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Literature on neurological effects of static and extremely-low frequency electromagnetic fields (2007-2019)

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- behavioral effect ; OX- oxidative changes; DE- development; MA- possible medical application; ND- neurodegenerative disease; EF- electric field.

E=212 (91%); NE=21 (9%)

(E) Afrasiabi A, Riazi GH, Abbasi S, Dadras A, Ghalandari B, Seidkhani H, Modaresi SM, Masoudian N, Amani A, Ahmadian S. Synaptosomal acetylcholinesterase activity variation pattern in the presence of electromagnetic fields. *Int J Biol Macromol.* 65:8-15, 2014. (CS, AE, CC, MA)

Acetylcholinesterase (AChE) is the enzyme that controls the acetylcholine (ACh) concentrations in cholinergic synaptic clefts by hydrolyzing ACh to choline and acetate. Cholinergic synapses are involved in important functions such as learning, memory and cognition. In this study, we investigated the effects of a wide range of extremely low frequency electromagnetic fields (ELF-EMFs) on synaptic ACh concentrations through AChE enzyme activity assay. Synaptosome suspensions were prepared as a neural terminus from cerebral cortex of sheep brain. Prepared synaptosomes were exposed to ELF-EMFs with frequency ranging from 50 Hz to 230 Hz for duration between 15 and 120 min and flux intensity between 0.1 mT and 1.7 mT. Consequently, AChE activity was measured by Ellman method. Raw data were analyzed by neural network based software, Inform 4.02, to predict AChE activity pattern through nine 3D curves. These curves showed that AChE activity decreases when exposed to ELF-EMFs of 1.2 mT to 1.7 mT intensity and 50 Hz to 90 Hz frequency. Thus, it is proposed that exposure to fields of in this range of frequency-intensity would be effective in clinical treatments of cholinergic disorders to increase synaptic ACh concentration. However, more in vivo experiments are needed to develop this suggested treatment.

(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) Akbarnejad Z, Esmaeilpour K, Shabani M, Asadi-Shekaari M, Saeedi-Goraghani M, Ahmadi M. Spatial memory recovery in Alzheimer's rat model by electromagnetic field exposure. Int J Neurosci. 128(8):691-696, 2018. (AS, CE, BE, CC, ND, MA)

INTRODUCTION: Although studies have shown a potential association between extremely low frequency electromagnetic fields (ELF-EMFs) exposure and Alzheimer's disease (AD), few studies have been conducted to investigate the effects of weak magnetic fields on brain functions such as cognitive functions in animal models. Therefore, this study aimed to investigate the effect of ELF-EMF exposure (50 Hz, 10 mT) on spatial learning and memory changes in AD rats. **METHODS:** Amyloid- β (A β) 1-42 (5 μ l/ bilateral, single-dose) was injected into lateral ventricle to establish an AD rat model. The rats were divided into six groups: Group I (control); Group II (surgical sham); Group III (AD) Alzheimer's rat model; Group IV (MF) rats exposed to ELF-MF for 14 consecutive days; Group V (A β injection+M) rats exposed to magnetic field for 14 consecutive days from day 0-14 days after the A β peptide injection; Group VI (AD+M) rats exposed to magnetic field for 14 consecutive days after 2 weeks of A β peptide injection from 14th to 28th day. Morris water maze investigation were implemented and performed 24 h after termination of ELF-MF, respectively. **RESULTS:** AD rats showed a significant impairment in learning and memory compared to control rats. The results showed that ELF-MF improved the learning and memory impairments in A β injection+M and AD+M groups. **CONCLUSION:** Our results showed that application of ELF-MF not only has improving effect on different cognitive disorder signs of AD animals, but also disrupts the processes of AD rat model formation.

(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) Alsaeed I, Al-Somali F, Sakhnini L, Aljarallah OS, Hamdan RM, Bubishate SA, Sarfaraz ZK, Kamal A. Autism-relevant social abnormalities in mice exposed perinatally to extremely low frequency electromagnetic fields. Int J Dev Neurosci. 2014 Jun 23. pii: S0736-5748(14)00092-6. doi: 10.1016/j.ijdevneu.2014.06.010. [Epub ahead of print]. (AS, CE, BE, DE)

The incidence of autism spectrum disorders (ASD) has been rising, but the causes of ASD remain largely unidentified. Collective data have implicated the increased human exposure to electromagnetic fields (EMF) in the increasing incidence of ASD. There are established biological effects of extremely low-frequency (ELF) EMF, but the relation to ASD is not investigated enough. In this study we examined the effects of perinatal exposure to ELF EMF on some ASD-relevant behavioral parameters in mice. The EMF was delivered via a Helmholtz coil pair. Male BALB/C mice were used and divided into exposed and control groups (n=8 and n=9, respectively). Tests were used to assess sociability, preference for social novelty, locomotion, anxiety, exploratory behavior, motor coordination, and olfaction. The examined mice were all males and exposed to EMF during the last week of gestation and for 7 days after delivery. The exposed mice demonstrated a lack of normal sociability and preference for social novelty while maintaining normal anxiety-like behavior, locomotion, motor coordination, and olfaction. Exposed mice also demonstrated decreased exploratory activity. We concluded that these results are supportive of the hypothesis of a causal link between exposure to ELF-EMF and ASD; however, replications of the study with further tests are recommended.

(E) Amara S, Douki T, Garel C, Favier A, Sakly M, Rhouma KB, Abdelmelek H. Effects of static magnetic field exposure on antioxidative enzymes activity and DNA in rat brain. Gen Physiol Biophys. 28(3):260-265, 2009. (AS, CE, OX)

The present study was undertaken in order to investigate the effects of static magnetic field (SMF) exposure on the antioxidative enzymes activity, malondialdehyde (MDA) concentration and DNA oxidation in male rat brain. The exposure of rats to SMF (128

mT, 1 h/day during 30 consecutive days) decreased the glutathione peroxidase (GPx; -39%, $p < 0.05$), CuZn superoxide dismutase (CuZn-SOD; -35%, $p < 0.05$) and catalase (-59%, $p < 0.05$) activities in frontal cortex. The same treatment decreased the CuZn-SOD (-51%, $p < 0.05$) and Mn-SOD (-13%, $p < 0.05$) activities in hippocampus. However, the glutathione levels remained unchanged in the both brain structures. In the hippocampus, SMF exposure increased MDA concentration (+32%, $p < 0.05$). Interestingly, exposed-rats to SMF displayed a significant increase of metallothioneins level in frontal cortex (+100%, $p < 0.05$), while the 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) concentration remained unaffected, indicating the absence of DNA oxidation. Our results indicated that sub-chronic exposure to SMF induced oxidative stress in rat hippocampus and frontal cortex. Metallothionein induction protected probably DNA against oxidative damage.

(E) Amara S, Douki T, Garrel C, Favier A, Ben Rhouma K, Sakly M, Abdelmelek H. Effects of static magnetic field and cadmium on oxidative stress and DNA damage in rat cortex brain and hippocampus. Toxicol Ind Health. 27(2):99-106, 2011. (AS, CE, OX)

The present study was undertaken to determine the effect of co-exposure to static magnetic field (SMF) and cadmium (Cd) on the antioxidant enzymes activity and DNA integrity in rat brain. Sub-chronic exposure to CdCl (CdCl₂, 40 mg/L, per os) for 30 days resulted in a significant reduction in antioxidant enzyme activity such as the glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) in frontal cortex and hippocampus. Total GSH were decreased in the frontal cortex of the Cd-exposed group. Cd exposure induced an increase in malondialdehyde (MDA) concentration in the frontal cortex and hippocampus. Moreover, the same exposure increased 8-oxo-7,8-dihydro-2-desoxyguanosine (8-oxodGuo) level in rat brain. Interestingly, the combined effect of SMF (128 mT, 1 hour/day for 30 consecutive days) and CdCl (40 mg/L, per os) decreased the SOD activity and glutathione level in frontal cortex as compared with the Cd group. Moreover, the association between SMF and Cd increased MDA concentration in frontal cortex as compared with Cd-exposed rats. DNA analysis revealed that SMF exposure failed to alter 8-oxodGuo concentration in Cd-exposed rats. Our data showed that Cd exposure altered the antioxidant enzymes activity and induced oxidative DNA lesions in rat brain. The combined effect of SMF and Cd increased oxidative damage in rat brain as compared with Cd-exposed rats.

(E) Ambalayam S, Jain S, Mathur R. Abnormal feeding behaviour in spinalised rats is mediated by hypothalamus: Restorative effect of exposure to extremely low frequency magnetic field. Spinal Cord. 2016 May 10. doi: 10.1038/sc.2016.32. [Epub ahead of print] (AS, CE, BE, MA)

STUDY DESIGN: Experimental study. OBJECTIVES: To investigate the role of hypothalamus in abnormal feeding behaviour after spinal cord injury (SCI) and the effect of exposure to extremely low frequency magnetic field (ELF-MF) on it. SETTING: India.

METHODS: Male Wistar rats (n=44) were divided into Sham (laminectomy), SCI (complete transection of T13 spinal cord), SCI+MF (ELF-MF exposure to SCI rats), VMHL (lesion of ventromedial hypothalamus; VMH), SCI+VMHL (VMHL after SCI) and SCI+VMHL+MF (ELF-MF exposure to SCI+VMHL rats) groups. Food intake (FI), water intake (WI), calorie intake (CI), body weight (BWT), taste preference and sucrose-induced biphasic (SIB) response to noxious stimulus were studied pre and post surgery. Neuronal activity at VMH was assessed by c-Fos immunohistochemistry. The extent of neuronal degeneration and regeneration in spinal cord was assessed microscopically. **RESULTS:** Data revealed post-SCI decrease in FI, WI, CI and BWT, preference for sodium chloride and citric acid, prolonged analgesic phase of SIB and increased c-Fos immunoreactivity in VMH of SCI rats vs Sham rats. VMH lesion increased FI, WI, CI, BW, preference for sweet tastants and abolished SIB, whereas in SCI+VMHL rats it abolished the effects of SCI on these parameters indicating probable involvement of VMH in SCI-induced alteration in feeding behaviour. Exposure to MF improved the study parameters in SCI rats and reduced the c-Fos immunoreactivity in VMH besides reduction in lesion volume, greater myelination and neuronal regeneration at SCI site. **CONCLUSION:** SCI influences VMH, leading to alteration in feeding behaviour, which is improved by exposure to ELF-MF.

(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 μ T) 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) Andrianome S, Hugueville L, de Seze R, Hanot-Roy M, Blazy K, Gamez C, Selmaoui B. Disturbed sleep in individuals with Idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF): Melatonin assessment as a biological marker. Bioelectromagnetics. 2016 Mar 10. doi: 10.1002/bem.21965. [Epub ahead of print] (HU, CC)

Individuals who suffer from idiopathic environmental intolerance attributed to electromagnetic fields (IEI-EMF) complain of a variety of adverse health effects. Troubled sleep remains a recurrent and common symptom in IEI-EMF individuals. Melatonin, a circadian hormone, plays a major role in the sleep process. In this study, we compared levels of melatonin between a sensitive group (IEI-EMF, n = 30) and a non-sensitive control group (non IEI-EMF, n = 25) without exposure to electromagnetic sources. Three questionnaires were used to evaluate the subjective quality and sleep quantity: the Epworth Sleepiness Scale, the Pittsburgh Sleep Quality Index and the Spiegel Sleep Inventory. Melatonin was quantified in saliva and its major metabolite 6-sulfatoxymelatonin (aMT6s) in urine. Melatonin levels were compared by a two-way analysis of variance at various times between the control and IEI-EMF group. Despite significantly different sleep scores between the two groups, with a lower score in the IEI-EMF group ($P < 0.001$), no statistical difference was found between the two groups for saliva melatonin ($P > 0.05$) and urine aMT6s ($P > 0.05$).

(E) Ansari AM, Farzampour S, Sadr A, Shekarchi B, Majidzadeh-A K. Effects of short term and long term extremely low frequency magnetic field on depressive disorder in mice: Involvement of nitric oxide pathway. Life Sci. 2016 Jan 4. pii: S0024-3205(15)30148-X. doi: 10.1016/j.lfs.2015.12.055. [Epub ahead of print] (AS, AE, CE, BE, OX)

AIMS: Previous reports on the possible effects of Extremely Low Frequency Magnetic Fields (ELF MF) on mood have been paradoxical in different settings while no study has yet been conducted on animal behavior. In addition, it was shown that ELF MF exposure makes an increase in brain nitric oxide level. Therefore, in the current study, we aimed to assess the possible effect(s) of ELF MF exposure on mice Forced Swimming Test (FST) and evaluate the probable role of the increased level of nitric oxide in the observed behavior. MAIN METHODS: Male adult mice NMRI were recruited to investigate the short term and long term ELF MF exposure (0.5 mT and 50Hz, single 2h and 2weeks 2h a day). Loco motor behavior was assessed by using Open-Field Test (OFT) followed by FST to evaluate the immobility time. Accordingly, N Ω -nitro-L-arginine methyl ester 30mg/kg was used to exert anti-depressant like effect. KEY FINDINGS: According to the results, short term exposure did not alter the immobility time, whereas long term exposure significantly reduces immobility time ($p < 0.01$). However, it was revealed that the locomotion did not differ among all experimental groups. Short term exposure reversed the anti-depressant like effect resulting from 30mg/kg of N Ω -nitro-L-arginine methyl ester ($p < 0.01$). SIGNIFICANCE: It has been concluded that long term exposure could alter the depressive disorder in mice, whereas short term exposure has no significant effect. Also, reversing the anti-depressant activity of L-NAME indicates a probable increase in the brain nitric oxide.

(E) Ayoobi F, Shamsizadeh A, Shafiei SA. The effect of local extremely low frequency magnetic field on student sleepiness. Neurol Res. 2017 Sep 27:1-6. doi: 10.1080/01616412.2017.1380931. [Epub ahead of print] (HU, AE, BE)

Objectives: Lack of high-quality sleep causes serious side effects. The current study aimed to investigate the impact of local extremely low frequency magnetic field (ELF-MF) on sleep and

drowsiness in healthy young adults. Methods: Sixty-five young adults (32 males and 31 females, aged 18-24, participated voluntarily in this randomized crossover clinical trial. 200 microTesla MF (3 minutes duration) at three frequencies (10, 14 and 18 Hz) was applied to the skull in areas C3, Cz and C4, respectively. The Stanford Sleepiness Scale (SSS) or Consciousness Test (CT), Epworth Sleepiness Scale (ESS) and Peabody Picture Vocabulary Test were used to evaluate drowsiness, sleepiness, and reaction time. These tests were done both before and after application of ELF-MF or sham operation. Results: Minimum reaction time after exposure to ELF-MF increased compared to that before exposure ($P = 0.03$), while it was not significant for the sham group ($P = 0.63$). From the ESS questionnaire, the results indicated that there was no significant difference for males or females between the exposure and sham groups. The mean of the SSS scores was no different compared to that before exposure. Conclusion: The results of this study demonstrated that exposure to ELF-MF may influence reaction time in young healthy people. However, as the results of ESS and SSS were not different between exposure and non-exposure groups, further studies using larger sample sizes are recommended in order to reach better interpretations of the effects of ELF-MF on sleepiness in young people.

(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 microT, 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) Ben Yakir-Blumkin M, Loboda Y, Schächter L, Finberg JP. Neuroprotective effect of weak static magnetic fields in primary neuronal cultures. Neuroscience. 278:313-326, 2014. (CS, CE, CC, MA, ND)

Low intensity static magnetic fields (SMFs) interact with various biological tissues including the CNS, thereby affecting key biological processes such as gene expression, cell proliferation and differentiation, as well as apoptosis. Previous studies describing the effect of SMFs on apoptotic cell death in several non-neuronal cell lines, emphasize the importance of such a potential modulation in the case of neurodegenerative disorders, where apoptosis constitutes a major route via which neurons degenerate and die. In this study, we examine the effect of SMFs on neuronal survival in primary cortical and hippocampal neurons that constitute a suitable experimental system for modeling the neurodegenerative state in vitro. We show that weak SMF exposure interferes with the apoptotic programming in rat primary cortical and hippocampal neurons, thereby providing protection against etoposide-induced apoptosis in a dose- and time-dependent manner. Primary cortical neurons exposed to SMF (50 G) for 7 days exhibited a $57.1 \pm 6.3\%$ decrease in the percentage of cells undergoing apoptosis induced by etoposide ($12 \mu\text{M}$), accompanied by a marked decrease in the expression of the pro-apoptotic markers: cleaved poly ADP ribose polymerase-1, cleaved caspase-3, active caspase-9 and the phospho- histone H2A variant (Ser 139) by $41.0 \pm 5.0\%$, $81.2 \pm 5.0\%$, $72.9 \pm 6.4\%$, $42.75 \pm 2.9\%$, respectively, and by a $57.2 \pm 1.0\%$ decrease in the extent of mitochondrial membrane potential collapse. Using the L-type voltage-gated Ca^{2+} channel inhibitor nifedipine, which is selective to Ca^{2+} influx through $\text{Ca}_v1.2$, we found that the anti-apoptotic effect of SMFs was mediated by Ca^{2+} influx through these channels. Our findings demonstrating altered Ca^{2+} -influx in response to thapsigargin stimulation in SMF-exposed cortical neurons, along with enhanced inhibition of KCl-induced Ca^{2+} -influx through $\text{Ca}_v1.2$ channels and enhanced expression of $\text{Ca}_v1.2$ and $\text{Ca}_v1.3$ channels, allude to the involvement of voltage and store operated Ca^{2+} channels in various aspects of the protective effect exerted by SMFs. These findings show the potential susceptibility of the CNS to weak SMF exposure and have implications for the design of novel strategies for the treatment and/or prevention of neurodegenerative diseases.

