorter 5-10 years*. Authors themselves recognize that this conclusion is not valid for longer latency periods, which are indeed predictable for gliomas and acoustic neuromas. Thus, authors do not take into account that radiation induced glioma (RIG) studies would reasonably not show so soon, given significantly higher latency periods. Common conclusions reached across diverse cases on RIG is that mean latency time was in the order of many years (*range: 9-17 years*) (Prasad and Haas-Kogan 2009). Thus, while the incidence rate has been shown to be increased in urban Shanghai, the conclusion of the authors on lack of association with mobile phones is flawed.

Australia

A multicenter study was performed to determine the brain cancer incidence in Australia (the state of New South Wales (NSW) and the Australian Capital Territory (ACT)) with age-, sex-, and benign-versus-malignant histology-specific analyses (Dobes, Shadbolt et al. 2011). One hundred percent of tumors were histologically confirmed. Data were weighted for patient outflow and *data completeness*. Incidence rates were age standardized and trends analyzed using joinpoint analysis. An overall significant increase in primary malignant brain tumors was observed over the study period from 2000 to 2008 (APC, 3.9; 95% CI, 2.4–5.4). Overall increasing trend in malignant tumors was consistent for both males (APC, 2.3; 95% CI, 0.4–4.2) and females (APC, 2.3; 95% CI, 0.3– 4.3). This increase appears to be largely due to an increase in malignant tumor incidence in the ≥ 65 -year age group. The same authors reported an analysis of incidence by tumor subtype (Dobes, Khurana et al. 2011). A significant increasing incidence in glioblastoma multiforme (GBM) was observed in the study period (annual percentage change [APC], 2.5; 95% confidence interval [CI], 0.4-4.6, n = 2275), particularly after 2006. In GBM patients in the ≥ 65 -year group, a significantly increasing incidence for men and women combined (APC, 3.0; 95% CI, 0.5-5.6) and men only (APC, 2.9; 95% CI, 0.1-5.8) was seen. Rising trends in incidence were also seen for meningioma in the total male population (APC, 5.3; 95% CI, 2.6-8.1, n = 515) and males aged 20-64 years (APC, 6.3; 95% CI, 3.8-8.8). Significantly decreasing incidence trends were observed for Schwannoma for the total study population (APC, -3.5; 95% CI, -7.2 to -0.2, n = 492), significant in women (APC, -5.3; 95% CI, -9.9 to -0.5) but not men.

Korea

Recent data from Korea has shown increase in brain cancer incidence (Jung, Won et al. 2013). Tumors of the brain and nervous system increased APC 1.0% per year for men and 0.5% per year for women during 1999 - 2010. The rate of increase was statistically significant for men ($p < 0.05\%$), while was not statistically significant for women. It should be noted that key parameters for the NT MW effects include sex and age (Belyaev 2010; IARC 2013). For both sexes, combined statistically significant rate of increase was 0.8% annually.

Nordic national cancer registers

In Denmark, the Danish cancer register has reported increase in brain cancer incidence of 40% in men, and by 29% in women during 2001-2010.

(<http://www.sst.dk/publ/Publ2011/DAF/Cancer/Cancerregisteret2010.pdf>)

Finland

In Finland, age-adjusted (world) brain cancer incidence rates per 100,000 person-years has not changed significantly since 1997

(<http://www.kreftregisteret.no/no/Registrene/Kreftstatistikk/>). Age-adjusted (world) incidence rates per 100 000 person-years by primary site and five-year period was in females 12,0 in 1992-96, 13,6 in 1997-01, 14,2 in 2002-06, 13,7 in 2007-11

(<http://stats.cancerregistry.fi/stats/eng/veng0006i0.html>)

Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in males 10,7 in 1992-96, 10,6 in 1997-01, 11,7 in 2002-06, 11,2 in 2007-11.

(<http://stats.cancerregistry.fi/stats/eng/veng0005i0.html>)

Norway

In Norway, age-adjusted (world) brain cancer incidence rates per 100 000 person-years has grown since 1997 (<http://www.kreftregisteret.no/no/Registrene/Kreftstatistikk/>).

Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in females 10.6 in 1992-96, 13.3 in 1997-01, 17.3 in 2002-06, and 16.4 in 2007-11. Age-adjusted (world) incidence rates per 100,000 person-years by primary site and five-year period was in males 10.7 in 1992-96, 12.2 in 1997-01, 14.1 in 2002-06, and 14.2 in 2007-11.