(E) Benassi B, Filomeni G, Montagna C, Merla C, Lopresto V, Pinto R, Marino C, Consales C. Extremely Low Frequency Magnetic Field (ELF-MF) Exposure Sensitizes SH-SY5Y Cells to the

Pro-Parkinson's Disease Toxin MPP. Mol Neurobiol. 2015 Jul 30. [Epub ahead of print] (CS, OX, ND)

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron loss, with an etiopathogenesis involving both genetic and environmental factors. The occupational/residential exposure to the electromagnetic fields has been recently associated with an increased risk of neurodegenerative diseases; it has been thus proposed that the extremely low frequency magnetic field (ELF-MF) may contribute to neurodegenerative etiopathogenesis, as its interaction with biological systems directly impairs redox homeostasis in specific areas of the brain. The molecular mechanisms elicited by ELF-MF, and their potential involvement in PD onset, still remain unclear. To this end, we set up a generator of ELF-MF able to stably and homogeneously reproduce environmental prolonged exposure to ELF-MF (50 Hz, 1 mT). Results obtained indicate that ELF-MF exposure alters cell response of SH-SY5Y cells to MPP⁺. We demonstrate that ELF-MF does not affect per se survival, shape, and morphology of both proliferating and differentiated SH-SY5Y cells but significantly impairs redox homeostasis and thiol content, triggering an increase in protein carbonylation. As a result, toxicity of MPP⁺, even at low doses, is highly enhanced in ELF-MF-exposed cells due to a significant increase in ROS levels, potentiation of oxidative damage, and induction of a caspase-dependent apoptosis. Pre-incubation with the thiol antioxidants N-acetyl-L-cysteine and GSH ethyl-ester significantly reduces the extent of oxidative damage and protects cells from death induced by the combined treatment ELF-MF/MPP⁺. Taken overall, our results demonstrate the redox-based molecular interaction between ELF-MF and PD neurotoxins in vitro, and open a new scenario for defining the synergy of environmental factors in PD onset.

(E) Bertolino G, Dutra Souza HC, de Araujo JE. Neuropathology and behavioral impairments in Wistar rats with a 6-OHDA lesion in the substantia nigra compacta and exposure to a static magnetic field. Electromagn Biol Med. 32(4):527-535, 2013. (AS, CE, BE, MC, ND, MA)

Studies have sought to assess various potential neuroprotective therapeutics in Parkinson's disease. The aim of this study was to evaluate the effects of static magnetic field stimulation 14 days after a 6-Hydroxydopamine (6-OHDA) substantia nigra compacta (SNc) lesion on motor behavior, as assessed by the rotarod (RR) test and brain tissue morphology. Forty male Wistar rats were used and were divided into five groups: control group, sham group (SG), lesion group (LG), lesion north pole group (LNPG) and lesion south pole group (LSPG). In groups with magnetic stimulation, a 3200-gauss magnet was fixed to the skull. After the experiments, the animals were anesthetized for brain perfusion. Coronal sections of the SNc were stained with Nissl. The RR test showed a decrease in the time spent on the apparatus in the LG compared with all groups. The LNPG and LSPG had significant increases in the time spent when compared to the LG. A morphometric analysis revealed a significant reduction in the number of neurons in the LG, LNPG and LSPG in relation to the SG. There were a higher number of neurons in the LNPG and LSPG than the LG, and a higher number of neurons in the LSPG than the LNPG. We observed that the LG, LNPG and LSPG showed a higher number of glial cells than the SG, and the LNPG and LSPG showed a lower number of glial cells than the LG. Our results demonstrate a potential therapeutic

use of static magnetic fields for the preservation of motor behavior and brain morphology in the SNc after 14 days with 6-OHDA lesion.

(E) Bobkova NV, Novikov VV, Medvinskaya NI, Aleksandrova IY, Nesterova IV, Fesenko EE. Effect of weak combined static and extremely low-frequency alternating magnetic fields on spatial memory and brain amyloid- β in two animal models of Alzheimer's disease. Electromagn Biol Med. 37(3):127-137, 2018. (AS, CE, BE, CC, MA, ND)

Subchronic effect of a weak combined magnetic field (MF), produced by superimposing a constant component, 42 μ T and an alternating MF of 0.08 μ T, which was the sum of two frequencies of 4.38 and 4.88 Hz, was studied in olfactory bulbectomized (OBE) and transgenic Tg (APPswe, PSEN1) mice, which were used as animal models of sporadic and heritable Alzheimer's disease (AD) accordingly. Spatial memory was tested in a Morris water maze on the following day after completion of training trials with the hidden platform removed. The amyloid- β (A β) level was determined in extracts of the cortex and hippocampus of mice using a specific DOT analysis while the number and dimensions of amyloid plaques were detected after their staining with thioflavin S in transgenic animals. Exposure to the MFs (4 h/day for 10 days) induced the decrease of A β level in brain of OBE mice and reduced the number of A β plaques in the cortex and hippocampus of Tg animals. However, memory improvement was revealed in Tg mice only, but not in the OBE animals. Here, we suggest that in order to prevent the A β accumulation, MFs could be used at early stage of neuronal degeneration in case of AD and other diseases with amyloid protein deposition in other tissues.

(E) Brouwer M, Koeman T, van den Brandt PA, Kromhout H, Schouten LJ, Peters S, Huss A, Vermeulen R. Occupational exposures and Parkinson's disease mortality in a prospective Dutch cohort. Occup Environ Med. 2015 Feb 23. pii:oemed-2014-102209. doi: 10.1136/oemed-2014-102209. [Epub ahead of print](HU, CE, ND)

OBJECTIVES: We investigated the association between six occupational exposures (ie, pesticides, solvents, metals, diesel motor emissions (DME), extremely low frequency magnetic fields (ELF-MF) and electric shocks) and Parkinson's disease (PD) mortality in a large population-based prospective cohort study. **METHODS:** The Netherlands Cohort Study on diet and cancer enrolled 58 279 men and 62 573 women aged 55-69 years in 1986. Participants were followed up for cause-specific mortality over 17.3 years, until December 2003, resulting in 402 male and 207 female PD deaths. Following a case-cohort design, a subcohort of 5 000 participants was randomly sampled from the complete cohort. Information on occupational history and potential confounders was collected at baseline. Job-exposure matrices were applied to assign occupational exposures. Associations with PD mortality were evaluated using Cox regression. **RESULTS:** Among men, elevated HRs were observed for exposure to pesticides (eg, ever high exposed, HR 1.27, 95% CI 0.86 to 1.88) and ever high exposed to ELF-MF (HR 1.54, 95% CI 1.00 to 2.36). No association with exposure duration or trend in cumulative exposure was observed for any of the occupational exposures. Results among women were unstable due to small numbers of high-exposed women. **CONCLUSIONS:** Associations with PD mortality were observed for occupational exposure to pesticides and ELF-MF. However, the weight given to these findings is limited by the absence of a monotonic trend with either duration or cumulative

exposure. No associations were found between PD mortality and occupational exposure to solvents, metals, DME or electric shocks.

(E) Bukia N, Butskhrikidze M, Machavariani L, Kekelia G, Svanidze M. POSSIBLE IMPLEMENTATION OF GABAERGIC AND GLUTAMATERGIC SYSTEMS IN REALIZATION OF ANTIEPILEPTIC EFFECTS OF ACOUSTIC RANGE ELECTRO - MAGNETIC FIELDS. Georgian Med News. 280-281:112-116, 2018. (AS, CE, BE, ND, MA)

Seizure is a clinical manifestation of a hyperexcitable neuronal network, in which, the electrical balance underlying the normal neuronal activity is altered pathologically-excitation (Glutamatergic activity) predominates over inhibition (GABAergic activity). Arresting of seizure activity is carried out by restoration of neurotransmitter balance. This process has a direct relation with ion channel permeability in cell and ion transmembrane movement. Low frequency EMS may have a neurostimulating and neuromodulating effect that is based on electromagnetic induction of electric field in the brain. Under the conditions of certain amplitude, frequency and relaxation time low-frequency electromagnetic field (EMF) induces depolarization of separate neurons, and changes the total cortical excitability in case of repeatedly carried out procedures. It was shown that the exposure of acoustic range EMS in GEPRs treated with GABA-A or GABA-B receptors antagonists decreased behavior seizure activity in response to audiogenic stimuli. Injection of Glutamate receptor agonist on background EMS causes seizure activity, but seizure manifestations have less degree compared to non-stimulated rats. Thus, in response to electromagnetic stimulation, the reduction or complete cramping of seizures can be explained by a change in the activity of the neurotransmitter systems.

(E) Calabrò E. Competition between hydrogen bonding and protein aggregation in neuronal-like cells under exposure to 50 Hz magnetic field. Int J Radiat Biol. 2016 May 13:1-9. [Epub ahead of print] (CS, AE, CC)

PURPOSE: To investigate the role of hydrogen bonding and protein unfolding in human SH-SY5Y neuronal-like cells under exposure to a 50 Hz magnetic field (MF) at the intensity of 1 mT. MATERIALS AND METHODS: Neuronal-like cells were placed into an incubator in a 5% CO₂/95% air humidified at the temperature of 37.1 °C and exposed for 4 h to a 50 Hz MF at 1 mT. The exposure system consisted of two Helmholtz coils driven by AC voltage at 50 Hz. Exposed and control samples were studied using Fourier Transform Infrared (FTIR) Spectroscopy. RESULTS: The vibration bands of the methylene group increased significantly after 4 h of exposure. A significant shift to low energies of the Amide I band and an increase in the intensity of the parallel and antiparallel β -sheet structures with respect to the α -helix component were observed after exposure. The Amide II frequency did not change significantly whereas a relative increase of its integrated area with respect to Amide I mode occurred after exposure. CONCLUSIONS: These results can be explained assuming that both the mechanisms of protein aggregation as well as the increase in hydrogen bonding occurred in neuronal-like cells under exposure to a 50 Hz MF.

(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 pentylenetetrazol (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) Carrasco-López C, Soto-León V, Céspedes V, Profice P, Strange BA, Foffani G, Oliviero A. Static Magnetic Field Stimulation over Parietal Cortex Enhances Somatosensory Detection in Humans. J Neurosci. 37(14):3840-3847, 2017. (HU, AE, EE, BE)

The role of neuronal oscillations in human somatosensory perception is currently unclear. To address this, here we use noninvasive brain stimulation to artificially modulate cortical network dynamics in the context of neurophysiological and behavioral recordings. We demonstrate that transcranial static magnetic field stimulation (tSMS) over the somatosensory parietal cortex increases oscillatory power specifically in the alpha range, without significantly affecting bottom-up thalamocortical inputs indexed by the early cortical component of somatosensory evoked potentials. Critically, we next show that parietal tSMS enhances the detection of near-threshold somatosensory stimuli. Interestingly, this behavioral improvement reflects a decrease of habituation to

somatosensation. Our data therefore provide causal evidence that somatosensory perception depends on parietal alpha activity. SIGNIFICANCE STATEMENT Artificially increasing alpha power by placing a powerful magnetic field over the somatosensory cortex overcomes the natural decline in detection probability of a repeated near-threshold sensory stimulus.

(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) Cheng Y, Dai Y, Zhu X, Xu H, Cai P, Xia R, Mao L, Zhao BQ, Fan W. Extremely low-frequency electromagnetic fields enhance the proliferation and differentiation of neural progenitor cells cultured from ischemic brains. Neuroreport. 26(15):896-902, 2015. (CS, CE, FC, MA)

In the mammalian brain, neurogenesis persists throughout the embryonic period and adulthood in the subventricular zone of the lateral ventricle and the granular zone (dentate gyrus) of the hippocampus. Newborn neural progenitor cells (NPCs) in the two regions play a critical role in structural and functional plasticity and neural regeneration after brain injury. Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) could promote osteogenesis, angiogenesis, and cardiac stem cells' differentiation, which indicates that ELF-EMF might be an effective tool for regenerative therapy. The present studies were carried out to examine the effects of ELF-EMF on hippocampal NPCs cultured from embryonic and adult ischemic brains. We found that exposure to ELF-EMF (50 Hz, 0.4 mT) significantly enhanced the proliferation capability both in embryonic NPCs and in ischemic NPCs. Neuronal differentiation was also enhanced after 7 days of cumulative ELF-EMF exposure, whereas glial differentiation was not influenced markedly. The expression of phosphorylated Akt increased during the proliferation process when ischemic NPCs were exposed to ELF-EMF. However, blockage of the Akt pathway abolished the ELF-EMF-induced proliferation of ischemic NPCs. These data show that ELF-EMF promotes

neurogenesis of ischemic NPCs and suggest that this effect may occur through the Akt pathway.

(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) Choi YK, Lee DH, Seo YK, Jung H, Park JK, Cho H. Stimulation of neural differentiation in human bone marrow mesenchymal stem cells by extremely low-frequency electromagnetic fields incorporated with MNPs. Appl Biochem Biotechnol. 2014 Aug 7. [Epub ahead of print] (CS, AE, MC, MA)

Human bone marrow-derived mesenchymal stem cells (hBM-MSCs) have been investigated as a new cell-therapeutic solution due to their capacity that could differentiate into neural-like cells. Extremely low-frequency electromagnetic fields (ELF-EMFs) therapy has emerged as a novel technique, using mechanical stimulus to differentiate hBM-MSCs and significantly enhance neuronal differentiation to affect cellular and molecular reactions. Magnetic iron oxide (Fe_3O_4) nanoparticles (MNPs) have recently achieved widespread use for biomedical applications and polyethylene glycol (PEG)-labeled nanoparticles are used to increase their circulation time, aqueous solubility, biocompatibility, and nonspecific cellular uptake as well as to decrease immunogenicity. Many studies have used MNP-labeled cells for differentiation, but there have been no reports of MNP-labeled neural differentiation combined with EMFs. In this study, synthesized PEG-phospholipid encapsulated magnetite (Fe_3O_4) nanoparticles are used on hBM-MSCs to improve their intracellular uptake. The PEGylated nanoparticles were exposed to the cells under 50 Hz of EMFs to improve neural differentiation. First, we measured cell viability and intracellular iron content in hBM-MSCs after treatment with MNPs. Analysis was conducted by RT-PCR, and immunohistological analysis using neural cell type-specific genes and antibodies after exposure to 50 Hz electromagnetic fields. These results suggest that electromagnetic fields enhance neural differentiation in hBM-MSCs incorporated with MNPs and would be an effective method for differentiating neural cells.

(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) Chung YH, Lee YJ, Lee HS, Chung SJ, Lim CH, Oh KW, Sohn UD, Park ES, Jeong JH. Extremely low frequency magnetic field modulates the level of neurotransmitters. Korean J Physiol Pharmacol. 19(1):15-20, 2015. (AS, CE, CC, OX)

This study was aimed to observe that extremely low frequency magnetic field (ELF-MF) may be relevant to changes of major neurotransmitters in rat brain. After the exposure to ELF-MF (60 Hz, 2.0 mT) for 2 or 5 days, we measured the levels of biogenic amines and their metabolites, amino acid neurotransmitters and nitric oxide (NO) in the cortex, striatum, thalamus, cerebellum and hippocampus. The exposure of ELF-MF for 2 or 5 days produced significant differences in norepinephrine and vanillyl mandelic acid in the striatum, thalamus, cerebellum and hippocampus. Significant increases in the levels of serotonin and 5-hydroxyindoleacetic acid were also observed in the striatum, thalamus or hippocampus. ELF-MF significantly increased the concentration of dopamine in the thalamus. ELF-MF tended to increase the levels of amino

acid neurotransmitters such as glutamine, glycine and γ -aminobutyric acid in the striatum and thalamus, whereas it decreased the levels in the cortex, cerebellum and hippocampus. ELF-MF significantly increased NO concentration in the striatum, thalamus and hippocampus. The present study has demonstrated that exposure to ELF-MFs may evoke the changes in the levels of biogenic amines, amino acid and NO in the brain although the extent and property vary with the brain areas. However, the mechanisms remain further to be characterized.

(E) Cichoń N, Bijak M, Miller E, Saluk J. Extremely low frequency electromagnetic field (ELF-EMF) reduces oxidative stress and improves functional and psychological status in ischemic stroke patients. Bioelectromagnetics. 38(5):386-396, 2017. (HU, CE, OX, BE, MA)

As a result of ischaemia/reperfusion, massive generation of reactive oxygen species occurs, followed by decreased activity of antioxidant enzymes. Extremely low frequency electromagnetic fields (ELF-EMF) can modulate oxidative stress, but there are no clinical antioxidant studies in brain stroke patients. The aim of our study was to investigate the effect of ELF-EMF on clinical and antioxidant status in post-stroke patients. Fifty-seven patients were divided into two groups: ELF-EMF and non-ELF-EMF. Both groups underwent the same 4-week rehabilitation program. Additionally, the ELF-EMF group was exposed to an ELF-EMF field of 40 Hz, 7 mT for 15 min/day for 4 weeks (5 days a week). The activity of catalase and superoxide dismutase was measured in hemolysates, and total antioxidant status (TAS) determined in plasma. Functional status was assessed before and after the series of treatments using Activities of Daily Living (ADL), Mini-Mental State Examination (MMSE), and Geriatric Depression Scale (GDS). Applied ELF-EMF significantly increased enzymatic antioxidant activity; however, TAS levels did not change in either group. Results show that ELF-EMF induced a significant improvement in functional (ADL) and mental (MMSE, GDS) status. Clinical parameters had positive correlation with the level of enzymatic antioxidant protection.

(E) Cichoń N, Czarny P, Bijak M, Miller E, Śliwiński T, Szemraj J, Saluk-Bijak J. Benign Effect of Extremely Low-Frequency Electromagnetic Field on Brain Plasticity Assessed by Nitric Oxide Metabolism during Poststroke Rehabilitation. Oxid Med Cell Longev. 2017:2181942, 2017. (HU, CE, OX, FC, MA)

Nitric oxide (NO) is one of the most important signal molecules, involved in both physiological and pathological processes. As a neurotransmitter in the central nervous system, NO regulates cerebral blood flow, neurogenesis, and synaptic plasticity. The aim of our study was to investigate the effect of the extremely low-frequency electromagnetic field (ELF-EMF) on generation and metabolism of NO, as a neurotransmitter, in the rehabilitation of poststroke patients. Forty-eight patients were divided into two groups: ELF-EMF and non-ELF-EMF. Both groups underwent the same 4-week rehabilitation program. Additionally, the ELF-EMF group was exposed to an extremely low-frequency electromagnetic field of 40 Hz, 7 mT, for 15 min/day. Levels of 3-nitrotyrosine, nitrate/nitrite, and TNF α in plasma samples were measured, and NOS2 expression was determined in whole blood samples. Functional status was evaluated before and after a series of treatments, using the Activity Daily Living, Geriatric Depression Scale, and Mini-Mental State Examination. We observed that application of ELF-EMF significantly increased 3-nitrotyrosine and nitrate/nitrite levels, while

expression of NOS2 was insignificantly decreased in both groups. The results also show that ELF-EMF treatments improved functional and mental status. We conclude that ELF-EMF therapy is capable of promoting recovery in poststroke patients.