Sweden

In Sweden, no statistically significant changes in brain cancer incidence per 100,000 person was shown in Cancer Register (Socialstyrelsens Cancerregister) during 1996 - 2011. (<http://www.socialstyrelsen.se/statistik/statistikdatabas/cancer>). There is a scientifically reasonable suspicion that underreporting of brain cancers masks the brain cancer incidence in Sweden (Barlow, Westergren et al. 2009).

All Nordic countries. NORDCAN

Nordic cancer register (NORDCAN) shows increases in brain cancer incidence. NORDCAN project presents the incidence, mortality, prevalence and survival statistics from 41 major cancers in the Nordic countries (<http://www-dep.iarc.fr/NORDCAN/english/frame.asp>). In Denmark, a statistically significant increase in incidence rate per year for brain and central nervous system tumors (combined) was seen during 2001-2011 both in men, *annual percentage change (APC)*, 3.77, [95% CI 2.90; 4.64] and in women 3.68, [95% CI 2.29; 5.10]. While no statistically significant changes are observed in incidence rate per year for brain and central nervous system tumors during last 10 years in other Nordic countries (Finland, Iceland, Norway, and Sweden), a statistically significant increase is seen during last 10 years in men 1.02, 95%CI [0.40;1.65] and women, 1.05, 95%CI [0.35;1.74] in all Nordic countries combined.

Quality and completeness of cancer registers

The SCENIHR preliminary Opinion reaches an indefensible and highly controversial conclusion on brain cancer: "*That renders all studies reporting increased risks of such magnitude implausible. The reason for the increases are methodological artefacts*". First, the time trends for brain cancer incidence is positive according to at least some data shown above. Second, it generally accepted that if two pieces of data do not fit each other both pieces should be scientifically analyzed. As a matter of fact, the utility of Cancer registries depends heavily on their quality including the *completeness* with which patients eligible for registration are ascertained (Bray and Parkin 2009; Parkin and Bray 2009). The *completeness* of cancer registry data – the extent to which all of the incident cancers occurring in the population are included in the registry database – is an extremely

important attribute of a cancer registry (Parkin and Bray 2009). However, registries rarely report their completeness because it is difficult to measure (Bullard, Coleman et al. 2000).

Incompleteness was found in the Swedish Cancer Register (Barlow, Westergren et al. 2009). Underreporting of brain cancers including gliomas in Swedish Cancer Register was about 3.7% of the cases reported in 1998 (Barlow, Westergren et al. 2009). It was estimated, that the Thames Cancer Registry (UK) attains 92.1% completeness 5 years after diagnosis for all cancers (Bullard, Coleman et al. 2000). Recent data have confirmed relatively low completeness of the Thames Cancer Registry with estimates ranging from around 78% (female melanoma) to 95% (female stomach cancer) (Robinson, Sankila et al. 2007). The Finnish data appeared to be more complete, with estimates ranging from around 96% completeness for prostate cancer to 100% for ovarian cancer (Robinson, Sankila et al. 2007).

The best characterized is the Cancer Register of Norway (CRN) (Larsen, Smastuen et al. 2009). A total of 93.8% of the cancer cases registered in the period 2001–2005 were morphologically verified. The proportion of DCO (death certificate only) cases 2001–2005 was only 0.9%, and only 2.2% were registered with primary site unknown (PSU). The overall completeness for the period 2001–2005, estimated by the capture/recapture method, was 98.8%. The lowest completeness was estimated for pancreas (95.7%), multiple myeloma (95.5%), leukemia (94.6%) and central nervous system (93.8%). Authors recognize that cancers of the central nervous system did not meet the highest standards. Nevertheless, recent registration data from Norway are among the most complete among the European Registries (Larsen, Smastuen et al. 2009).

Recent study has indicated the US cancer registries data may be incomplete as related to cancer mortality (German, Fink et al. 2011). Confirmation rate was estimated as 93.4 (95% CI, 92.6–94.2) (per 100 deaths) = the number of individuals who died sometime in 2002–2004 and had been diagnosed with brain cancer sometime in 1993–2004 for whom the cancer site listed in the population-based cancer registry matched the site (underlying cause) on their death certificate, divided by the total number of these decedents (both matched and unmatched). Detection rate was estimated 93.7 (95% CI, 90.5–96.9) = the number of individuals diagnosed with brain cancer (ICD-10, the International Statistical Classification of Diseases and Related Health Problems, 10th Revision) sometime in 1993–1995 who died sometime in 1993–2004 for whom the cancer site listed in the population-based cancer registry matched the site (underlying cause) on their death certificate, divided by the total number of these decedents (both matched and unmatched).

Similar incompleteness has been reported by Meguerditchian et al for the National Cancer Data Base (NCDB) (Meguerditchian, Stewart et al. 2010). Claims for patients with breast cancer surgery from one payer in Western New York (WNY) were matched with NCDB for participating hospitals for 2001–2003 using available identifiers (reporting hospital, gender, birth date, ZIP code). Four hundred seventy patients with health insurance provided by IHA with a breast procedure and a diagnosis code for breast

cancer between January 1, 2001 and January 1, 2003 at the participating institutions were identified by ICD-9 and CPT codes. These patients were matched to all breast cancers reported to the NCDB from the CoC-approved hospitals during the same period and in the same geographic area. The final match rate between the two datasets was 93.4% (430 patients). Forty cases identified by IHA remained unmatched to the registries.

The time trends for *incompleteness* of the Cancer Registers is not known. Finally, Cancer Register's data should be questioned if no consistence is observed between them and epidemiological data on mobile phone usage.

Conclusion on brain cancer time trend data and mobile phones

Cancer incidence data are derived from cancer registries and quality of these data dependent on quality and completeness of cancer registers. Completeness and quality of most cancer registries are not comprehensively characterized and vary between cancer registers. At least some cancer registries including better described Nordic Cancer Register show increased time trends in brain cancer incidence, especially in those parts of brain which are mostly exposed to radiation from mobile phones. Taking into account the IARC statement regarding the role of incidence data in phone risk assessment, the incidence data do not contradict to the increased cancer risk seen in epidemiological studies at latencies more than 10-25 years (Carlberg, Soderqvist et al. 2013; Hardell and Carlberg 2013; Hardell, Carlberg et al. 2013; Hardell, Carlberg et al. 2013). The IARC Working Group further noted that these descriptive analyses would be null if an excess in cancer risk from mobile-phone use became manifest only decades after phone use began, or if an increase affected only a small proportion of the cases by location.

On page 68 the SCENIHR report states: "it appears the evidence for glioma became weaker". This conclusion is in evident contradiction with available data. Recent publications including those omitted in the SCENHIR report and mentioned in these comments make this evidence much stronger than during the last IARC meeting in 2011 and demands IARC classification "carcinogen, group 1" for EMF exposures from mobile phones.

In vivo studies

Similar to other parts of this report, the conclusions from *In vivo* studies, p 68- , are fundamentally flawed because they are not based on mechanistic studies and consideration of important physical and biological parameters (IARC 2013). As a matter of fact, only negligible amount of real signals (frequency, modulation, polarizaton) were tested in mentioned *in vivo* studies. Thus, the statement, p 68, "Overall, it was concluded that RFR fields such as those emitted by mobile phones were not carcinogenic in laboratory rodents" may be relevant only to these limited number of tested signals. Similarly the statement: " Overall, because a considerable number of well-performed studies using a wide variety of animal models have been mostly negative in outcome, the animal studies are considered to provide strong evidence for the absence of an effect" deals with only minority of real signals and cannot be used as an argument against overwhelming evidence for increased cancer risks following from epidemiological studies, which involved all possible signals. What is even more

important, most positive studies involved exposure to the more realistic exposure that includes combined signals from real mobile phones. These are the most relevant for health risk assessment, but were omitted in the SCENIHR report (see below). It is fundamentally flawed to question results of epidemiological studies obtained with exposure to all signals from mobile phones by *in vivo* or *in vitro* negative studies obtained with negligible number of mobile phone-like signals.

Genotoxic RFR effects, p. 70

These studies were omitted from review in the preliminary Opinion and should be incorporated. Positive studies on RFR/mobile phone genotoxicity include but are not limited to (Guler, Tomruk et al. 2010; Cam and Seyhan 2012; Guler, Tomruk et al. 2012; Karaca, Durmaz et al. 2012; Sekeroglu, Akar et al. 2012; Atasoy, Gunal et al. 2013; Atli Şekeroğlu, Akar et al. 2013; Hanci, Odaci et al. 2013; Liu, Duan et al. 2013; Liu, Gao et al. 2013; Pesnya and Romanovsky 2013).