(E) Cichoń N, Bijak M, Czarny P, Miller E, Synowiec E, Sliwinski T, Saluk-Bijak J. Increase in Blood Levels of Growth Factors Involved in the Neuroplasticity Process by Using an Extremely Low Frequency Electromagnetic Field in Post-stroke Patients. Front Aging Neurosci. 10:294, 2018. (HU, CE BE, CC, MA)

Background: Neuroplasticity ensures the improvement of functional status in patients after stroke. The aim of this study was to evaluate the effect of extremely low-frequency electromagnetic field therapy (ELF-EMF) on brain plasticity in the rehabilitation of patients after stroke. Methods: Forty-eight patients were divided into two groups underwent the same rehabilitation program, but in the study group, the patients additionally were exposed to a standard series of 10 ELF-EMF treatments. To determine the level of neuroplasticity, we measured the plasma level of the brain-derived neurotrophic factor (BDNF), the vascular-endothelial growth factor, as well as BDNF mRNA expression. Additionally, we determined the molecule levels for hepatocyte growth factor, stem cell factor, stromal cell-derived factor 1 α , nerve growth factor β , and leukemia inhibitory factor, using 5plex cytokine panel in plasma. After 4 weeks, during which patients had undergone neurorehabilitation and neurological examinations, we assessed functional recovery using the Barthel Index, Mini-Mental State Examination (MMSE), Geriatric Depression Scale, National Institutes of Health Stroke Scale (NIHSS), and the modified Rankin Scale (mRS). Results: We observed that ELF-EMF significantly increased growth factors and cytokine levels involved in neuroplasticity, as well as promoted an enhancement of functional recovery in post-stroke patients. Additionally, we presented evidence that these effects could be related to the increase of gene expression on the mRNA level. Moreover, a change of BDNF plasma level was positively correlated with the Barthel Index, MMSE, and negatively correlated with GDS. Conclusion: Extremely low-frequency electromagnetic field therapy improves the effectiveness of rehabilitation of post-stroke patients by improving neuroplasticity processes.

(E) Cichon N, Bijak M, Synowiec E, Miller E, Sliwinski T, Saluk-Bijak J. Modulation of antioxidant enzyme gene expression by extremely low frequency electromagnetic field in post-stroke patients. Scand J Clin Lab Invest. 78(7-8):626-631, 2018. (HU, CE, OX, MA)

Oxidative stress plays the most important role in the pathogenesis of stroke. Extremely low frequency electromagnetic field (ELF-EMF) therapy may be complementary in post-stroke therapy, as it modulates oxidative stress. The aim of this study was to evaluate the messenger ribonucleic acid (mRNA) levels of certain antioxidant genes in post-stroke patients given ELF-EMF therapy. Forty-eight post-stroke patients were divided into two groups: an ELF-EMF group and a non-ELF-EMF group. All patients underwent the same program of physical therapy, but the ELF-EMF group was additionally given ELF-EMF treatment. In order to determine the level of gene expression, we evaluated the level of mRNA expression of catalase, superoxide dismutase, and glutathione peroxidase. We observed that after ELF-EMF therapy, the mRNA expression of the studied genes (CAT, SOD1, SOD2, GPx1, and GPx4) significantly increased, which enhanced the antioxidant defence of the body. ELF-EMF therapy intensifies the endogenous antioxidant system by increasing the mRNA expression of genes encoding antioxidant enzymes and enhances the effectiveness of post-stroke patient therapy.

(E) Cichoń N, Rzeźnicka P, Bijak M, Miller E, Miller S, Saluk J. Extremely low frequency electromagnetic field reduces oxidative stress during the rehabilitation of post-acute stroke patients. Adv Clin Exp Med. 27(9):1285-1293, 2018. (HU, CE, BE, OX, MA)

BACKGROUND: One of the therapeutic methods used in stroke rehabilitation is magnetotherapy using extremely low frequency and variable pulse shape electromagnetic field (ELF-EMF). OBJECTIVES: The aim of our study was to investigate the effect of magnetotherapy on the condition of postacute stroke patients, as measured by plasma oxidative stress markers and clinical parameters which show the progress of rehabilitation. MATERIAL AND METHODS: The selected 57 post-stroke patients were divided into 2 groups, those with ELFEMF therapy and those without. The level of oxidative stress in the plasma was estimated by typical markers: thiobarbituric acid reactive substances (TBARS), thiol groups, and carbonyl groups. The effect of ELF-EMF on the course of the patients' rehabilitation following ischemic stroke was evaluated with the use of scales of physical activity and mental state: Activities of Daily Living (ADL), Mini-Mental State Examination (MMSE) and Geriatric Depression Scale (GDS). RESULTS: Our comparative analysis showed that all parameters of oxidative stress are significantly reduced during rehabilitation using ELF-EMF, compared to the control group rehabilitated only by kinesiotherapy. We also recorded much higher therapeutic benefits using magnetotherapy, which revealed a significant improvement of clinimetric parameters. CONCLUSIONS: The ELF-EMF therapy meaningfully improves the overall condition of patients through a decrease of oxidative stress markers and it significantly affects the psychophysical abilities of patients after stroke. The change in carbonyl group level correlates with the change in the degree of physical and mental disability; therefore, it could be a marker for the effectiveness of rehabilitation.

(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 (H_2O_2), 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 H_2O_2 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) Clarke D, Penrose MA, Penstone T, Fuller-Carter PI, Hool LC , Harvey AR, Rodger J, Bates KA. Frequency-specific effects of repetitive magnetic stimulation on primary astrocyte cultures. Restor Neurol Neurosci. 35(6):557-569, 2017. (CS, AE, FC)

BACKGROUND: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique that uses magnetic pulses over the cranium to induce electrical currents in underlying cortical tissue. Although rTMS has shown clinical utility for a number of neurological conditions, we have only limited understanding of how rTMS influences cellular function and cell-cell interactions. **OBJECTIVE:** In this study, we sought to investigate whether repeated magnetic stimulation (rMS) can influence astrocyte biology in vitro. **METHOD:** We tested four different rMS frequencies and measured the calcium response in primary neonatal astrocyte cultures. We also tested the effect of rMS on astrocyte migration and proliferation in vitro. We tested 3 to 4 culture replicates and 17 to 34 cells for each rMS frequency (sham, 1 Hz, cTBS, 10 Hz and biomimetic high frequency stimulation - BHFS). **RESULTS:** Of all frequencies tested, 1 Hz stimulation resulted in a statistically significant rise in intracellular calcium in the cytoplasmic and nuclear compartments of the cultured astrocytes. This calcium rise did not affect migration or proliferation in the scratch assay, though astrocyte hypertrophy was reduced in response to 1 Hz rMS, 24 hours post scratch injury. **CONCLUSION:** Our results provide preliminary evidence that rMS can influence astrocyte physiology, indicating the potential for a novel mechanism by which rTMS can influence brain activity.

(E) Consales C, Cirotti C, Filomeni G, Panatta M, Butera A, Merla C, Lopresto V, Pinto R, Marino C, Benassi B. Fifty-Hertz Magnetic Field Affects the Epigenetic Modulation of the miR-34b/c in Neuronal Cells. Mol Neurobiol. 55(7):5698-5718, 2018. (CS, AE, OX, CC, ND)

The exposure to extremely low-frequency magnetic fields (ELF-MFs) has been associated to increased risk of neurodegenerative diseases, although the underlying molecular mechanisms are still undefined. Since epigenetic modulation has been recently encountered among the key events leading to neuronal degeneration, we here aimed at assessing if the control of gene expression mediated by miRNAs, namely miRs-34, has any roles in driving neuronal cell response to 50-Hz (1 mT) magnetic field in vitro. We demonstrate that ELF-MFs drive an early reduction of the expression level of miR-34b and miR-34c in SH-SY5Y human neuroblastoma cells, as well as in mouse primary cortical neurons, by affecting the transcription of the common pri-miR-34. This modulation is not p53 dependent, but attributable to the hyper-methylation of the CpG island mapping within the miR-34b/c promoter. Incubation with N-acetyl-l-cysteine or glutathione ethyl-ester fails to restore miR-34b/c expression, suggesting that miRs-34 are not responsive to ELF-MF-induced oxidative stress. By contrast, we show that miRs-34 control reactive oxygen species production and affect mitochondrial oxidative stress

triggered by ELF-MFs, likely by modulating mitochondria-related miR-34 targets identified by in silico analysis. We finally demonstrate that ELF-MFs alter the expression of the α -synuclein, which is specifically stimulated upon ELF-MFs exposure via both direct miR-34 targeting and oxidative stress. Altogether, our data highlight the potential of the ELF-MFs to tune redox homeostasis and epigenetic control of gene expression in vitro and shed light on the possible mechanism(s) producing detrimental effects and predisposing neurons to degeneration.

(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, Balabanlı 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) Davarpanah Jazi S, Modolo J, Baker C, Villard S, Legros A. Effects of A 60 Hz Magnetic Field of Up to 50 milliTesla on Human Tremor and EEG: A Pilot Study. Int J Environ Res Public Health. 2017 Nov 24;14(12). pii: E1446. doi: 10.3390/ijerph14121446. (HU, AE, EE)

Humans are surrounded by sources of daily exposure to power-frequency (60 Hz in North America) magnetic fields (MFs). Such time-varying MFs induce electric fields and currents in living structures which possibly lead to biological effects. The present pilot study examined possible extremely low frequency (ELF) MF effects on human neuromotor control in general, and physiological postural tremor and electroencephalography (EEG) in particular. Since the EEG cortical mu-rhythm (8-12 Hz) from the primary motor cortex and physiological tremor are related, it was hypothesized that a 60 Hz MF exposure focused on this cortical region could acutely modulate human physiological tremor. Ten healthy volunteers (age: 23.8 ± 4 SD) were fitted with a MRI-compatible EEG cap while exposed to 11 MF conditions (60 Hz, 0 to 50 mT_{rms}, 5 mT_{rms} increments). Simultaneously, physiological tremor (recorded from the contralateral index finger) and EEG (from associated motor and somatosensory brain regions) were measured. Results showed no significant main effect of MF exposure conditions on any of the analyzed physiological tremor characteristics. In terms of EEG, no significant effects of the MF were observed for C1, C3, C5 and CP1 electrodes. However, a significant main effect was found for CP3 and CP5 electrodes, both suggesting a decreased mu-rhythm spectral power with increasing MF flux density. This is however not confirmed by Bonferroni corrected pairwise comparisons. Considering both EEG and tremor findings, no effect of the MF exposure on human motor control was observed. However, MF exposure had a subtle effect on the mu-rhythm amplitude in the brain region involved in tactile perception. Current findings are to be considered with caution due to the small size of this pilot work, but they provide preliminary insights to international agencies establishing guidelines regarding electromagnetic field exposure with new experimental data acquired in humans exposed to high mT-range MFs.

(NE) de Groot MW, Kock MD, Westerink RH. Assessment of the neurotoxic potential of exposure to 50Hz extremely low frequency electromagnetic fields (ELF-EMF) in naïve and

chemically-stressed PC12 cells. Neurotoxicology. 2014 Aug 8. pii: S0161-813X(14)00138-7. doi: 10.1016/j.neuro.2014.07.009. [Epub ahead of print] (CS, AE, CC, OX)

Increasing exposure to extremely low frequency electromagnetic fields (ELF-EMF), generated by power lines and electric appliances, raises concern about potential adverse health effects of ELF-EMF. The central nervous system is expected to be particularly vulnerable to ELF-EMF as its function strongly depends on electrical excitability. We therefore investigated effects of acute (30min) and sub-chronic (48h) exposure to 50Hz ELF-EMF on naïve and chemically-stressed pheochromocytoma (PC12) cells. The latter have higher levels of iron and/or reactive oxygen species (ROS) and display increased vulnerability to environmental insults. Effects of ELF-EMF on Ca^{2+} -homeostasis, ROS production and membrane integrity were assessed using Fura-2 single cell fluorescence microscopy, H_2 -DCFDA and CFDA assays, respectively. Our data demonstrate that acute exposure of naïve PC12 cells to 50 Hz ELF-EMF up to 1000 μT fails to affect basal or depolarization-evoked $[\text{Ca}^{2+}]_i$. Moreover, sub-chronic ELF-EMF exposure up to 1000 μT has no consistent effects on Ca^{2+} -homeostasis in naïve PC12 cells and does not affect ROS production and membrane integrity. Notably, in chemically-stressed PC12 cells both acute and sub-chronic ELF-EMF exposure also failed to exert consistent effects on Ca^{2+} -homeostasis, ROS production and membrane integrity. Our combined findings thus indicate that exposure to 50Hz ELF-EMF up to 1000 μT , i.e. 10,000 times above background exposure, does not induce neurotoxic effects in vitro, neither in naïve nor in chemically-stressed PC12 cells. Though our data require confirmation, e.g. in developing neuronal cells in vitro or (developing) animals, it appears that the neurotoxic risk of ELF-EMF exposure is limited.

(E) de Groot MW, van Kleef RG, de Groot A, Westerink RH. In vitro developmental neurotoxicity following chronic exposure to 50 Hz extremely low frequency electromagnetic fields (ELF-EMF) in primary rat cortical cultures. Toxicol Sci. 2015 Nov 15. pii: kfv242. [Epub ahead of print] (CS, CE, CC)

Exposure to 50-60 Hz extremely low frequency electromagnetic fields (ELF-EMFs) has increased considerably over the last decades. Several epidemiological studies suggested that ELF-EMF exposure is associated with adverse health effects, including neurotoxicity. However, these studies are debated as results are often contradictory and the possible underlying mechanisms are unknown. Since the developing nervous system is particularly vulnerable to insults, we investigate effects of chronic, developmental ELF-EMF exposure in vitro. Primary rat cortical neurons received 7 days developmental exposure to 50 Hz block-pulsed ELF-EMF (0-1000 μT) to assess effects on cell viability (Alamar Blue/CFDA assay), calcium homeostasis (single cell fluorescence microscopy), neurite outgrowth (β (III)-Tubulin immunofluorescent staining) and spontaneous neuronal activity (multi-electrode arrays [MEAs]). Our data demonstrate that cell viability is not affected by developmental ELF-EMF (0-1000 μT) exposure. Depolarization- and glutamate-evoked increases in intracellular calcium concentration ($[\text{Ca}^{2+}]_i$) are slightly increased at 1 μT , whereas both basal and stimulation-evoked $[\text{Ca}^{2+}]_i$ show a modest inhibition at 1000 μT . Subsequent morphological analysis indicated that neurite length is unaffected up to 100 μT , but increased at 1000 μT . However, neuronal activity appeared largely unaltered following chronic ELF-EMF exposure up to 1000 μT . The effects of ELF-EMF

exposure were small and largely restricted to the highest field strength (1000 μ T), i.e. 10,000 times above background exposure and well above current residential exposure limits. Our combined data therefore indicate that chronic ELF-EMF exposure has only limited (developmental) neurotoxic potential in vitro.

(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 Abeta 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 (Abeta) secretion, including the isoform Abeta 1-42, without affecting cell survival. These findings show for the first time that exposure to LF-EMF stimulates Abeta 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) Dey S, Bose S, Kumar S, Rathore R, Mathur R, Jain S. Extremely low frequency magnetic field protects injured spinal cord from the microglia- and iron-induced tissue damage. Electromagn Biol Med. 2017 Nov 15:1-11. doi: 10.1080/15368378.2017.1389750. [Epub ahead of print] (AS, CE, FC, MA)

Spinal cord injury (SCI) is insult to the spinal cord, which results in loss of sensory and motor function below the level of injury. SCI results in both immediate mechanical damage and secondary tissue degeneration. Following traumatic insult, activated microglia release proinflammatory cytokines and excess iron due to hemorrhage, initiating oxidative stress that contributes to secondary degeneration. Literature suggests that benefits are visible with the reduction in concentration of iron and activated microglia in SCI. Magnetic field attenuates oxidative stress and promotes axonal regeneration in vitro and in vivo. The present study demonstrates the potential of extremely low frequency magnetic field to attenuate microglia- and iron-induced secondary injury in SCI rats. Complete transection of the spinal cord (T13 level) was performed in male Wistar rats and subsequently exposed to magnetic field (50 Hz, 17.96 μ T) for 2 h daily for 8 weeks. At the end of the study period, spinal cords were dissected to quantify microglia, macrophage, iron content and study the architecture of lesion site. A significant improvement in locomotion was observed in rats of the SCI + MF group as compared to those in the SCI group. Histology, immunohistochemistry and flow cytometry revealed significant reduction in lesion volume, microglia, macrophage, collagen tissue and iron content, whereas, a significantly higher vascular endothelial growth factor expression around the epicenter of the lesion in SCI + MF group as compared to SCI group. These novel findings suggest that exposure to ELF-MF reduces lesion volume, inflammation and iron content in addition to facilitation of angiogenesis following SCI.

(E) Di G, Kim H, Xu Y, Kim J, Gu X. A comparative study on influences of static electric field and power frequency electric field on cognition in mice. Environ Toxicol Pharmacol. 66:91-95, 2019. (AS, CE, BE, EE)

Recently, electromagnetic fields around ultra-high voltage transmission lines have received considerable attentions for their potential biological effects. This study aimed to investigate the effects of static electric field (SEF) and power frequency electric field (PFEF) on cognition. Mice were exposed to SEF and PFEF with the same strength (35 kV/m) for 49 days, respectively. Behaviors in Morris water maze test and amino acid neurotransmitter levels in hippocampus were examined during exposure. Results indicated that the exposure of 35 kV/m SEF would not cause significant influences on learning and memory ability in mice, while the exposure of 35 kV/m PFEF would cause significant positive effects on learning and memory ability in mice on day 33. This difference in effects from SEF and PFEF on cognition was possibly induced by the difference in the degree of molecular polarization and ion migration in organisms under exposure of two kinds of electric fields with different frequency.

(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) Dileone M, Carrasco-López MC, Segundo-Rodriguez JC, Mordillo-Mateos L, López-Ariztegui N, Alonso-Frech F, Catalan-Alonso MJ, Obeso JA, Oliviero A, Foffani G, Dopamine-dependent changes of cortical excitability induced by transcranial static magnetic field stimulation in Parkinson's disease. Sci Rep. 7(1):4329, 2017. (HU, AE, ND, CE, EE, MA)

Transcranial static magnetic field stimulation (tSMS) is a recent low-cost non-invasive brain stimulation technique that decreases cortical excitability in healthy subjects. The objective of the present study was to test the ability of tSMS to modulate cortical excitability in patients with Parkinson's disease. We performed a randomized double-blind sham-controlled cross-over study to assess cortical excitability before and immediately after tSMS (or sham) applied for 10 min to the more affected motor cortex of patients with Parkinson's disease. Cortical excitability was quantified by the amplitude of motor evoked potentials (MEPs) elicited by single-pulse transcranial magnetic stimulation (TMS). tSMS significantly decreased MEP amplitudes in patients OFF medication (after overnight withdrawal of dopaminergic drugs), but not ON medication (after an acute dose of levodopa). The between-patients variability of tSMS-induced changes was significantly greater ON medication. The variability ON medication could be partly explained by disease progression, i.e. the more advanced the patient, the more likely it was to observe a switch from inhibitory tSMS plasticity OFF medication to paradoxical facilitatory plasticity ON medication. These results suggest that tSMS

induces dopamine-dependent changes of cortical excitability in patients with Parkinson's disease.

(E) Dileone M, Mordillo-Mateos L, Oliviero A, Foffani G. Long-lasting effects of transcranial static magnetic field stimulation on motor cortex excitability. Brain Stimul. 11(4):676-688, 2018. (HU, AE, EE)

BACKGROUND: Transcranial static magnetic field stimulation (tSMS) was recently added to the family of inhibitory non-invasive brain stimulation techniques. However, the application of tSMS for 10-20 min over the motor cortex (M1) induces only short-lasting effects that revert within few minutes. **OBJECTIVE:** We examined whether increasing the duration of tSMS to 30 min leads to long-lasting changes in cortical excitability, which is critical for translating tSMS toward clinical applications. **METHODS:** The study comprised 5 experiments in 45 healthy subjects. We assessed the impact of 30-min-tSMS over M1 on corticospinal excitability, as measured by the amplitude of motor evoked potentials (MEPs) and resting motor thresholds (RMTs) to single-pulse transcranial magnetic stimulation (TMS) (experiments 1-2). We then assessed the impact of 30-min-tSMS on intracortical excitability, as measured by short-interval intracortical facilitation (SICF) and short-interval intracortical inhibition (SICI) using paired-pulse TMS protocols (experiments 2-4). We finally assessed the impact of 10-min-tSMS on SICF and SICI (experiment 5). **RESULTS:** 30-min-tSMS decreased MEP amplitude compared to sham for at least 30 min after the end of the stimulation. This long-lasting effect was associated with increased SICF and reduced SICI. 10-min-tSMS -previously reported to induce a short-lasting decrease in MEP amplitude- produced the opposite changes in intracortical excitability, decreasing SICF while increasing SICI. **CONCLUSIONS:** These results suggest a dissociation of intracortical changes in the consolidation from short-lasting to long-lasting decrease of corticospinal excitability induced by tSMS. The long-lasting effects of 30-min-tSMS open the way to the translation of this simple, portable and low-cost technique toward clinical trials.

(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) Dinčić M, Krstić DZ, Čolović MB, Nešović Ostojić J, Kovačević S, De Luka SR, Djordjević DM, Ćirković S, Brkić P, Todorović J. Modulation of rat synaptosomal ATPases and acetylcholinesterase activities induced by chronic exposure to the static magnetic field. Int J Radiat Biol. 94(11):1062-1071, 2018. (AS, CE, OX, CC, MA)

PURPOSE: It is considered that exposure to static magnetic fields (SMF) may have both detrimental and therapeutic effect, but the mechanism of SMF influence on the living organisms is not well understood. Since the adenosine triphosphatases (ATPases) and acetylcholinesterase (AChE) are involved in both physiological and pathological processes, the modulation of Na⁺/K⁺-ATPase, ecto-ATPases and AChE activities, as well as oxidative stress responses were followed in synaptosomes isolated from rats after chronic exposure toward differently oriented SMF. **MATERIAL AND METHODS:** Wistar albino rats were randomly divided into three experimental groups (six animals per group): Up and Down group - exposed to upward and downward oriented SMF, respectively, and Control group. After 50 days, the rats were sacrificed, and synaptosomes were isolated from the whole rat brain and used for testing the enzyme activities and oxidative stress parameters. **RESULTS:** Chronic exposure to 1 mT SMF significantly increased ATPases, AChE activities, and malondialdehyde (MDA) level in both exposed groups, compared to control values. The significant decrease in synaptosomal catalase activity (1.48 ± 0.17 U/mg protein) induced by exposure to the downward oriented field, compared to those obtained for Control group (2.60 ± 0.29 U/mg protein), and Up group (2.72 ± 0.21 U/mg protein). **CONCLUSIONS:** It could be concluded that chronic exposure to differently oriented SMF increases ATPases and AChE activities in rat synaptosomes. Since brain ATPases and AChE have important roles in the pathogenesis of several neurological diseases, SMF influence on the activity of these enzymes may have potential therapeutic importance.

(E) Djordjevic NZ, Paunović MG, Peulić AS. Anxiety-like behavioural effects of extremely low-frequency electromagnetic field in rats. Environ Sci Pollut Res Int. 2017 Jul 29. doi: 10.1007/s11356-017-9710-1. [Epub ahead of print] (AS, CE, BE, OX)

In recent years, extremely low-frequency electromagnetic field (ELF-EMF) has received considerable attention for its potential biological effects. Numerous studies have shown the role of ELF-EMF in behaviour modulation. The aim of this study was to investigate the effect of short-term ELF-EMF (50 Hz) in the development of anxiety-like behaviour in rats through change hypothalamic oxidative stress and NO. Ten adult male rats (Wistar albino) were divided in two groups: control group-without exposure to ELF-EMF and experimental group-exposed to ELF-EMF during 7 days. After the exposure, time open field test and elevated plus maze were used to evaluate the anxiety-like behaviour

of rats. Upon completion of the behavioural tests, concentrations of superoxide anion ($O_2^{\cdot-}$), nitrite (NO_2^- , as an indicator of NO) and peroxynitrite ($ONOO^-$) were determined in the hypothalamus of the animals. Obtained results show that ELF-EMF both induces anxiety-like behaviour and increases concentrations of $O_2^{\cdot-}$ and NO, whereas it did not effect on $ONOO^-$ concentration in hypothalamus of rats. In conclusion, the development of anxiety-like behaviour is mediated by oxidative stress and increased NO concentration in hypothalamus of rats exposed to ELF-EMF during 7 days.

(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) Duan Y, Wang Z, Zhang H, He Y, Fan R, Cheng Y, Sun G, Sun X. Extremely low frequency electromagnetic field exposure causes cognitive impairment associated with alteration of the glutamate level, MAPK pathway activation and decreased CREB phosphorylation in mice hippocampus: reversal by procyanidins extracted from the lotus seedpod. Food Funct. 5(9):2289-2297, 2014. (AS, CE, FC, CC)

Lotus seedpod procyanidins (LSPCs) could effectively prevent learning and memory damage and oxidative damage caused by extremely low frequency electromagnetic field (ELF-EMF) exposure. However, LSPCs protect neurons from ELF-EMF-induced damage by mechanisms currently not clear. An excessive release of glutamate is considered to be one of the molecular

mechanisms of neuronal damage in several neurological diseases. In this study we determined whether the ELF-EMF (50 Hz, 8 mT, 28 days) exposure induced alterations of glutamate release in mice hippocampus and explored the possible mechanism, and if LSPC treatment normalized its alterations. The results showed that ELF-EMF exposure induced the increased contents of glutamate, GABA, excessively activated NMDA receptors, increasing the number of NMDA receptor 2B (NR2B) and intracellular Ca^{2+} concentration $[\text{Ca}^{2+}]_i$ in hippocampus. In addition, ELF-EMF exposure decreased the ERK1/2 and CREB phosphorylation, which suggested that the Ca^{2+} influx induced by the ELF-EMF exposure stimulated activity of the ERK, in turn, influences the expression of downstream proteins in this signaling pathway. Besides, ELF-EMF exposure also increased JNK1/2 phosphorylation through the activated ASK1, which plays a pivotal role in hippocampal neuronal cell death. However, oral administration of LSPCs (especially 60 and 90 mg kg^{-1}) markedly improved expressions of p-CREB, p-ERK1/2 and p-JNK1/2, accompanied by decreased levels of glutamate, GABA, $[\text{Ca}^{2+}]_i$ and NR2B. Thus, the results from the present study suggest that p-ERK1/2, p-JNK1/2, $[\text{Ca}^{2+}]_i$ and p-CREB expression normalized, possibly via a NMDA receptor-channel through the changes of GABA, glutamate and NR2B, which might be responsible for the neuroprotective or memory enhancing effects of LSPCs.

(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) Elferchichi M, Ammari M, Maaroufi K, Sakly M, Abdelmelek H. Effects of exposure to static magnetic field on motor skills and iron levels in plasma and brain of rats. Brain Inj. 25(9):901-908, 2011. (AS, CE, CC, BE)

PRIMARY OBJECTIVE: The present work investigated the behavioural and biochemistry effects of moderate exposure to a static magnetic field (SMF) in rats. SMF effects were evaluated in sham- and SMF-exposed rats. **METHODS:** Adult Wistar rats were exposed for 1 hour per day for 5 consecutive days to 128 millitesla (mT) SMF.

Then, their motor skills were tested using a Stationary beam and Suspended string test. Iron level in plasma and brain (i.e. frontal cortex, basal ganglia, hippocampus and cerebellum) was measured. **RESULTS:** No significant change was observed between sham and SMF-exposed rats in the Stationary beam and Suspended string test. However, the same treatment induced an increase in plasma transferrin content (+25.4%) and decreased the iron level in plasma (-16.2%). The SMF treatment failed to alter the iron concentration in the brain. **CONCLUSION:** The findings indicate that SMF exposure induced iron deficiency in plasma but did not induce motor-skills deficit.

(E) Erdal ME, Yılmaz SG, Gürgül S, Uzun C, Derici D, Erdal N. miRNA expression profile is altered differentially in the rat brain compared to blood after experimental exposure to 50 Hz and 1 mT electromagnetic field. Prog Biophys Mol Biol. 132:35-42, 2018. (AS, CE, CC)

Common complex diseases are a result of host and environment interactions. One such putative environmental factor is the electromagnetic field exposure, especially the occupational extremely low frequency (ELF) magnetic field, 50 Hz, 1 mT, whose neurobiological relevance remains elusive. We evaluated the effects of long-term (60 days) ELF-MF exposure on miRNAs previously related to brain and human diseases (miR-26b-5p, miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p). A total of 64 young (3 weeks-old) and mature (10 weeks-old) male/female Wistar-Albino rats were divided into sham and ELF-MF exposed groups. After sacrifice of the animals, blood samples from rat's tail vein and brain tissues were collected. The expression levels of miRNAs were investigated with Real-Time PCR technique and TaqMan probe Technology. All miRNA expression levels of the young female rats show a significant decrease in blood according to brain samples ($p < 0.05$), but fewer miRNAs displayed a similar significant decrease in the blood. In conclusion, these new observations might inform future clinical biological psychiatry studies of long-term electromagnetic field exposure, and the ways in which host-environment interactions contribute to brain diseases.

(E) Esmaeilpour K, Sheibani V, Shabani M, Mirnajafi-Zadeh J, Akbarnejad Z. Low Frequency Stimulation Reverses the Kindling-Induced Impairment of Learning and Memory in the Rat Passive-avoidance Test. Basic Clin Neurosci. 9(1):51-58, 2018. (AS, CE, BE, MA)

INTRODUCTION: The life quality of patients with epileptic seizures is highly affected by cognitive deficits. Low Frequency Stimulation (LFS) is a novel approach for the treatment of pharmacoresistant epilepsy. The main goal of this research is investigating the possible effect of LFS on seizure-induced cognitive dysfunction. **METHODS:** To this end, the kindled animal were prepared via CA1 electrical stimulation in a semi-rapid way (12 stimulations/day). A group of animals were stimulated with LFS, 4 times at 30 s, 6 h, 24 h, and 30 h after the last kindling stimulation. Applied LFS was administered in 4 packages every 5 minutes. The packages were designed with 200 monophasic 200 monophasic square wave pulses of 0.1 ms duration at 1 Hz. The passive-avoidance test was conducted on all animals in order to measure the learning and memory

behavior. **RESULTS:** Hippocampal kindled rats showed deficits in learning and memory when passive avoidance test was performed. Application of LFS reversed the impairment in learning and memory behavior in kindled rats. At the same time, LFS markedly diminished kindling-induced neuronal loss and atrophy in the hippocampus. **CONCLUSION:** LFS may have some protection against seizure-induced cognitive damage in kindled rats.

(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, Marigliò 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) Falone S, Santini S Jr, Di Loreto S, Cordone V, Grannonico M, Cesare P, Cacchio M, Amicarelli F. Improved Mitochondrial and Methylglyoxal-Related Metabolisms Support Hyperproliferation Induced by 50 Hz Magnetic Field in Neuroblastoma Cells. J Cell Physiol. 2016 Jan 12. doi: 10.1002/jcp.25310. [Epub ahead of print] (CS, AE, FC)

Extremely low frequency magnetic fields (ELF-MF) are common environmental agents that are suspected to promote later stages of tumorigenesis, especially in brain-derived malignancies. Even though ELF magnetic fields have been previously linked to increased proliferation in neuroblastoma cells, no previous work has studied whether ELF-MF exposure may change key biomolecular features, such as anti-glycative defence and energy re-programming, both of which are currently considered as crucial factors involved in the phenotype and progression of many malignancies. Our study investigated whether the hyperproliferation that is induced in SH-SY5Y human neuroblastoma cells by a 50 Hz, 1 mT ELF magnetic field is supported by an improved defense towards methylglyoxal (MG), which is an endogenous cancer-static and glyating α -oxoaldehyde, and by rewiring of energy metabolism. Our findings show that not only the ELF magnetic field interfered with the biology of neuron-derived malignant cells, by de-differentiating further the cellular phenotype and by increasing the proliferative activity, but also triggered cytoprotective mechanisms through the enhancement of the defense against MG, along with a more efficient management of metabolic energy, presumably to support the rapid cell outgrowth. Intriguingly, we also revealed that the MF-induced bioeffects took place after an initial imbalance of the cellular homeostasis, which most likely created a transient unstable milieu. The biochemical pathways and molecular targets revealed in this research could be exploited for future approaches aimed at limiting or suppressing the deleterious effects of ELF magnetic fields.

(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) Frilot C 2nd, Carrubba S, Marino AA. Sensory transduction of weak electromagnetic fields: role of glutamate neurotransmission mediated by NMDA receptors. Neuroscience. 258:184-191, 2014. (AS, AE, EE)

Subliminal electromagnetic fields (EMFs) triggered nonlinear evoked potentials in awake but not anesthetized animals, and increased glucose metabolism in the hindbrain. Field detection occurred somewhere in the head and possibly was an unrecognized function of sensory neurons in facial skin, which synapse in the trigeminal nucleus and project to the thalamus via glutamate-dependent pathways. If so, anesthetic agents that antagonize glutamate neurotransmission would be expected to degrade EMF-evoked potentials (EEPs) to a greater extent than agents having different pharmacological effects. We tested the hypothesis using ketamine which blocks N-methyl-d-aspartate (NMDA) receptors (NMDARs), and xylazine which is an α_2 -adrenoreceptor agonist. Electroencephalograms (EEGs) of rats were examined using recurrence analysis to observe EEPs in the presence and absence of ketamine and/or xylazine anesthesia. Auditory evoked potentials (AEPs) served as positive controls. The frequency of observation of evoked potentials in a given condition (wake or anesthesia) was compared with that due to chance using the Fisher's exact test. EEPs were observed in awake rats but not while

they were under anesthesia produced using a cocktail of xylazine and ketamine. In another experiment each rat was measured while awake and while under anesthesia produced using either xylazine or ketamine. EEPs and AEPs were detected during wake and under xylazine ($P < 0.05$ in each of the four measurements). In contrast, neither EEPs nor AEPs were observed when anesthesia was produced partly or wholly using ketamine. The duration and latency of the EEPs was unaltered by xylazine anesthesia. The afferent signal triggered by the transduction of weak EMFs was likely mediated by NMDAR-mediated glutamate neurotransmission.

(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.

(E) Gallasch E, Rafolt D, Postruznik M, Fresnoza S, Christova M. Decrease of motor cortex excitability following exposure to a 20 Hz magnetic field as generated by a rotating permanent magnet. Clin Neurophysiol. 129(7):1397-1402, 2018. (HU, AE, EE)

OBJECTIVES: Rotation of a static magnet over the motor cortex (MC) generates a transcranial alternating magnetic field (tAMF), and a linked alternating electrical field. The aim of this transcranial magnetic stimulation (TMS) study is to investigate whether such fields are able to influence MC excitability, and whether there are parallels to tACS induced effects. **METHODS:** Fourteen healthy volunteers received 20 Hz tAMF stimulation over the MC, over the vertex, and 20 Hz tACS over the MC, each with a duration of 15 min. TMS assessments were performed before and after the interventions. Changes in motor evoked potentials (MEP), short interval intra-cortical inhibition (SICI) and intra-cortical facilitation (ICF) were evaluated. **RESULTS:** The tACS and the tAMF stimulation over the MC affected cortical excitability in a different way. After tAMF stimulation MEP amplitudes and ICF decreased and the effect of SICI increased. After tACS MEP amplitudes increased and there were no effects on SICI and ICF. **CONCLUSIONS:** The recorded single and paired pulse MEPs indicate a general decrease of MC excitability following 15 min of tAMF stimulation. **SIGNIFICANCE:** The effects demonstrate that devices based on rotating magnets are potentially suited to become a novel brain stimulation tool in clinical neurophysiology.