Considering Belyaev's group studies (Belyaev, Markova et al. 2009; Markova, Malmgren et al. 2010) the SCENIHR preliminary opinion stated, page 72, that effects at 905 MHz were inconsistent. It should be noted that this "inconsistency" was actually individual variability, which nature has recently been established to be dependant on individual state of chromatin at time of exposure (Sarimov, Alipov et al. 2011). One of the main results following from the Belyaev's group studies including those unmentioned neither in this nor in previous SCENIHR report (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005) is strong dependence of effects from mobile phones on carrier frequency/frequency channel. Effects at 905 MHz/GSM channel 74 on DNA repair foci were consistently lower compared to effects at 915 MHz/GSM channel 124 regardless cell type, human lymphocytes, fibroblasts or stem cells. In addition, the data indicated stronger effects of exposure to RF from UMTS mobile phone at frequency at 1947.4 MHz, middle channel. Importantly, human stem cells (not "stem cells" as spelled in the SCENIHR preliminary opinion on page 72, line 16) were most sensitive to MW exposure providing a mechanistic link to carcinogenesis. This is because stem cells are the generally accepted cellular target for origination of different types of tumors and leukemia. These data provided evidence that different frequency channels of different types of mobile communications should be separately tested for health effects and that primary human stem cells are an key cellular focus for *in vitro* EMF studies dealing with carcinogenesis.

Mechanisms for non-thermal MW effects below ICNIRP safety levels

It is generally accepted now that MW induce effects under non-thermal intensities which are generally called non-thermal effects. The SCENIHR preliminary opinion states that: "*(In view of the lack of verification of any proposed non-thermal interaction mechanism, established knowledge does not suggest effects accumulating with time*".

First, this statement is in contradiction with generally accepted Bradford Hill criteria: "*Plausibility: It will be helpful if the causation we suspect is biologically plausible. But this is a feature I am convinced we cannot demand. What is biologically plausible depends upon the biological knowledge of the day.*" . . . no biological knowledge to

support (or to refute) Pott's observation in the 18th century of the excess of cancer in chimney sweeps. It was lack of biological knowledge in the 19th that led a prize essayist writing on the value and the fallacy of statistics to conclude, amongst other "absurd" associations, that "*it could be no more ridiculous for the stranger who passed the night in the steerage of an emigrant ship to ascribe the typhus, which he there contracted, to the vermin with which bodies of the sick might be infected*". And coming to nearer times, in the 20th century there was no biological knowledge to support the evidence against rubella.' the association we observe may be one new to science or medicine and we must not dismiss it too light-heartedly as just too odd. As Sherlock Holmes advised Dr Watson, '*when you have eliminated the impossible, whatever remains, however improbable, must be the truth.*' "(Hill 1965).

Second, there are a number of studies showing accumulation of effects with time (Belyaev 2010).

Third, the majority of scientists consider NT MW effects within the frame of mechanisms using quantum mechanics and physics of nonlinear systems in biological non-equilibrium systems, which are relevant for mechanisms of NT MW in biological systems (Belyaev 2010). It is generally accepted that more than one physical theory may describe the same phenomena (compare for example Debye model of phonons in a box and Einstein model of quantum harmonic oscillators for solids). Thus, the demand of a generally accepted mechanism is not scientifically justified and represents methodological flaw. Most representative so far international IARC expert panel has concluded: "*Although it has been argued that RF radiation cannot induce physiological effects at exposure intensities that do not cause an increase in tissue temperature, it is likely that not all mechanisms of interaction between weak RF-EMF (with the various signal modulations used in wireless communications) and biological structures have been discovered or fully characterized*", see page 104 (IARC 2013). Thus, the IARC Working Group does not reject physical mechanisms for mobile phone exposure and recognizes that either new mechanisms may come or already known mechanisms may be better characterized to explain the non-thermal effects.

Among other mechanisms, radical pairs mechanisms is widely accepted. In many recent reports unmentioned by the SCENIHR preliminary opinion it has been shown that ROS may be involved in radical pair reactions, thus, radical pairs may be considered one of the mechanisms of transduction able to initiate cell oxidative stress (Georgiou 2010; Apollonio, Liberti et al. 2013; Boderá, Stankiewicz et al. 2013; Burlaka, Tsybulin et al. 2013).

Furthermore, many of the changes observed in RF-exposed cells were prevented by (pre)treatment with antioxidants (IARC 2013). In addition, recent review has summarized studies on EMFs exposure and oxidative stress in brain (Consales, Merla et al. 2012). While the data from different studies should be compared with care in view of variation in physical and biological parameters, most part of collected data have shown effects of ELF and RF EMF on oxidative stress in brain (Consales, Merla et al. 2012). IARC monograph states: "*even small effects on radical concentration could potentially affect multiple biological functions*", page 103 (IARC 2013).

One of the main arguments against NT MW effects, so called kT-paradox, has further been challenged by consideration of biological processes far from thermodynamic equilibrium (Cifra, Fields et al. 2011). Subculture structures such as molecular motors operate, in general, under conditions far from thermodynamic equilibrium and, therefore, the formalism of non-equilibrium thermodynamics, which was generally used in critics of mechanisms for NT MW effects, for coupled mechano-chemical processes is not applicable (Chowdhury 2013). Therefore, one has to use the more sophisticated toolbox of stochastic processes and nonequilibrium statistical mechanics for theoretical treatment of molecular motors.