(E) Gao X, Wang X, Chen F, Qi H, Wang X, Ming D, Zhou P. Research on brain induced effect by extremely low frequency pulsed magnetic stimulation. Conf Proc IEEE Eng Med Biol Soc. 2014:2613-2616, 2014. (HU, AE, EE)

In order to evaluate the influence of extremely low frequency pulsed magnetic fields (ELF PMF) on human brain, we conducted the magnetic stimulation experiments (1 Hz, 10 mT, 20 min), and analyzed the changes of spontaneous EEG activity from 10 subjects. Compared with sham exposure group, the EEG power of theta band (3.5-7.5 Hz) and lower-alpha band (7.5-10 Hz) from the stimulation group increased significantly after magnetic stimulation. By analyzing the latency period and amplitude of P300 in auditory oddball task, we found that the latency period extended and the amplitude decreased. We suggested that these results might be explained via event-related synchronization induced by magnetic stimulation.

(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.

(E) Gholami D, Riazi G, Fathi R, Sharafi M, Shahverdi A. Comparison of polymerization and structural behavior of microtubules in rat brain and sperm affected by the extremely low-frequency electromagnetic field. BMC Mol Cell Biol. 2019 Aug 29;20(1):41. doi: 10.1186/s12860-019-0224-1. (AS, CE, CC, MC)

BACKGROUND: Microtubule proteins are able to produce electromagnetic fields and have an important role in memory formation, and learning. Therefore, microtubules have the potential to be affected by exogenous electromagnetic fields. This study aimed to examine the comparison of microtubule polymerization and its structural behavior in brain and sperm affected by 50 Hz extremely low-frequency electromagnetic field (ELEF). **RESULTS:** Twenties adult male rats were randomly and equally divided into control and experimental groups, to evaluate the effect of 50 Hz ELEF on the sperm and brain functions. Plus-maze, serum testosterone and corticosterone, and sperm evaluation were performed. Next, the semen and brain samples were

obtained, and they were divided into four experimental groups for investigation of microtubule polymerization. There was no significant difference in testosterone and, corticosterone levels, anxiety behaviors, and sperm morphology between control and ELEF-exposure groups. The sperm viability, total and progressive motility were significantly higher in the ELEF-exposed group than that of the control group. The microtubule polymerization in sperm ELEF was significantly higher than in other groups. The secondary and tertiary structures of tubulins were significantly affected in the brain, and sperm ELEF groups. CONCLUSION: It seems that the polymerization of microtubules and conformational changes of tubulin dimers are improved by ELEF application

(E) Giorgetto C, Silva EC, Kitabatake TT, Bertolino G, de Araujo JE. Behavioural profile of Wistar rats with unilateral striatal lesion by quinolinic acid (animal model of Huntington disease) post-injection of apomorphine and exposure to static magnetic field. Exp Brain Res. 233(5):1455-1462, 2015. (AS, CE, BE, ND, MA)

We analysed the motor behaviour of Wistar rats after 7 days lesion in the left striatum, injected with apomorphine (APO) and stimulated by a continuous magnetic field of 3,200 Gauss. For the behaviour assessment, we utilised the activity cage test and the rotarod test. Sixty-eight male Wistar rats were divided into six groups: control, sham, sham magnetic, lesion, and stimulated South and North Poles. After the experiments, coronal sections of the striatum were taken and stained with Nissl for analysis of the lesion. In the activity cage test for distance ($F = 3.19$), time of activity ($F = 5.46$) and crossings ($F = 3.31$) in all groups, except for the North Pole-stimulated group, we observed a significant increase in these behaviours when compared to the control group. Considering the number of counterclockwise turns, we observed a significant increase in the lesion in the South and North Pole stimulation groups compared with the control group. Highlighting the minor number of counterclockwise turns observed in the North Pole-stimulated group in relation to the South Pole-stimulated and Lesion groups ($F = 16.01$). The rotarod test revealed a decrease in the time spent in this apparatus for the Lesion group when compared to all other groups ($F = 5.46$). The morphometric analysis showed a reduction in the number of neurons in the Lesion group in relation to all other groups ($F = 5.13$). Thus, the results suggest that the static magnetic field north and south promoted a distinct behavioural profile and morphological preservation after 7 days of lesion with quinolinic acid associated with APO.

(NE) Giorgi G, Lecciso M, Capri M, Lukas Yani S, Virelli A, Bersani F, Del Re B. An evaluation of genotoxicity in human neuronal-type cells subjected to oxidative stress under an extremely low frequency pulsed magnetic field. Mutat Res Genet Toxicol Environ Mutagen. 775-776:31-37, 2014.

The possible genotoxicity of extremely low frequency magnetic field (ELF-MF) exposure is still a controversial topic. The most of the reported data suggests that it alone does not affect DNA integrity, but several recent reports have suggested that sinusoidal ELF-MF may increase the effect of known genotoxic agents. Only a few studies deal with non sinusoidal ELF-MF, including pulsed magnetic field (PMF), which are produced by several devices. The aim of this study is to investigate whether PMF exposure can interfere with DNA damage and repair in the presence of a genotoxic oxidative agent in neuronal type cells. To this purpose gamma-H2AX

foci formation, which is a sensitive marker of DNA double strand breaks (DSB), was investigated at different points of time (1, 24, 48, 72h) after the H₂O₂ treatment (300μM for 1h) under PMF exposure (1mT, 50Hz) in human neuroblastoma BE(2)C cells. Moreover, cytotoxicity evaluation, by MTT assay and cell cycle analysis, was performed at various points of time after the treatment. Taken together, results suggest that PMF exposure does not interfere with genotoxicity and cytotoxicity induced by oxidative stress.

(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) Gok DK, Akpinar D, Hidisoglu E, Ozen S, Agar A, Yargicoglu P. The developmental effects of extremely low frequency electric fields on visual and somatosensory evoked potentials in adult rats. Electromagn Biol Med. 35(3):245-259, 2016. (AS, CE, EE, OX, EF)

The purpose of our study was to investigate the developmental effects of extremely low frequency electric fields (ELF-EFs) on visual evoked potentials (VEPs) and somatosensory-evoked potentials (SEPs) and to examine the relationship between lipid peroxidation and changes of these potentials. In this context, thiobarbituric acid reactive substances (TBARS) levels were determined as an indicator of lipid peroxidation. Wistar albino female rats were divided into four groups; Control (C), gestational (prenatal) exposure (Pr), gestational+ postnatal exposure (PP) and postnatal exposure (Po) groups. Pregnant rats of Pr and PP groups were exposed to 50 Hz electric field (EF) (12 kV/m; 1 h/day), while those of C and Po groups were placed in an inactive system during pregnancy. Following parturition, rats of PP and Po groups were exposed to ELF-EFs whereas rats of C and Pr groups were kept under the same experimental conditions without being exposed to any EF during 68 days. On postnatal day 90, rats were prepared for VEP and SEP recordings. The latencies of VEP components in all experimental groups were significantly prolonged versus C group. For SEPs, all components of PP group, P2, N2 components of Pr group and P1, P2, N2 components of Po group were delayed versus C group. As brain TBARS levels were significantly increased in Pr and Po groups, retina TBARS levels were significantly elevated in all experimental groups versus C group. In conclusion, alterations seen in evoked potentials, at least partly, could be explained by lipid peroxidation in the retina and brain.

Guerriero F, Ricevuti G. Extremely low frequency electromagnetic fields stimulation modulates autoimmunity and immune responses: a possible immuno-modulatory therapeutic effect in neurodegenerative diseases. Neural Regen Res. 11(12):1888-1895, 2016. (Review)

Increasing evidence shows that extremely low frequency electromagnetic fields (ELF-EMFs) stimulation is able to exert a certain action on autoimmunity and immune cells. In the past, the efficacy of pulsed ELF-EMFs in alleviating the symptoms and the progression of multiple sclerosis has been supported through their action on neurotransmission and on the autoimmune mechanisms responsible for demyelination. Regarding the immune system, ELF-EMF exposure contributes to a general activation of macrophages, resulting in changes of autoimmunity and several immunological reactions, such as increased reactive oxygen species-formation, enhanced phagocytic activity and increased production of chemokines. Transcranial electromagnetic brain stimulation is a non-invasive novel technique used recently to treat different neurodegenerative disorders, in particular Alzheimer's disease. Despite its proven value, the mechanisms through which EMF brain-stimulation exerts its beneficial action on neuronal function remains unclear. Recent studies have shown that its beneficial effects may be due to a neuroprotective effect on oxidative cell damage. On the basis of *in vitro* and clinical studies on brain activity, modulation by ELF-EMFs could possibly counteract the aberrant pro-inflammatory responses present in neurodegenerative disorders reducing their severity and their onset. The objective of this review is to provide a systematic overview of the published literature on EMFs and outline the most promising effects of ELF-EMFs in developing treatments of neurodegenerative disorders. In this regard, we review data supporting the role of ELF-EMF in generating immune-modulatory responses, neuromodulation, and potential neuroprotective benefits. Nonetheless, we reckon that the underlying mechanisms of interaction between EMF and the immune system are still to be completely understood and need further studies at a molecular level.

(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) Gupta SK, Mesharam MK, Krishnamurthy S. Electromagnetic radiation 2450 MHz exposure causes cognition deficit with mitochondrial dysfunction and activation of intrinsic pathway of apoptosis in rats. J Biosci. 43(2):263-276, 2018. (AS, CE, BE, MA, CC)

Electromagnetic radiation (EMR) can induce or modulate several neurobehavioral disorders. Duration and frequency of exposure of EMR is critical to develop cognitive disorders. Even though EMR-2450 is widely used, its effects on cognition in relation to mitochondrial function and apoptosis would provide better understanding of its pathophysiological effects. Therefore, a comparative study of different frequencies of EMR exposure would give valuable information on effects of discrete frequencies of EMR on cognition. Male rats were exposed to EMR (900, 1800 and 2450 MHz) every day for 1 h for 28 consecutive days. The cognitive behavior in terms of novel arm entries in Y-maze paradigm was evaluated every week after 1 h to last EMR exposure. Animals exposed to EMR-2450 MHz exhibited significant cognitive deficits. EMR-2450 MHz caused loss of mitochondrial function and integrity, an increase in amyloid beta expression. There was release of cytochrome-c and activation of apoptotic factors such as caspase-9 and -3 in the hippocampus. Further, there was decrease in levels of acetylcholine, and increase in activity of acetyl cholinesterase, indicating impairment of cholinergic system. Therefore, exposure of EMR-2450 in rats caused cognitive deficit with related pathophysiological changes in mitochondrial and cholinergic function, and amyloidogenesis.

(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) Haghighat N, Abdolmaleki P, Parnian J, Behmanesh M. The expression of pluripotency and neuronal differentiation markers under the influence of electromagnetic field and nitric oxide. Mol Cell Neurosci. 85:19-28, 2017. (CS, AE, MC)

Nitric oxide (NO) is a diatomic free radical compound that as a secondary messenger contributes to cell physiological functions and its variations influence proteins activity and triggering intracellular signaling cascades. Low frequency electromagnetic field (EMF) alters the cell biology such as cell differentiation by targeting the plasma membrane and entering force to the ions and small electrical ligands. The effect of these chemical (NO) and physical (EMF) factors on the expression of the stemness and neuronal differentiation markers in rat bone marrow mesenchymal stem cells (BMSC) was investigated. The cells were treated with low (50micromolar) and high (1mM) concentrations of Deta-NO as a NO donor molecule and 50Hz low frequency EMF. The expression of pluripotency and neuronal differentiation genes and proteins was investigated using real time qPCR and Immunocytochemistry techniques. The simultaneous treatment of EMF with NO (1mM) led to the down-regulation of stemness markers expression and up-regulation of neuronal differentiation markers expression. Cell proliferation decreased and cell morphology changed which caused the majority of cells obtains neuronal protein markers in their cytoplasm. The decrease in the expression of neuronal differentiation Nestin and DCX markers without any change in the expression of pluripotency Oct4 marker (treated with low concentration of NO) indicates protection of stemness state in these cells. Treatment with NO demonstrated a double behavior. NO low concentration helped the cells protect the stemness state but NO high concentration plus EMF pushed cells into differentiation pathway.

Hales CG. The origins of the brain's endogenous electromagnetic field and its relationship to provision of consciousness. J Integr Neurosci. 2014 Jun;13(2):313-61. doi: 10.1142/S0219635214400056. Epub 2014 Jun 24. (opinion)

As a potential source of consciousness, the brain's endogenous electromagnetic (EM) field has much to commend it. Difficulties connecting EM phenomena and consciousness have been exacerbated by the lack of a specific conclusive biophysically realistic mechanism originating the EM field, its form and dynamics. This work explores a potential mechanism: the spatial and temporal coherent action of transmembrane ion channel currents which simultaneously produce electric and magnetic fields that dominate all other field sources. Ion channels, as tiny current filaments, express, at a distance, the electric and magnetic fields akin to those of a short (transmembrane) copper wire. Following assembly of appropriate formalisms from EM field theory, the paper computationally explores the scalar electric potential produced by the current filaments responsible for an action potential (AP) in a realistic hippocampus CA1 pyramidal neuron. It reveals that AP signaling can impress a highly structured, focused and directed "sweeping-lighthouse beam" that "illuminates" neighbors at mm scales. Ion channel currents thereby provide a possible explanation for both EEG/MEG origins and recently confirmed functional EM coupling effects. Finally, a physically plausible EM field decomposition is

posited. It reveals objective and subjective perspectives intrinsic to the membrane-centric field dynamics. Perceptual "fields" can be seen to operate as the collective action of virtual EM-boson composites (called qualeons) visible only by "being" the fields, yet objectively appear as the familiar EM field activity. This explains the problematic evidence presentation and offers a physically plausible route to a solution to the "hard problem".

(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.

(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) Hernádi L, László JF. Pharmacological analysis of response latency in the hot plate test following whole-body static magnetic field-exposure in the snail *Helix pomatia*. Int J Radiat Biol. 90(7):547-553, 2014. (AS, AE, CE, BE)

Purpose: The effect of single, 30 min long, whole-body static magnetic field (SMF)-exposure on the response latency of the snail *Helix pomatia* has been the focus of this study. Materials and methods: The response was investigated using the hot plate test. Results: The effect caused by exposure to homogeneous SMF (147±3 mT) was compared to sham-exposure and resulted in significant differences (up to 47.1%, p<0.001). The response latency depended on the day-night cycle; response latency was higher by 51.2% (p<0.001) during the night. This trend also held for SMF-exposure (28.6%, p<0.001). Serotonin alone increased response latency (55.7%, p<0.001), whereas serotonin antagonist tryptamine decreased it (-97.8%, p<0.001). Using naloxone, response latency decreased (-52.5%, p<0.001); however both SMF-exposure and serotonin in combination with naloxone rose it back to above the control level (116. 9%, p<0.001 or 150.2%, p<0.001, respectively). Conclusions: This study provides evidence that SMF-exposure mediates peripheral thermal nociceptive threshold by affecting the serotonerg as well as the opioiderg system.

(E) Hong I, Garrett A, Maker G, Mullaney I, Rodger J, Etherington SJrkip. Repetitive low intensity magnetic field stimulation in a neuronal cell line: a metabolomics study. PeerJ. 2018 Mar 12;6:e4501. (CS, AE, CC)

Low intensity repetitive magnetic stimulation of neural tissue modulates neuronal excitability and has promising therapeutic potential in the treatment of neurological disorders. However, the underpinning cellular and biochemical mechanisms remain poorly understood. This study investigates the behavioural effects of low intensity repetitive magnetic stimulation (LI-rMS) at a cellular and biochemical level. We delivered LI-rMS (10 mT) at 1 Hz and 10 Hz to B50 rat neuroblastoma cells *in vitro* for 10 minutes and measured levels of selected metabolites immediately after stimulation. LI-rMS at both frequencies depleted selected tricarboxylic acid (TCA) cycle metabolites without affecting the main energy supplies. Furthermore, LI-rMS effects were frequency-specific with 1 Hz stimulation having stronger effects than 10 Hz. The observed depletion of metabolites suggested that higher spontaneous activity may have led to an increase in GABA release. Although the absence of organised neural circuits and other cellular contributors (e.g., excitatory neurons and glia) in the B50 cell line limits the degree to which our results can be extrapolated to the human brain, the changes we describe provide novel insights into how LI-rMS modulates neural tissue.

(E) Hu Y, Lai J, Wan B, Liu X, Zhang Y, Zhang J, Sun D, Ruan G, Liu E, Liu GP, Chen C, Wang DW. Long-term exposure to ELF-MF ameliorates cognitive deficits and attenuates tau hyperphosphorylation in 3xTg AD mice. Neurotoxicology. 53:290-300, 2016. (AS, CE, BE, CC, MA, ND)

Although numerous studies have reported the influence of extremely low frequency magnetic field (ELF-MF) exposure on human health, its effects on cognitive deficits in Alzheimer's disease (AD) have remained under debate. Moreover, the influence of ELF-MF on hyperphosphorylated tau, which is one of the most common pathological hallmarks of AD, has not been reported to date. Therefore, transgenic mice (3xTg) were used in the present study. 3xTg mice, which express an APP/PS1 mutation combined with a tau (P301L) mutation and that develop cognitive deficits at 6 months of age, were subjected to ELF-MF (50Hz, 500 μ T) exposure or sham exposure daily for 3 months. We discovered that ELF-MF exposure ameliorated cognitive deficits and increased synaptic proteins in 3xTg mice. The protective effects of ELF-MF exposure may have also been caused by the inhibition of apoptosis and/or decreased oxidative stress levels that were observed in the hippocampus tissues of treated mice. Furthermore, tau hyperphosphorylation was decreased *in vivo* because of ELF-MF exposure, and this decrease was induced by the inhibition of GSK3 β and CDK5 activities and activation of PP2Ac. We are the first to report that exposure to ELF-MF can attenuate tau phosphorylation. These findings suggest that ELF-MF exposure could act as a valid therapeutic strategy for ameliorating cognitive deficits and attenuating tau hyperphosphorylation in AD.

(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) Jakubowska M, Urban-Malinga B, Otremba Z, Andruliewicz E. Effect of low frequency electromagnetic field on the behavior and bioenergetics of the polychaete Hediste diversicolor. Marine Environ Res. 2019 Aug 3;150:104766. doi: 10.1016/j.marenvres.2019.104766. (AS, CE, FC, BE)

The aim of the present study was to determine the effect of an electromagnetic field (EMF) of value typically recorded in the vicinity of submarine cables (50 Hz, 1 mT) on the behavior and bioenergetics of the polychaete Hediste diversicolor. No avoidance or attraction behavior to EMF was shown, **but the burrowing activity of H. diversicolor was enhanced in EMF treatment, indicating a stimulating effect on bioturbation potential.** The polychaete maintained a positive energy balance and high amount (85% of assimilated energy) of energy available for individual production (scope for growth) after exposure to EMF. Food consumption and respiration rates were not affected but ammonia excretion rate was significantly reduced in EMF-exposed animals compared to the control conditions (geomagnetic field). The mechanisms behind this effect remain, however, unclear. This is the first study demonstrating the effects of environmentally realistic EMF value on the behavior and physiology of marine invertebrates, thus there is a need for more research.

(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 D(1) and D(2) 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 D(1) and D(2) 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) Jankowska M, Pawlowska-Mainville A, Stankiewicz M, Rogalska J, Wyszowska J. Exposure to 50 Hz electromagnetic field changes the efficiency of the scorpion alpha toxin. J Venom Anim Toxins Incl Trop Dis. 2015 Sep 30;21:38. doi: 10.1186/s40409-015-0040-9. eCollection 2015. (AS, CS, AE, FC)

BACKGROUND: Extremely low-frequency (50 Hz) electromagnetic field (ELF-EMF) is produced by electric power transmission lines and electronic devices of everyday use. Some phenomena are proposed as "first effects" of ELF-EMF: the discrete changes in the membrane potential and the increase of the calcium channel activity as well as the intracellular concentration of Ca^{2+} . Interaction of the scorpion alpha toxin with the sodium channel depends on the orientation of the charges and may be perturbed by changes in the membrane polarization. The toxin induces overexcitability in the nervous system and an increase in the neurotransmitters released with different consequences, mainly the paralysis of muscles. We assumed that the exposure to ELF-EMF 0.7 mT will change the effects of the insect selective scorpion alpha toxin (recombinant Lqh α IT from *Leiurus quinquestriatus hebraeus*) at the level of the cercal nerve function, the synaptic transmission and on the level of entire insect organism. Taking into account the compensatory mechanisms in organisms, we tested in addition ten times higher ELF-EMF on whole insects. **METHODS:** Experiments were performed in vivo on cockroaches (*Periplaneta americana*) and in vitro - on isolated cockroach abdominal nerve cord with cerci. In biotests, the effects of Lqh α IT (10^{-8} M) were estimated on the basis of the insect ability to turn back from dorsal to ventral side. Three groups were compared: the control one and the two exposed to ELF-EMF - 0.7 and 7 mT. Bioelectrical activity of the cercal nerve and of the connective nerve that leaves the terminal abdominal ganglion was recorded using extracellular electrodes. Lqh α IT (5×10^{-8} M) induced modifications of neuronal activity that were observed in the control cockroach preparations and in the ones exposed to ELF-EMF (0.7 mT). The exposure to ELF-EMF was carried out using coils with a size appropriate to the examined objects. **RESULTS:** The exposure to ELF-EMF (0.7 mT) modified the effects of Lqh α IT (5×10^{-8} M) on activity of the cercal nerve and of the connective nerve. We observed a decrease of the toxin effect on the cercal nerve activity, but the toxic effect of Lqh α IT on the

connective nerve was increased. Biotests showed that toxicity of Lqh α IT (10⁻⁸ M) on cockroaches was reduced by the exposure to ELF-EMF (0.7 and 7 mT). **CONCLUSIONS:** The exposure to 50 Hz ELF-EMF modified the mode of action of the anti-insect scorpion alpha toxin Lqh α IT at cellular level of the cockroach nervous system and in biotests. Toxin appeared as a usefull tool in distinguishing between the primary and the secondary effects of ELF-EMF.

(E) Kantar Gok D, Akpinar D, Yargicoglu P, Ozen S, Aslan M, Demir N, Derin N, Agar A. Effects of extremely low-frequency electric fields at different intensities and exposure durations on mismatch negativity. Neuroscience. 272C:154-166, 2014. (EE, AS, CE, EE, OX)

The effects of extremely low-frequency electric fields (ELF-EFs, 3-300Hz) on lipid peroxidation levels and antioxidant enzyme activities have been shown in many tissues and plasma after exposure to 50-Hz alternating current (AC) electric fields. However, similar studies investigating brain lipid peroxidation status are limited. Moreover and as far as we know, no study has been conducted to examine mismatch negativity (MMN) response in rats following exposure to a 50-Hz AC electric field. Therefore, the purpose of the study was to investigate different intensities and exposure durations of ELF-EFs on MMN component of event-related potentials (ERPs) as well as apoptosis and oxidative brain damage in rats. Ninety male rats, aged 3months were used in our study. A total of six groups, composed of 15 animals each, was formed as follows: sham-exposed rats for 2weeks (C2), sham-exposed rats for 4weeks (C4), rats exposed to 12-kV/m and 18-kV/m electric fields for 2weeks (E12-2 and E18-2), rats exposed to 12- and 18-kV/m electric fields for 4weeks (E12-4 and E18-4). At the end of the experimental period, MMN responses were recorded in urethane-anesthetized rats by electrodes positioned stereotaxically to the surface of the dura. After MMN recordings, animals were killed by exsanguination and their brain tissues were removed for 4-hydroxy-2-nonenal (4-HNE), protein carbonyl and TUNEL analysis. In the current study, different change patterns in ERP parameters were observed dependent on the intensity and exposure duration of ELF-EFs. There were differences in the amplitudes of ERP between the responses to the standard and the deviant tones in all groups. When peak-to-peak amplitude of the difference curves was evaluated, MMN amplitude was significantly decreased in the E18-4 group compared with the C4 group. Additionally, the amount of 4-HNE was increased in all experimental groups compared with the control group. Consequently, it could be concluded that electric field decreased MMN amplitudes possibly induced by lipid peroxidation.

(E) Kapri-Pardes E, Hanoch T, Maik-Rachline G, Murbach M, Bounds PL, Kuster N, Seger R. Activation of Signaling Cascades by Weak Extremely Low Frequency Electromagnetic Fields. Cell Physiol Biochem. 2017 Oct 16;43(4):1. doi: 10.1159/000481977. [Epub ahead of print] (CS, AE, CC, OX)

Results from recent studies suggest that extremely low frequency magnetic fields (ELF-MF) interfere with intracellular signaling pathways related to proliferative control. The mitogen-activated protein kinases (MAPKs), central signaling components that regulate essentially all stimulated cellular processes, include the extracellular signal-regulated kinases 1/2 (ERK1/2) that are extremely sensitive to extracellular cues. Anti-phospho-ERK antibodies serve as a

readout for ERK1/2 activation and are able to detect minute changes in ERK stimulation. The objective of this study was to explore whether activation of ERK1/2 and other signaling cascades can be used as a readout for responses of a variety of cell types, both transformed and non-transformed, to ELF-MF. METHODS: We applied ELF-MF at various field strengths and time periods to eight different cell types with an exposure system housed in a tissue culture incubator and followed the phosphorylation of MAPKs and Akt by western blotting. RESULTS: We found that the phosphorylation of ERK1/2 is increased in response to ELF-MF. However, the phosphorylation of ERK1/2 is likely too low to induce ELF-MF-dependent proliferation or oncogenic transformation. The p38 MAPK was very slightly phosphorylated, but JNK or Akt were not. The effect on ERK1/2 was detected for exposures to ELF-MF strengths as low as 0.15 μ T and was maximal at \sim 10 μ T. We also show that ERK1/2 phosphorylation is blocked by the flavoprotein inhibitor diphenyleneiodonium, indicating that the response to ELF-MF may be exerted via NADP oxidase similar to the phosphorylation of ERK1/2 in response to microwave radiation. CONCLUSIONS: Our results further indicate that cells are responsive to ELF-MF at field strengths much lower than previously suspected and that the effect may be mediated by NADP oxidase. However, the small increase in ERK1/2 phosphorylation is probably insufficient to affect proliferation and oncogenic transformation. Therefore, the results cannot be regarded as proof of the involvement of ELF-MF in cancer in general or childhood leukemia in particular.

(E) Kesari KK, Juutilainen J, Luukkonen J, Naarala J. Induction of micronuclei and superoxide production in neuroblastoma and glioma cell lines exposed to weak 50 Hz magnetic fields. J R Soc Interface. 2016 Jan;13(114). pii: 20150995. doi: 10.1098/rsif.2015.0995. (CS, AE, MC, OX)

Extremely low-frequency (ELF) magnetic fields (MF) have been associated with adverse health effects in epidemiological studies. However, there is no known mechanism for biological effects of weak environmental MFs. Previous studies indicate MF effects on DNA integrity and reactive oxygen species, but such evidence is limited to MFs higher (greater than or equal to 100 μ T) than those generally found in the environment. Effects of 10 and 30 μ T fields were studied in SH-SY5Y and C6 cells exposed to 50-Hz MFs for 24 h. Based on earlier findings, menadione (MQ) was used as a cofactor. Responses to MF were observed in both cell lines, but the effects differed between the cell lines. Micronuclei were significantly increased in SH-SY5Y cells at 30 μ T. This effect was largest at the highest MQ dose used. Increased cytosolic and mitochondrial superoxide levels were observed in C6 cells. The effects on superoxide levels were independent of MQ, enabling further mechanistic studies without co-exposure to MQ. The micronucleus and mitochondrial superoxide data were consistent with a conventional rising exposure-response relationship. For cytosolic superoxide, the effect size was unexpectedly large at 10 μ T. The results indicate that the threshold for biological effects of ELF MFs is 10 μ T or less.

(E) Kazemi M, Sahraei H, Aliyari H, Tekieh E, Saberi M, Tavacoli H, Meftahi GH, Ghanaati H, Salehi M, Hajnasrollah M. Effects of the Extremely Low Frequency Electromagnetic Fields on NMDA-Receptor Gene Expression and Visual Working

Memory in Male Rhesus Macaques. Basic Clin Neurosci. 9(3):167-176, 2018. (AS, CE, BE, CC, MA)

INTRODUCTION: The present research aimed to examine Visual Working Memory (VWM) test scores, as well as hormonal, genomic, and brain anatomic changes in the male rhesus macaques exposed to Extremely Low Frequency Magnetic Field (ELF-MF). **METHODS:** Four monkeys were exposed to two different ELF-MF frequencies: 1 Hz (control) and 12 Hz (experiment) with 0.7 μ T (magnitude) 4 h/d for 30 consecutive days. Before and after the exposure, VWM test was conducted using a coated device on a movable stand. About 10 mL of the animals' blood was obtained from their femoral vein and used to evaluate their melatonin concentration. Blood lymphocytes were used for assaying the expressions of N-Methyl-D-aspartate NMDA-receptor genes expression before and after ELF exposure. Anatomical changes of hippocampus size were also assessed using MRI images. **RESULTS:** Results indicated that VWM scores in primates exposed to 12 Hz frequency ELF increased significantly. Plasma melatonin level was also increased in these animals. However, these variables did not change in the animals exposed to 1 Hz ELF. At last, expression of the NMDA receptors increased at exposure to 12 Hz frequency. However, hippocampal volume did not increase significantly in the animals exposed to both frequencies. **CONCLUSION:** In short, these results indicate that ELF (12 Hz) may have a beneficial value for memory enhancement (indicated by the increase in VWM scores). This may be due to an increase in plasma melatonin and or expression of NMDA glutamate receptors. However, direct involvement of the hippocampus in this process needs more research.

(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 $\text{Ca}(2+)$ regulation, which is a critical component of neurodegeneration. We also observed that the intracellular $\text{Ca}(2+)$ 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) Kirimoto H, Tamaki H, Otsuru N, Yamashiro K, Onishi H, Nojima I, Oliviero A. Transcranial Static Magnetic Field Stimulation over the Primary Motor Cortex Induces Plastic Changes in Cortical Nociceptive Processing. Front Hum Neurosci. 12:63, 2018. (HU, AE, EE, MA)

Transcranial static magnetic field stimulation (tSMS) is a novel and inexpensive, non-invasive brain stimulation (NIBS) technique. Here, we performed non-invasive modulation of intra-epidermal electrical stimulation-evoked potentials (IES-EPs) by applying tSMS or sham stimulation over the primary motor (M1) and somatosensory (S1) cortices in 18 healthy volunteers for 15 min. We recorded EPs after IES before, right after, and 10 min after tSMS. The IES-EP amplitude was significantly reduced immediately after tSMS over M1, whereas tSMS over S1 and sham stimulation did not affect the IES-EP amplitude. Thus, tSMS may affect cortical nociceptive processing. Although the results of intervention for experimental acute pain in healthy subjects cannot be directly translated into the clinical situation, tSMS may be a potentially useful NIBS method for managing chronic pain, in addition to standard of care treatments.

(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) Koeman T, Schouten LJ, van den Brandt PA, Slottje P, Huss A, Peters S, Kromhout H, Vermeulen R. Occupational exposures and risk of dementia-related mortality in the prospective Netherlands Cohort Study. Am J Ind Med. 58(6):625-635, 2015. (HU, CE, ND)

BACKGROUND: Occupational exposures may be associated with non-vascular dementia.

METHODS: We analyzed the effects of occupational exposures to solvents, pesticides, metals, extremely low frequency magnetic fields (ELF-MF), electrical shocks, and diesel motor exhaust on non-vascular dementia related mortality in the Netherlands Cohort Study (NLCS). Exposures were assigned using job-exposure matrices. After 17.3 years of follow-up, 682 male and 870 female cases were available. Analyses were performed using Cox regression. RESULTS:

Occupational exposure to metals, chlorinated solvents and ELF-MF showed positive associations with non-vascular dementia among men, which seemed driven by metals (hazard ratio ever high vs. background exposure: 1.35 [0.98-1.86]). Pesticide exposure showed statistically significant, inverse associations with non-vascular dementia among men. We found no associations for shocks, aromatic solvents, and diesel motor exhaust. CONCLUSIONS: Consistent positive associations were found between occupational exposure to metals and non-vascular dementia. The finding on pesticides is not supported in the overall literature.

(E) Koeman T, Slottje P, Schouten LJ, Peters S, Huss A, Veldink JH, Kromhout H, van den Brandt PA, Vermeulen R. Occupational exposure and amyotrophic lateral sclerosis in a prospective cohort. Occup Environ Med. 2017 Mar 29. pii: oemed-2016-103780. doi: 10.1136/oemed-2016-103780. [Epub ahead of print] (HU, ND)

OBJECTIVE: To prospectively study suspected occupational risk factors for amyotrophic lateral sclerosis (ALS). METHODS: For this case-cohort analysis within the prospective Netherlands Cohort Study, 58 279 men and 62 573 women aged 55-69 years at enrolment in 1986 were followed up for 17.3 years on ALS mortality. Information on occupational history and potential confounders were collected at baseline through a self-administered questionnaire and entered for a random subcohort (2092 men and 2074 women) and ALS deaths (76 men and 60 women). Occupational exposure to solvents, pesticides, metals, extremely low frequency magnetic fields (ELF-MFs) and electrical shocks was estimated by means of job exposure matrices (JEMs). Associations between ever/never occupationally exposed and cumulative exposure and ALS mortality were analysed by gender using Cox regression. RESULTS: Occupational exposure to ELF-MF showed a possible association with ALS mortality among men: HR for ever holding a job with high exposure versus background 2.19 (95% (CI): 1.02 to 4.73) and HR for the highest tertile of cumulative exposure versus background 1.93 (95% CI 1.05 to 3.55). INTERPRETATION: These results strengthen the evidence suggesting a positive association between ELF-MF exposure and ALS. We did not replicate earlier positive findings for other occupational exposures.

(E) Komaki A, Khalili A, Salehi I, Shahidi S, Sarihi A. Effects of exposure to an extremely low frequency electromagnetic field on hippocampal long-term potentiation in rat. Brain Res. 2014 Apr 10. pii: S0006-8993(14)00419-3. doi: 10.1016/j.brainres.2014.03.041. [Epub ahead of print] (AS, CE, EE)

Modern lifestyle exposes nearly all humans to electromagnetic fields, particularly extremely low frequency electromagnetic field (ELF-EMF). Prolonged exposure to ELF-EMF induces persistent changes in neuronal activity. However, the modulation of synaptic efficiency by ELF-EMF in vivo is still unclear. In the present study, we investigated whether ELF-EMF can change induction of long-term potentiation (LTP) and paired-pulse ratio (PPR) in rat hippocampal area. Twenty-nine adult male Wistar rats were divided into 3 groups (ELF-EMF exposed, sham and control groups). ELF-EMF group was exposed to the magnetic field for 90 consecutive days (2 hours/day). ELF-EMF was produced by a circular coil (50Hz, 100 micro Tesla). The sham-exposed controls were placed in an identical chamber with no electromagnetic field. After this period, rats were deeply anesthetized with urethane (2.0mg/kg) and then a bipolar stimulating and recording electrode was implanted into the perforant pathway (PP) and dentate gyrus (DG), respectively. LTP in hippocampal area was induced by high-frequency stimulation (HFS). Prolonged exposure to ELF-EMF increased LTP induction. There was a significant difference in the slope of EPSP and amplitude of PS between the ELF-EMF and other groups. In conclusion, our data suggest that exposure to ELF-EMF produces a marked change in the synaptic plasticity generated in synapses of the PP-DG. No significant difference in PPR of ELF-EMF group before and after HFS suggests a postsynaptic expression site of LTP.

(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).

Kumar S, Dey S, Jain S. Extremely low-frequency electromagnetic fields: A possible non-invasive therapeutic tool for spinal cord injury rehabilitation. Electromagn Biol Med. 36(1):88-101, 2017. (Review)

Traumatic insults to the spinal cord induce both immediate mechanical damage and subsequent tissue degeneration. The latter involves a range of events namely cellular disturbance, homeostatic imbalance, ionic and neurotransmitters derangement that ultimately result in loss of sensorimotor functions. The targets for improving function after spinal cord injury (SCI) are mainly directed toward limiting these secondary injury events. Extremely low-frequency electromagnetic field (ELF-EMF) is a possible non-invasive therapeutic intervention for SCI rehabilitation which has the potential to constrain the secondary injury-induced events. In the present review, we discuss the effects of ELF-EMF on experimental and clinical SCI as well as on biological system.

(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 ($38 \pm 0.5(^{\circ})\text{C}$, 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 histopathology and process of apoptosis. The brains of other embryos were prepared for Scanning Electron 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.