Theoretical studies by Srobar in development of fundamental theory by H. Fröhlich have not been considered neither in this nor in previous SCENIHR Opinion on EMF (Srobar 2009; Srobar 2009).

Effects of RFR exposure on oxidative stress, p 177

This chapter provides a biased record of very minor part of oxidative stress studies without definition how these studies have been chosen for analysis. Recent positive studies on RF/mobile phone oxidative stress and genotoxicity have not been included (Haghani, Shabani et al. 2013) (Tomruk, Guler et al. 2010; Esmekaya, Ozer et al. 2011; Kumar, Behari et al. 2012) (Lu, Huang et al. 2012) (Tkalec, Stambuk et al. 2013) (Deshmukh, Banerjee et al. 2013) (Shahin, Singh et al. 2013) (Eser, Songur et al. 2013) (Burlaka, Tsybulin et al. 2013) (Esmekaya, Aytakin et al. 2011) (Avci, Akar et al. 2012) (Ceyhan, Akkaya et al. 2012) (Sokolovic, Djordjevic et al. 2013) (Oksay, Naziroglu et al. 2012) (Sisodia, Rifat et al. 2013) (Liu, Duan et al. 2013) (Jelodar, Akbari et al. 2013) (Liu, Gao et al. 2013) (Ghanbari, Mortazavi et al. 2013) (Guler, Tomruk et al. 2010; Guler, Tomruk et al. 2012) (Imge, Kilicoglu et al. 2010) (Jelodar, Akbari et al. 2013) (Liu, Duan et al. 2013) (Naziroglu, Cig et al. 2012) (Ni, Yu et al. 2013) (Ozgun, Gler et al. 2010) (Park, Seo et al. 2013)

Replication studies

The most representative so far international IARC panel have included in the RF monograph, pages 101-102: "*The reproducibility of reported effects may be influenced by exposure characteristics (including SAR or power density, duration of exposure, carrier frequency, type of modulation, polarization, continuous versus intermittent exposures, pulsed-field variables, and background electromagnetic environment), biological parameters (including cell type, growth phase, cell density, sex, and age) and environmental conditions (including culture medium, aeration, and antioxidant levels)*" (IARC 2013). IARC admits also that some of the discrepancies between EMF replication studies could be due to differences in species, page 416 (IARC 2013). And at the page 104: "*Biological systems are complex and factors such as metabolic activity, growth phase, cell density, and antioxidant level might alter the potential effects of RF radiation*". Physical factors that affect interpretation of study results are considered in the IARC monograph in more detail on pages 385-387 (IARC 2013).

The SCENIHR preliminary Opinion requires "*replication studies in a strict sense*" for positive findings (page 101). Furthermore, those studies which consistently showed positive findings were criticized for deviations in protocols (p 101, lines 41-49). No such

criticism was applied to studies which failed to "replicate" original positive finding (for example page 102, lines 39-49) even if the key parameters of experiments were or might be different between original studies and "replications". At many occasions, the SCENIHR preliminary Opinion states that replication of positive findings is essential before weight is given to positive results. However, the SCENIHR preliminary Opinion has never applied the same criteria to negative studies even if statistical power was not evaluated in most of them and thus the value of possibly missed effects is not known. As a matter of fact, not one of the negative studies has been replicated "*in a strict sense*" and not one of positive studies has been "unreplicated"/dismissed in "*in a strict sense*". Application of double standards for assessment of positive and negative studies is methodologically flawed and makes the SCENIHR preliminary Opinion internally inconsistent.

The SCENIHR report missed successful replications of positive studies (Grigoriev, Grigoriev et al. 2010; Havas and Marrongelle 2013).

In addition to aforementioned omitted studies reporting positive effects, this preliminary Opinion omitted many other recent positive studies which include but not limited to:

(Fragopoulou, Samara et al. 2012) (Karaca, Durmaz et al. 2012) (Dasdag, Akdag et al. 2012) (Celikozlu, Ozyurt et al. 2012) (Sharma, Sisodia et al. 2013) (Lv, Chen et al. 2014) (Jin, Zong et al. 2012) (Trivino Pardo, Grimaldi et al. 2012) (Aboul Ezz, Khadrawy et al. 2013) (Kesari, Kumar et al. 2011) (Redmayne, Smith et al. 2013) (Deshmukh, Banerjee et al. 2013; Deshmukh, Megha et al. 2013) (Aboul Ezz, Khadrawy et al. 2013) (Cam and Seyhan 2012) (Cervellati, Valacchi et al. 2013) (Finnie, Cai et al. 2010) (Jorge-Mora, Misa-Agustino et al. 2011) (Kwon, Vorobyev et al. 2011) (Panagopoulos, Chavdoula et al. 2010; Panagopoulos and Margaritis 2010; Panagopoulos and Margaritis 2010) (Shckorbatov, Pasiuga et al. 2010) (Suhhova, Bachmann et al. 2013) (Vishnu, Nithyaja et al. 2011) (Sun, Shen et al. 2013) (Tomruk, Guler et al. 2010) (Wu, Wang et al. 2012) (Xu, Chen et al. 2013)

Negative studies were preferentially included into the report even if the same group published both positive and negative studies analyzing different endpoints. An example is the group of Lopez-Martin, which has published negative study on apoptosis in adult male Sprague-Dawley rats exposed for 1 hour to 900 MHz. This negative study was included to the SCENIHR report on page 157. However, the same group has published study revealing that similar exposure at 900 MHz and intensities lower than those from mobile phones induces c-fos proto-oncogene and glial fibrillary acid protein (GFAP) marker in brain of exposed male Sprague-Dawley rats (Carballo-Quintas, Martinez-Silva et al. 2011). This positive study has not been included in the SCENIHR report.

Omission of positive studies showing detrimental effects of RFR exposure and their possible mechanisms especially negatively affects conclusions of the SCENIHR report. An example is data from by Deshmukh et al., which show effects of RFR on cognitive function, DNA damage and oxidative stress in rats exposed under the same conditions (Deshmukh, Banerjee et al. 2013; Deshmukh, Megha et al. 2013).

Exclusion of positive studies questions the conclusions of the SCENIHR report on RFR health effects because some of them describe critical effects which were not considered by the SCENIHR report. Example is study by Aboul Ezz (Aboul Ezz, Khadrawy et al. 2013) which investigated the effect of RFR (frequency 1800 MHz, specific absorption rate 0.843 W/kg, power density 0.02 mW/cm², modulated at 217 Hz) on the concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the hippocampus, hypothalamus, midbrain and medulla oblongata of adult rats. Adult rats were exposed daily to EMR and sacrificed after 1, 2 and 4 months of daily RFR exposure and 1 month after 4 months of daily RFR exposure. RFR exposure induced significant changes in DA, NE and 5-HT in all studied areas of adult rat brain. The authors concluded that exposure of adult rats to RFR may cause disturbances in monoamine neurotransmitters and this may underlie many of the adverse effects reported after RFR including memory, learning, and stress. In a recent German study, 24 out of 60 participants were exposed to MW from a base station (cell tower) at a power density of < 60 $\mu\text{W}/\text{m}^2$, 20 participants to 60 - 100 $\mu\text{W}/\text{m}^2$, and 16 participants to more than 100 $\mu\text{W}/\text{m}^2$ (Buchner and Eger 2011). The values of the stress hormones adrenaline and noradrenalin increased significantly during the first 6 months after exposure to the GSM base station; the values of the precursor substance dopamine substantially decreased in this time period. The subject's initial endocrine state was not restored even after 1.5 years. Due to the non-regulable chronic difficulties of the stress balance, the phenylethylamine levels dropped until the end of the investigation period. These effects show a dose response relationship.

Provocation studies, p. 108

In view of complex dependence of NT MEW effects on physiological state of the object, individual sensitivity, physical parameters of exposure, duration and time after exposure the provocation studies should not be considered as informative regarding exposure to all real mobile communication systems including cellphones because only minor part of these parameters (frequency, modulation, duration of exposure et cetera) have been analyzed.

Conclusions on symptoms. p. 115

Similar to other conclusions on RFR health effects, conclusions on symptoms on page 115 do not take into account dependence of RFR effects on physical parameters such as frequency and modulation. In contrast to this flawed approach by the SCENIHR report, in recent study Redmayne et al. evaluated associations between New Zealand early-adolescents' subjective well-being and self-reported use of, or exposure to different types of wireless phones and internet technology (Redmayne, Smith et al. 2013). In this cross-sectional survey, participants completed questionnaires in class about their cellphone and cordless phone use, their self-reported well-being, and possible confounding information such as whether they had had influenza recently or had a television in the bedroom. Parental questionnaires provided data on whether they had WiFi at home and cordless phone ownership and model. Data were analysed with Ordinal Logistic Regression adjusting for common confounders. Odds ratios (OR) and 95% confidence intervals were calculated. The number and duration of cellphone and cordless phone calls were associated with increased risk of headaches (>6 cellphone calls over 10 minutes weekly,