(NE) Lai J, Zhang Y, Liu X, Zhang J, Ruan G, Chaugai S, Chen C, Wang DW. Effects of extremely low frequency electromagnetic fields (100 μT) on behaviors in rats. Neurotoxicology. 2015 Nov 21. pii: S0161-813X(15)30026-7. doi: 10.1016/j.neuro.2015.11.010. [Epub ahead of print] (AS, CE, BE, MC)

Recently, extremely low frequency electromagnetic fields (ELF-EMF) have received considerable attentions for their potential pathogenicity. In the present study, we explored the effects of ELF-EMF on behaviors of adult male rats. Sixty adult male rats were randomly divided into two groups, the sham exposure group and the 50Hz/100 μT ELF-EMF exposure group. During the 24 weeks exposure, body weights, as well as food and water intake were recorded. Results showed that food and water intake and the body weight of the rats were not affected by the exposure. After 24 weeks exposure, open field test and elevated plus maze were conducted to evaluate the anxiety-like behavior, the tail suspension test and forced swim test were conducted to evaluate depression-like behavior and Morris water maze and fear conditioning tests were used to evaluate the cognitive and memory ability. Exposure to ELF-EMF did not induce any anxiety-like or depression-like behaviors compared with the sham exposure. Moreover, the cognitive and memory ability was not impaired by the ELF-EMF exposure. Furthermore, ELF-EMF exposure did not affect the morphology and histology of the brain. In conclusion, 24 weeks exposure to 50Hz/100 μT ELF-EMF had no effect on the behaviors of the adult male rats.

(E) Laszlo AM, Ladanyi M, Boda K, Csicsman J, Bari F, Serester A, Molnar Z, Sepp K, Galfi M, Radacs M. Effects of extremely low frequency electromagnetic fields on turkeys. Poult Sci. 97(2):634-642, 2018. (AS, CE, FC, CC)

Several studies have examined the potential biological effects of electromagnetic fields (EMF) on birds; however, little attention has been paid to the extremely low frequency (ELF; 0-300 Hz; 0-50 μ T) radiation found in an urbanized environment. For monitoring the effects of ELF EMF, we used a turkey (*Meleagris gallopavo*) model, because the nucleated erythrocytes of turkeys contain β -adrenoceptors, and norepinephrine- (NE-) activated β -adrenoceptors have an important role in physiological and behavioral processes. Our aims were the following: 1) to investigate the intracellular mechanisms; 2) to compare the intracellular mechanisms in the treated and control groups over time, considering inter-individual differences and intra-subject correlations; 3) and to study the reversible nature of the response. The turkeys in the treatment group were treated in vivo with ELF EMF (50 Hz; 10 μ T) for 3 wk after a 1-wk-long adaptation period. The animals were not exposed to ELF EMF during the regeneration period (5 wk following the exposure). The NE-activated β -adrenoceptor function was detected by measuring the amount of 3'5'-cyclic-adenosine-monophosphate (cAMP), and the biochemical enzyme parameters were defined. Repeated measurements of cAMP levels were analyzed using marginal models and a piecewise linear mixed model to compare treatment and control groups over time. According to our results, NE-activated β -adrenoceptor function was decreased in the treated birds in a time-dependent manner, while there were no differences between toxicological parameters in the serum, compared to the normal ranges. The decreased NE-dependent β -adrenoceptor function could be compensated by the homeostatic complex during the 5-wk regeneration period. Extended experimental periods and more sophisticated analysis methods may help prevent harmful environmental effects on birds; furthermore, these findings could affect public health and the economy.

(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) Legros A, Modolo J, Brown S, Roberston J, Thomas AW. Effects of a 60 Hz Magnetic Field Exposure Up to 3000 μ T on Human Brain Activation as Measured by Functional Magnetic Resonance Imaging. PLoS One. 2015 Jul 27;10(7):e0132024. (HU, AE, BE, FC)

Several aspects of the human nervous system and associated motor and cognitive processes have been reported to be modulated by extremely low-frequency (ELF, < 300 Hz) time-varying Magnetic Fields (MF). Due to their worldwide prevalence; power-line frequencies (60 Hz in North America) are of particular interest. Despite intense research efforts over the last few decades, the potential effects of 60 Hz MF still need to be elucidated, and the underlying mechanisms to be understood. In this study, we have used functional Magnetic Resonance Imaging (fMRI) to characterize potential changes in functional brain activation following human exposure to a 60 Hz MF through motor and cognitive tasks. First, pilot results acquired in a first set of subjects (N=9) were used to demonstrate the technical feasibility of using fMRI to detect subtle changes in functional brain activation with 60 Hz MF exposure at 1800 μ T. Second, a full study involving a larger cohort of subjects tested brain activation during 1) a finger tapping task (N=20), and 2) a mental rotation task (N=21); before and after a one-hour, 60 Hz, 3000 μ T MF exposure. The results indicate significant changes in task-induced functional brain activation as a consequence of MF exposure. However, no impact on task performance was found. These results illustrate the potential of using fMRI to identify MF-induced changes in functional brain activation, suggesting that a one-hour 60 Hz, 3000 μ T MF exposure can modulate activity in specific brain regions after the end of the exposure period (i.e., residual effects). We discuss the possibility that MF exposure at 60 Hz, 3000 μ T may be capable of modulating cortical excitability via a modulation of synaptic plasticity processes.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbatì 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.

(E) Li C, Xie M, Luo F, He C, Wang J, Tan G, Hu Z. The extremely low-frequency magnetic field exposure differently affects the AMPAR and NMDAR subunit expressions in the hippocampus, entorhinal cortex and prefrontal cortex without effects on the rat spatial learning and memory. Environ Res. 2014 Jul 18;134C:74-80. doi: 10.1016/j.envres.2014.06.025. [Epub ahead of print] (AS; CE; CC)

In the present study, we investigated the effects of chronic exposure (14 and 28 days) to a 50Hz, 0.5mT extremely low-frequency magnetic field (ELF-MF) on the NMDAR and AMPAR subunit expressions and rat spatial learning and memory. Using the Western blotting method, we found ELF-MF exposure specifically decreased the expressions of GluA2 in the EC post 28 day exposure and GluA3 of AMPAR subunits in the PFC after 14 day exposure, while it did not affect the AMPAR subunit expression in the hippocampus at both time points. As for NMDAR subunits, 14 day ELF-MF exposure significantly increased the levels of GluN2A and GluN2B in the hippocampus. Moreover, the levels of GluN1 and GluN2A were enhanced in the EC and PFC after two weeks of ELF-MF exposure. Interestingly, 28 day ELF-MF exposure induced a different expression pattern for NMDAR subunits. The increased GluN2A expression observed at 14 day post ELF-MF exposure was recovered after prolonged exposure in the hippocampus and PFC. In the EC, the increased expression of GluN1 achieved to control level and, specifically, a decrease in GluN2A level was observed. Surprisingly, neither 14 nor 28 day ELF-MF did affect the rat spatial reference memory as assessed by water maze. These results indicate that the dynamic and brain-region specific changes in ionotropic glutamate receptor expression induced by ELF-MF are insufficient to influence the rat spatial learning ability.

(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.

(E) Li Y, Yan X, Liu J, Li L, Hu X, Sun H, Tian J. Pulsed electromagnetic field enhances brain-derived neurotrophic factor expression through L-type voltage-gated calcium channel- and Erk-dependent signaling pathways in neonatal rat dorsal root ganglion neurons. *Neurochem Int.* 75:96-104, 2014. (CS, AE, CC)

Although pulsed electromagnetic field (PEMF) exposure has been reported to promote neuronal differentiation, the mechanism is still unclear. Here, we aimed to examine the effects of PEMF exposure on brain-derived neurotrophic factor (Bdnf) mRNA expression and the correlation between the intracellular free calcium concentration ($[Ca^{2+}]_i$) and Bdnf mRNA expression in cultured dorsal root ganglion neurons (DRGNs). Exposure to 50Hz and 1mT PEMF for 2h increased the level of $[Ca^{2+}]_i$ and Bdnf mRNA expression, which was found to be mediated by increased $[Ca^{2+}]_i$ from Ca^{2+} influx through L-type voltage-gated calcium channels (VGCCs). However, calcium mobilization was not involved in the increased $[Ca^{2+}]_i$ and BDNF expression, indicating that calcium influx was one of the key factors responding to PEMF exposure. Moreover, PD098059, an extracellular signal-regulated kinase (Erk) inhibitor, strongly inhibited PEMF-dependant Erk1/2 activation and BDNF expression, indicating that Erk activation is required for PEMF-induced upregulation of BDNF expression. These findings indicated that PEMF exposure increased BDNF expression in DRGNs by activating Ca^{2+} - and Erk-dependent signaling pathways.

(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) Li Y, Zhang Y, Wang W, Zhang Y, Yu Y, Cheing GL, Pan W. Effects of pulsed electromagnetic fields on learning and memory abilities of STZ-induced dementia rats. Electromagn Biol Med. 2019 Mar 17:1-8. doi: 10.1080/15368378.2019.1591437. [Epub ahead of print] (AS, CE, BE, ND, MA)

INTRODUCTION: Recent studies have shown that pulsed electromagnetic field (EMF) has therapeutic potential for dementia, but the associated neurobiological effects are unclear. This study aimed to determine the effects of pulsed EMF on Streptozotocin (STZ)-induced dementia rats. **METHODS:** Forty Sprague-Dawley rats were randomly allocated to one of the four groups: (i) control, (ii) normal saline injection (sham group), (iii) STZ injection (STZ group) and (iv) STZ injection with pulsed EMF exposure (PEMF, 10 mT at 20 Hz) (STZ + MF group). Morris water maze was used to assess the learning and memory abilities. Insulin growth factors 1 and 2 (IGF-1 and IGF-2) gene expression were determined by quantitative PCR. **RESULTS:** The results showed that the mean escape latency in STZ-induced dementia rats was reduced by 66% under the exposure of pulsed EMF. Compared with the STZ group, the swimming distance and the time for first crossing the platform decreased by 55 and 41.6% in STZ + MF group, respectively. Furthermore, the IGF-2 gene expression significantly increased compared to that of the STZ group. **CONCLUSIONS:** Our findings indicate that the pulsed EMF exposure can improve the ability of learning and memory in STZ-induced dementia rats and this effect may be related to the process of IGF signal transduction, suggesting a potential role for the pulsed EMF for the amelioration of cognition impairment.

Liboff AR. The electromagnetic basis of social interactions. Electromagn Biol Med. 2017;36(2):177-181. (opinion)

It has been established that living things are sensitive to extremely low-frequency magnetic fields at vanishingly small intensities, on the order of tens of nT. We hypothesize, as a consequence of this sensitivity, that some fraction of an individual's central nervous system activity can be magnetically detected by nearby individuals. Even if we restrict the information content of such processes to merely simple magnetic cues that are unconsciously received by individuals undergoing close-knit continuing exposure to these cues, it is likely that they will tend to associate these cues with the transmitting individual, no less than would occur if such signals were visual or auditory. Furthermore, following what happens when one experiences prolonged exposure to visual and like sensory inputs, it can be anticipated that such association occurring magnetically will eventually also enable the receiving individual to bond to the transmitting individual. One can readily extrapolate from single individuals to groups, finding

reasonable explanations for group behavior in a number of social situations, including those occurring in families, animal packs, gatherings as found in concerts, movie theaters and sports arenas, riots and selected predatory/prey situations. The argument developed here not only is consistent with the notion of a magnetic sense in humans, but also provides a new approach to electromagnetic hypersensitivity, suggesting that it may simply result from sensory overload.

Liboff AR. Magnetic correlates in electromagnetic consciousness. Electromagn Biol Med. 2016;35(3):228-236. (opinion)

We examine the hypothesis that consciousness is a manifestation of the electromagnetic field, finding supportive factors not previously considered. It is not likely that traditional electrophysiological signaling modes can be readily transmitted throughout the brain to properly enable this field because of electric field screening arising from the ubiquitous distribution of high dielectric lipid membranes, a problem that vanishes for low-frequency magnetic fields. Many reports over the last few decades have provided evidence that living tissue is robustly sensitive to ultraslow (1-100 nT) ELF magnetic fields overlapping the γ -frequency range often associated with awareness. An example taken from animal behavior (coherent bird flocking) lends support to the possibility of a disembodied electromagnetic consciousness. In contrast to quantum consciousness hypotheses, the present approach is open to experimental trial.

Liboff AR. Is the geomagnetic map imprinted in pre-emergent egg? Electromagn Biol Med. 2016;35(2):167-169. (opinion)

Although it is well-accepted that the geomagnetic field (GMF) plays an important role in animal navigation and migration, key problems remain unanswered. To explain the puzzling ability of hatchlings to embark on unexplored migrational journeys we hypothesize that mothers who have previously navigated the trip enable their offspring by direct transfer of route information to their eggs prior to hatching. The freshly hatched animal registers the local GMF as a reference point before embarking on the journey the mother has prepared for it. This process represents a novel type of biological cycle that finesses the need to treat questions such as natal homing and route parameters separately.

(E) Liu DD, Ren Z, Yang G, Zhao QR, Mei YA. Melatonin protects rat cerebellar granule cells against electromagnetic field-induced increases in Na⁺ currents through intracellular Ca²⁺ release. J Cell Mol Med. 2014 Feb 18. doi: 10.1111/jcmm.12250. [Epub ahead of print] (CS, AE, CC, OX)

Although melatonin (MT) has been reported to protect cells against oxidative damage induced by electromagnetic radiation, few reports have addressed whether there are other protective mechanisms. Here, we investigated the effects of MT on extremely low-frequency electromagnetic field (ELF-EMF)-induced Na_v activity in rat cerebellar granule cells (GCs).

Exposing cerebellar GCs to ELF-EMF for 60 min. significantly increased the I_{Na_v} current (I_{Na}) densities by 62.5%. MT (5 μ M) inhibited the ELF-EMF-induced I_{Na} increase. This inhibitory effect of MT is mimicked by an MT_2 receptor agonist and was eliminated by an MT_2 receptor antagonist. The Na_v channel steady-state activation curve was significantly shifted towards hyperpolarization by ELF-EMF stimulation but remained unchanged by MT in cerebellar GC that were either exposed or not exposed to ELF-EMF. ELF-EMF exposure significantly increased the intracellular levels of phosphorylated PKA in cerebellar GCs, and both MT and IIK-7 did not reduce the ELF-EMF-induced increase in phosphorylated PKA. The inhibitory effects of MT on ELF-EMF-induced Na_v activity was greatly reduced by the calmodulin inhibitor KN93. Calcium imaging showed that MT did not increase the basal intracellular Ca^{2+} level, but it significantly elevated the intracellular Ca^{2+} level evoked by the high K^+ stimulation in cerebellar GC that were either exposed or not exposed to ELF-EMF. In the presence of ruthenium red, a ryanodine-sensitive receptor blocker, the MT-induced increase in intracellular calcium levels was reduced. Our data show for the first time that MT protects against neuronal I_{Na} that result from ELF-EMF exposure through Ca^{2+} influx-induced Ca^{2+} release.

(E) Liu H, Chen G, Pan Y, Chen Z, Jin W, Sun C, Chen C, Dong X, Chen K, Xu Z, Zhang S, Yu Y. (2014) Occupational Electromagnetic Field Exposures Associated with Sleep Quality: A Cross-Sectional Study. PLoS ONE 9(10): e110825. doi:10.1371/journal.pone.0110825. (HU, CE, BE)

BACKGROUND: Exposure to electromagnetic field (EMF) emitted by mobile phone and other machineries concerns half the world's population and raises the problem of their impact on human health. The present study aims to explore the effects of electromagnetic field exposures on sleep quality and sleep duration among workers from electric power plant. METHODS: A cross-sectional study was conducted in an electric power plant of Zhejiang Province, China. A total of 854 participants were included in the final analysis. The detailed information of participants was obtained by trained investigators using a structured questionnaire, which including socio-demographic characteristics, lifestyle variables, sleep variables and electromagnetic exposures. Physical examination and venous blood collection were also carried out for every study subject. RESULTS: After grouping daily occupational electromagnetic exposure into three categories, subjects with long daily exposure time had a significantly higher risk of poor sleep quality in comparison to those with short daily exposure time. The adjusted odds ratios were 1.68 (95%CI: 1.18, 2.39) and 1.57 (95%CI: 1.10, 2.24) across tertiles. Additionally, among the subjects with long-term occupational exposure, the longer daily occupational time apparently increased the risk of poor sleep quality (OR (95%CI): 2.12 (1.23~3.66) in the second tertile; 1.83 (1.07~3.15) in the third tertile). There was no significant association of long-term occupational exposure duration, monthly electric fee or years of mobile-phone use with sleep quality or sleep duration. CONCLUSIONS: The findings showed that daily occupational EMF exposure was positively associated with poor sleep quality. It implies EMF exposure may damage human sleep quality rather than sleep duration.

(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) Liu X, Zuo H, Wang D, Peng R, Song T, Wang S, Xu X, Gao Y, Li Y, Wang S, Wang L, Zhao L. Improvement of spatial memory disorder and hippocampal damage by exposure to electromagnetic fields in an Alzheimer's disease rat model. PLoS One. 10(5):e0126963, 2015. (AS, CE, BE, FC, OX, MA)

Although some epidemiological investigations showed a potential association between long-term exposure of extremely low frequency electromagnetic fields (ELF-EMF) and Alzheimer's disease (AD), no reasonable mechanism can explain this association, and the related animal experiments are rare. In this study, ELF-EMF exposure (50 Hz 400 μ T 60 d) combined with D-galactose intraperitoneal (50 mg/kg, q.d., 42 d) and A β 25-35 hippocampal (5 μ l/unilateral, bilateral, single-dose) injection was implemented to establish a complex rat model. Then the effects of ELF-EMF exposure on AD development was studied by using the Morris water maze, pathological analysis, and comparative proteomics. The results showed that ELF-EMF exposure delayed the weight gain of rats, and partially improved cognitive and clinicopathologic symptoms of AD rats. The differential proteomic analysis results suggest that synaptic transmission, oxidative stress, protein degradation, energy metabolism, Tau aggregation, and

inflammation involved in the effects mentioned above. Therefore, our findings indicate that certain conditions of ELF-EMF exposure could delay the development of AD in rats.