adjusted OR 2.4, CI 1.2-4.8; >15 minutes cordless use daily adjusted OR 1.74, CI 1.1-2.9). Using a wired cellphone headset was associated with tinnitus (adjusted OR 1.8, CI 1.0-3.3), while wireless headsets were associated with headache (adjusted OR 2.2, CI 1.1-4.5), feeling down/depressed (adjusted OR 2.0, CI 1.1-3.8), and waking in the night (adjusted OR 2.4, CI 1.2-4.8). Several cordless phone frequencies bands were related to tinnitus, feeling down/depressed and sleepiness at school, while the last of these was also related to modulation. The only significant negative regression was less likely Waking nightly for those with Wi-Fi at home (adjusted OR 0.7, CI 0.4-0.99). Being woken at night by a cellphone was strongly related to tiredness at school (OR 3.49, CI 1.97-6.2). There were more statistically significant associations (36%) than could be expected by chance (5%). Several were dose-dependent relationships. The obtained data were in line with previous findings of others and suggested limiting use of cellphones and cordless phones to less than 15 minutes daily, and employing a speaker-phone device for longer daily use.

Methodological flaw in assessment

In contrast to generally accepted methodology used by IARC, this SCENIHR report subjectively divides studies into informative and non-informative (page 83-84). As a result the same studies SCENIHR report assess differently as compared to IARC : "*For in vivo studies our assessment of evidence is weaker than IARC, based on the same studies as used in the IARC evaluation*". While the SCENIHR report requires statistical power for negative studies (page 17), the majority of negative studies which the preliminary Opinion relies upon did not analyze statistical power and were not able to determine at what level of sensitivity the RFR effects might be missed. It is not stated in the SCENIHR preliminary Opinion how many experts evaluated each study and whether experts were allowed to evaluate own studies.

The SCENIHR report inconsistently uses criteria for replication studies and verification of results. Strict following to generally accepted key biological and physical parameters the conditions is demanded at some occasions of the SCENIHR report. On the other hand, the effects of gender and biological efficiency of low SAR values is used to question validity of results (lines 3-4, page 103). Effects of low SARs and gender were described in many papers (Belyaev 2010; IARC 2013) and thus cannot be used as argument against NT MW effects.

Exclusion of studies with exposure to real mobile phones, which are most relevant for assessment of health effects from mobile telephony p. 117

On Page 117 the SCENIHR report states that studies with exposure to real mobile phones "*are of no use for health risk assessment, as the exposures would have been highly complex and very variable, especially if the animals were unrestrained and free to move in their cages*". This is fundamentally flawed statement which results in excluding mostly important for health risk assessment studies and thus masking health risks from mobile communication. As a matter of fact, the studies with real mobile phones, given the EMF field was measured from the phone, represent most valuable type of studies for assessment of risks from mobile telephony. The reasons were recently analyzed in review by Belyaev that has not been included in the SCENIHR report (Belyaev 2010). In brief, real signals contain multiple (hundreds and even thousands, in dependence on type

of mobile communication) components, such as carrier frequencies or frequency bands, different types of modulations. It is generally accepted that all these parameters are important for effects of MW (IARC 2013). Exposure to mobile phone may reproduce the majority of real signals during the same exposure session and thus provide the best possibility to assess detrimental effects from mobile telephony. Another type of exposure, to which the SCENIHR report has chosen to rely upon, is exposure to one fixed frequency and fixed modulation which reproduces one from thousands possible signals. While one RFR frequency/frequency band/modulation can induce detrimental effect, another one can be inactive (Belyaev 2010). In addition, mobile phones emit not only MW but also ELF fields, which have also been shown to produce detrimental effects (www.bioinitiative.org) and to interfere with MW effects (Belyaev 2010; Sun, Shen et al. 2013).

Importantly, most of aforementioned studies with mobile phones as source of EMF exposure and omitted by the SCENIHR report show detrimental effects and most importantly indicate mechanism of these effects based on induction of ROS.

Data obtained with selected frequency/frequency band/modulation provides possibility to assess only this specific signal and may be important for consideration of biophysical mechanisms for NT MW effects. However, these studies are evidently less important for health risk assessment by the reasons provided above.

Recommendations

The main issue of further research is to promote studies on biophysical mechanisms that will provide a mechanistic basis for risk assessment. Such parameters as frequency, modulation, polarization should be given priority for mechanistic studies so that physical and biological variables that influence study outcome can be taken into account. For risks assessment in laboratory studies, the complexity and interplay of variables from real systems of mobile communication should also be taken into account. In other words, to assess health risks from any type of mobile communication, all specific frequency channels and all specific modulations should be investigated in combinations as at real exposures.

Recent studies indicated that financial interests may affect the outcome of EMF laboratory studies (Huss, Egger et al. 2007; Huss, Egger et al. 2008). Also recent review reports that the negative results produced by studies funded by the cell-phone companies are affected by many biases and flaws, giving rise to a systematic underestimate of the risk (Levis, Minicuci et al. 2011). On the contrary, studies producing positive results - without errors and financial conditioning - indicate a cause/effect relationship supported by biological plausibility (Levis, Minicuci et al. 2011). In view of these facts, it is recommended to take into account the source of funding in evaluation of the results.

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Exhibit G: Mitochondrial Dysfunction and Disruption of Electrophysiology

Mitochondria are broadly vulnerable, in part because the integrity of their membranes is vital to their optimal functioning – including channels and electrical gradients, and their membranes can be damaged by free radicals which can be generated in myriad ways. Moreover, just about every step in their metabolic pathways can be targeted by environmental agents, including toxicants and drugs, as well as mutations [1]. This supports a cumulative allostatic load model for conditions in which mitochondrial dysfunction is an issue, which includes autism as well as myriad other chronic conditions.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network [2]. [2]. It is also the case that *“The physiological domain is characterized by small-amplitude oscillations in mitochondrial membrane potential ($\Delta\psi(m)$) showing correlated behavior over a wide range of frequencies.... Under metabolic stress, when the balance between ROS [reactive oxygen species, or free radicals] generation and ROS scavenging [as by antioxidants] is perturbed, the mitochondrial network throughout the cell locks to one main low-frequency, high-amplitude oscillatory mode. This behavior has major pathological implications because the energy dissipation and cellular redox changes that occur during $\Delta\psi(m)$ depolarization result in suppression of electrical excitability and Ca^{2+} handling...”* [3]. These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by EMF/RFR.

Other types of mitochondrial damage have been documented in at least some of the studies that have examined the impacts of EMF/RFR upon mitochondria. These include reduced or absent mitochondrial cristae [4-6], mitochondrial DNA damage [7], swelling and crystallization [5], alterations and decreases in various lipids suggesting an increase in their use in cellular energetics [8], damage to mitochondrial DNA [7], and altered mobility and lipid peroxidation after exposures [9]. Also noted has been enhancement of brain mitochondrial function in Alzheimer’s transgenic mice and normal mice [10]. The existent of positive as well as negative effects gives an indication of the high context dependence of exposure impacts, including physical factors such as frequency, duration, and tissue characteristics [11].

Secondary mitochondrial dysfunction (i.e. environmentally triggered rather than rooted directly in genetic mutations) [15-18] could result among other things from the already discussed potential for EMF/RFR to damage channels, membranes and mitochondria themselves as well as from toxicant exposures and immune challenges. In a meta-analysis of studies of children with ASC and mitochondrial disorder, the spectrum of severity varied, and 79% of the cases were identified by laboratory findings without associated genetic abnormalities [16].

Electrophysiology

Nervous system electrophysiology when disrupted by ELF-EMF and RFR can produce alterations in molecular, cellular and systems physiological function. It occurs in the brain as well as in the body, and impacts the transduction into the electrical signaling activities of the brain and nervous

system. If the cells responsible for generating synapses and oscillatory signaling are laboring under cellular and oxidative stress, lipid peroxidation, impaired calcium and other signaling system abnormalities, then mitochondrial metabolism will fall short, all the more so because of the challenges from the immune system which in turn be triggered to a major extent by environment. How well will synaptic signals be generated? How well will immune-activated and thereby distracted glial cells be able to modulate synaptic and network activity? [19-22] Microglial activation can impact excitatory neurotransmission mediated by astrocytes [23]. Cortical innate immune response increases local neuronal excitability and can lead to seizures [24,25]. Inflammation can play an important role in epilepsy [26].

Seizures and epilepsy

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma [27-31], which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if a BBB is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact [32,33], suggesting that such exposures can further exacerbate vicious circles already underway. The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling [34,35]. In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment [36], suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

One critical issue here is nonlinearity and context and parameter sensitivity of impact. In one study, rat brain slices exposed to EMF/RFR showed reduced synaptic activity and diminution of amplitude of evoked potentials, while whole body exposure to rats led to synaptic facilitation and increased seizure susceptibility in the subsequent analysis of neocortical slices [37]. Another study unexpectedly identified enhanced rat pup post-seizure mortality after perinatal exposure to a specific frequency and intensity of exposure, and concluded that apparently innocuous exposures during early development might lead to vulnerability to stimuli presented later in development [38].

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