(E) Lozano-Soto E, Soto-León V, Sabbarese S, Ruiz-Alvarez L, Sanchez-Del-Rio M, Aguilar J, Strange BA, Foffani G, Oliviero A. Transcranial static magnetic field stimulation (tSMS) of the visual cortex decreases experimental photophobia. Cephalalgia. 38(8):1493-1497, 2018. (HU, AE, BE, MA)

Background Transcranial static magnetic field stimulation (tSMS) reduces cortical excitability in humans. Methods The objective of this study was to determine whether tSMS over the occipital cortex is effective in reducing experimental photophobia. In a sham-controlled double-blind crossover study, tSMS (or sham) was applied for 10 minutes with a cylindrical magnet on the occiput of 20 healthy subjects. We assessed subjective discomfort induced by low-intensity and high-intensity visual stimuli presented in a dark room before, during and after tSMS (or sham). Results Compared to sham, tSMS significantly reduced the discomfort induced by high-intensity light stimuli. Conclusions The visual cortex may contribute to visual discomfort in experimental photophobia, providing a rationale for investigating tSMS as a possible treatment for photophobia in migraine.

(E) Lundberg L, Sienkiewicz Z, Anthony DC, Broom KA. Effects of 50 Hz magnetic fields on circadian rhythm control in mice. Bioelectromagnetics. 40(4):250-259, 2019. (AS, AE, BE)

Artificial light and power frequency magnetic fields are ubiquitous in the built environment. Light is a potent zeitgeber but it is unclear whether power frequency magnetic fields can influence circadian rhythm control. To study this possibility, 8-12-week-old male C57BL/6J mice were exposed for 30 min starting at zeitgeber time 14 (ZT14, 2 h into the dark period of the day) to 50 Hz magnetic fields at 580 μ T using a pair of Helmholtz coils and/or a blue LED light at 700 lux or neither. Our experiments revealed an acute adrenal response to blue light, in terms of increased adrenal *per1* gene expression, increased serum corticosterone levels, increased time spent sleeping, and decreased locomotor activity (in all cases, $P < 0.0001$) compared to an unexposed control group. There appeared to be no modulating effect of the magnetic fields on the response to light, and there was also no effect of the magnetic fields alone (in both cases, $P > 0.05$) except for a decrease in locomotor activity ($P < 0.03$). Gene expression of the cryptochromes *cry1* and *cry2* in the adrenals, liver, and hippocampus was also not affected by exposures (in all cases, $P > 0.05$). In conclusion, these results suggest that 50 Hz magnetic fields do not significantly affect the acute light response to a degree that can be detected in the adrenal response.

(E) Ma Q, Chen C, Deng P, Zhu G, Lin M, Zhang L, Xu S, He M, Lu Y, Duan W, Pi H, Cao Z, Pei L, Li M, Liu C, Zhang Y, Zhong M, Zhou Z, Yu Z. Extremely Low-Frequency Electromagnetic Fields Promote In Vitro Neuronal Differentiation and Neurite Outgrowth of Embryonic Neural Stem Cells via Up-Regulating TRPC1. PLoS One. 11(3):e0150923, 2016. (AS, CE, MC, DE)

Exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) can enhance hippocampal neurogenesis in adult mice. However, little is focused on the effects of ELF-EMFs on embryonic neurogenesis. Here, we studied the potential effects of ELF-EMFs on embryonic neural stem cells (eNSCs). We exposed eNSCs to ELF-EMF (50 Hz, 1 mT) for 1, 2, and 3 days with 4 hours per day. We found that eNSC proliferation and maintenance were significantly enhanced after ELF-EMF exposure in proliferation medium. ELF-EMF exposure increased the ratio of differentiated neurons and promoted the neurite outgrowth of eNSC-derived neurons without influencing astrocytes differentiation and the cell apoptosis. In addition, the expression of the proneural genes, NeuroD and Ngn1, which are crucial for neuronal differentiation and neurite outgrowth, was increased after ELF-EMF exposure. Moreover, the expression of transient receptor potential canonical 1 (TRPC1) was significantly up-regulated accompanied by increased the peak amplitude of intracellular calcium level induced by ELF-EMF. Furthermore, silencing TRPC1 expression eliminated the up-regulation of the proneural genes and the promotion of neuronal differentiation and neurite outgrowth induced by ELF-EMF. These results suggest that ELF-EMF exposure promotes the neuronal differentiation and neurite outgrowth of eNSCs via up-regulation the expression of TRPC1 and proneural genes (NeuroD and Ngn1). These findings also provide new insights in understanding the effects of ELF-EMF exposure on embryonic brain development.

(E) Madjid Ansari A, Farzampour S, Sadr A, Shekarchi B, Majidzadeh-A K. Effects of short term and long term Extremely Low Frequency Magnetic Field on depressive disorder in mice: Involvement of nitric oxide pathway. Life Sci. 146:52-57, 2016. (AS, AE, CE, BE, OX)

AIMS: Previous reports on the possible effects of Extremely Low Frequency Magnetic Fields (ELF MF) on mood have been paradoxical in different settings while no study has yet been conducted on animal behavior. In addition, it was shown that ELF MF exposure makes an increase in brain nitric oxide level. Therefore, in the current study, we aimed to assess the possible effect(s) of ELF MF exposure on mice Forced Swimming Test (FST) and evaluate the probable role of the increased level of nitric oxide in the observed behavior. MAIN METHODS: Male adult mice NMRI were recruited to investigate the short term and long term ELF MF exposure (0.5 mT and 50 Hz, single 2h and 2 weeks 2h a day). Locomotor behavior was assessed by using open-field test (OFT) followed by FST to evaluate the immobility time. Accordingly, N Ω -nitro-L-arginine methyl ester 30 mg/kg was used to exert anti-depressant like effect. KEY FINDINGS: According to the results, short term exposure did not alter the immobility time, whereas long term exposure significantly reduces immobility time ($p < 0.01$). However, it was revealed that the locomotion did not differ among all experimental groups. Short term exposure reversed the anti-depressant like effect resulting from 30 mg/kg of N Ω -nitro-L-arginine methyl ester ($p < 0.01$). SIGNIFICANCE: It has been concluded that long term exposure could alter the depressive disorder in mice, whereas short term exposure has no significant effect. Also, reversing the anti-depressant activity of L-NAME indicates a probable increase in the brain nitric oxide.

(E) Maes A, Anthonissen R, Wambacq S, Simons K, Verschaeve L. The Cytome Assay as a Tool to Investigate the Possible Association Between Exposure to

Extremely Low Frequency Magnetic Fields and an Increased Risk for Alzheimer's Disease. J Alzheimers Dis. 50(3):741-749, 2016. (CS, AE, MC, ND)

Exposure to extremely low frequency magnetic fields (ELF-MF) has been identified as one of the potential environmental risk factors for Alzheimer's disease (AD). However, this is far from being established. So far there is no experimental evidence supporting this alleged association. We have performed an in vitro cytogenetic laboratory investigation to explore the plausibility of such association. Our investigation was based on possible similarities found in cells from AD patients and in cells exposed to ELF-MF. We especially found that 50Hz ELF-MF increase the frequency of cells with (large) micronuclei and nuclear buds indicating that fields above 50 μ T may induce chromosome instabilities as those found in AD patients. It should be stressed yet that results from the few published experimental studies on ELF-MF and AD are rather reassuring. Thus, our findings certainly do not prove anything. They only suggest that further investigations might be necessary.

(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) Mahdavi SM, Sahraei H, Yaghmaei P, Tavakoli H. Effects of Electromagnetic Radiation Exposure on Stress-Related Behaviors and Stress Hormones in Male Wistar Rats. Biomol Ther (Seoul). 22(6):570-576, 2014. (AS, AE, CE, CC, BE)

Studies have demonstrated that electromagnetic waves, as the one of the most important physical factors, may alter cognitive and non-cognitive behaviors, depending on the frequency and energy. Moreover, non-ionizing radiation of low energy waves e.g. very low frequency waves could alter this phenomenon via alterations in neurotransmitters and neurohormones. In this study, short, medium, and long-term exposure to the extremely low frequency electromagnetic field (ELF-EMF) (1 and 5 Hz radiation) on behavioral, hormonal, and metabolic changes in male Wistar rats (250 g) were studied. In addition, changes in plasma concentrations for two main stress hormones, noradrenaline and adrenocorticotrophic hormone (ACTH) were evaluated. ELF-EMF exposure did not alter body weight, and food and water intake. Plasma glucose level was increased and decreased in the groups which exposed to the 5 and 1Hz wave, respectively. Plasma ACTH concentration increased in both using frequencies, whereas nor-adrenaline concentration showed overall reduction. At last, numbers of rearing, sniffing, locomotor activity was increased in group receiving 5 Hz wave over the time. In conclusions, these data showed that the effects of 1 and 5 Hz on the hormonal, metabolic and stress-like behaviors may be different. Moreover, the influence of waves on stress system is depending on time of exposure.

(E) Mahdavi SM, Sahraei H, Rezaei-Tavirani M, Najafi Abedi A. Common behaviors alterations after extremely low-frequency electromagnetic field exposure in rat animal model. Electromagn Biol Med. 2015 Jul 16:1-6. [Epub ahead of print] (AS, CE, BE)

Naturally, the presence of electromagnetic waves in our living environment affects all components of organisms, particularly humans and animals, as the large part of their body consists of water. In the present study, we tried to investigate the relation between exposure to the extremely low-frequency electromagnetic field (ELF-EMF) and common behaviors such as body weight, food and water intake, anorexia (poor appetite), plasma glucose concentration, movement, rearing and sniffing in rats. For this purpose, rats were exposed to 40 Hz ELF-EMF once a day for 21 days, then at days 1, 3, 7, 14 and 21 after exposure, any changes in the above-mentioned items were assessed in the exposed rats and compared to the non-exposed group as control. Body weight of irradiated rats significantly increased only a week after exposure and decreased after that. No significant change was observed in food and water intake of irradiated rats compared to the control, and the anorexia parameter in the group exposed to ELF-EMF was significantly decreased at one and two weeks after irradiation. A week after exposure, the level of glucose was significantly increased but at other days these changes were not significant. Movements, rearing and sniffing of rats at day 1 after exposure were significantly decreased and other days these changes did not follow any particular pattern. However, the result of this study demonstrated that exposure to ELF-EMF can alter the normal condition of animals and may represent a harmful impact on behavior.

(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 μ T) 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 μ T than at 50 μ T. 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 \times 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) Marchesi N, Osera C, Fassina L, Amadio M, Angeletti F, Morini M, Magenes G, Venturini L, Biggiogera M, Ricevuti G, Govoni S, Caorsi S, Pascale A, Comincini S. Autophagy is modulated in human neuroblastoma cells through direct exposition to low frequency electromagnetic fields. J Cell Physiol. 229(11):1776-1786, 2014. (CS, AE, CC, ND, MA)

In neurogenerative diseases, comprising Alzheimer's (AD), functional alteration in autophagy is considered one of the pathological hallmarks and a promising therapeutic target. Epidemiological investigations on the possible causes undergoing these diseases have suggested that electromagnetic fields (EMF) exposition can contribute to their etiology. On the other hand, EMF have therapeutic implications in reactivating neuronal functionality. To partly clarify this dualism, the effect of low-frequency EMF (LF-EMF) on the modulation of autophagy was investigated in human neuroblastoma SH-SY5Y cells, which were also subsequently exposed to A β peptides, key players in AD. The results primarily point that LF-EMF induce a significant reduction of microRNA 30a (miR-30a) expression with a concomitant increase of Beclin1 transcript (BECN1) and its corresponding protein. Furthermore, LF-EMF counteract the induced miR-30a up-regulation in the same cells transfected with miR-30a mimic precursor molecules and, on the other side, rescue Beclin1 expression after BECN1 siRNA treatment. The expression of autophagy-related markers (ATG7 and LC3B-II) as well as the dynamics of autophagosome formation were also visualized after LF-EMF exposition. Finally, different protocols of repeated LF-EMF treatments were assayed to contrast the effects of A β peptides in vitro administration. Overall, this research demonstrates, for the first time, that specific LF-EMF treatments can modulate in vitro the expression of a microRNA sequence, which in turn affects autophagy via Beclin1 expression. Taking into account the pivotal role of autophagy in the clearance of protein aggregates within the cells, our results indicate a potential cytoprotective effect exerted by LF-EMF in neurodegenerative diseases such as AD.

(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.

(E) Masoudian N, Riazi GH, Afrasiabi A, Modaresi SM, Dadras A, Raffei S, Yazdankhah M, Lyaghi A, Jarah M, Ahmadian S, Seidkhani H. Variations of Glutamate Concentration Within Synaptic Cleft in the Presence of Electromagnetic Fields: An Artificial Neural Networks Study. Neurochem Res. 2015 Jan 13. [Epub ahead of print](CS,AE, CC, MA)

Glutamate is an excitatory neurotransmitter that is released by the majority of central nervous system synapses and is involved in developmental processes, cognitive functions, learning and memory. Excessive elevated concentrations of Glu in synaptic cleft results in neural cell apoptosis which is called excitotoxicity causing neurodegenerative diseases. Hence, we investigated the possibility of extremely low frequency electromagnetic fields (ELF-EMF) as a risk factor which is able to change Glu concentration in synaptic cleft. Synaptosomes as a model of nervous terminal were exposed to ELF-EMF for 15-55 min in flux intensity range from 0.1 to 2 mT and frequency range from 50 to 230 Hz. Finally, all raw data by INForm v4.02 software as an artificial neural network program was analyzed to predict the effect of whole mentioned range spectra. The results showed the tolerance of all effects between the ranges from -35 to +40 % compared to normal state when glutamatergic systems exposed to ELF-EMF. It indicates that glutamatergic system attempts to compensate environmental changes through release or reuptake in order to keep the system safe. Regarding the wide range of ELF-EMF acquired in this study, the obtained outcomes have potential for developing treatments based on ELF-EMF for some neurological diseases; however, in vivo experiments on the cross linking responses between glutamatergic and cholinergic systems in the presence of ELF-EMF would be needed. No DNA damage response and negligible genome-wide transcriptional changes in human embryonic stem cells exposed to terahertz radiation.

(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) Medina-Fernandez FJ, Escribano BM, Agüera E, Aguilar-Luque M, Feijoo M, Luque E, Garcia-Maceira FI, Pascual-Leone A, Drucker-Colin R, Tunes I. Effects of transcranial magnetic stimulation on oxidative stress in experimental autoimmune encephalomyelitis. Free Radic Res. 2017 May;51(5):460-469, 2017. (AS, CE, MC, OX, ND, MA)

Experimental autoimmune encephalomyelitis (EAE) reproduces a multiple sclerosis (MS)-like experimental model. The main objective was to evaluate the effect of extremely low-frequency electromagnetic fields (EL-EMF) application, like a paradigm of transcranial magnetic stimulation (TMS) in the development of EAE. Rats were injected with a single dose of 150 µg of myelin oligodendrocyte glycoprotein (MOG, fragment 35-55) to produce experimental MS. To assess the effect of TMS application in EAE, the rats were treated with TMS (60 Hz and 0.7 mT) for 2 h in the morning, once a day, 5 days a week, during 3 weeks. TMS was applied to the head. The effect of TMS on EAE was evaluated as motor symptoms and, oxidative and cell damage. The data showed that MOG induced motor symptoms as tail paralysis and limb paresis/paralysis, oxidative stress and cell death similar to MS when compared with control animals. Importantly, TMS application attenuated motor symptoms, oxidative and cell damage, whereas it increased antioxidant system. Our findings suggest that: (i) MOG reproduces an experimental model of MS characterised by oxidative and cell damage; and (ii) TMS application decreases oxidative stress and cell death induced by MOG.

(E) Monazzam MR, Hosseini M, Matin LF, Aghaei HA, Khosroabadi H, Hesami A. Sleep quality and general health status of employees exposed to extremely low frequency magnetic fields in a petrochemical complex. Journal of Environmental Health Science and Engineering 2014, 12:78. (CE, HU, BE)

Background. Advances in science and technology of electrical equipment, despite increasing human welfare in everyday life, have increased the number of people exposed to Electro-Magnetic Fields (EMFs). Because of possible adverse effects on the health of exposed individuals, the EMFs have been the center of attention. This study was performed to determine possible correlation between Extremely Low Frequency Electro-Magnetic Fields (ELF EMFs) and sleep quality and public health of those working in substation units of a petrochemical complex in southern Iran. Materials and method. To begin with, magnetic flux density was measured at different parts of a Control Building and two substations in accordance with IEEE std 644 1994. Subsequently, the questionnaires Pittsburgh Sleep Quality Index (PSQI) and General Health Quality (GHQ) were used to investigate relationship between ELF exposure level and sleep quality and public health, respectively. Both questionnaires were placed at disposal of a total number of 40 workers at the complex. The filled out questionnaires were analyzed by T-test, Duncan and the Chi-square tests. Results. The obtained results revealed that 28% of those in case group suffered from poor health status and 61% were diagnosed with a sleep disorder. However, all members in control group were in good health condition and only 4.5% of them had undesirable sleep quality. Conclusion. In spite of a significant difference between the case and control groups in terms of sleep quality and general health, no significant relationship was found between the exposure level and sleep quality and general health. It is worth noting that the measured EMF values were lower than the standard limits recommended by American Conference of Industrial Hygienists (ACGIH). However, given the uncertainties about the pathogenic effects caused by exposure to ELF EMFs, further epidemiological studies and periodic testing of personnel working in high voltage substations are of utmost importance.

(E) Murugan NJ, Persinger MA. Comparisons of Responses by Planarian to Micromolar to Attomolar Dosages of Morphine or Naloxone and/or Weak Pulsed Magnetic Fields: Revealing Receptor Subtype Affinities and Nonspecific Effects. Int J Radiat Biol. 2014 Apr 10. [Epub ahead of print] (AS, AE, BE)

Purpose: The behavioral responses of planaria to the exposures of a range of concentrations of morphine (μM to attoM) or the μ -opiate antagonist naloxone or to either of these compounds and a burst-firing magnetic field ($5 \mu\text{T}$) were studied. Material and Methods: The locomotor velocity (LMV) of planaria was measured after individual worms were exposed to increasing concentrations from attomolar to micromolar of morphine or naloxone, physiologically patterned magnetic fields or a combination of the two. Results: Compared to spring water controls, the two-hour exposure to the patterned magnetic field before measurement reduced activity by about 50% which was comparable to the non-specific effects of morphine and naloxone across all dosages except 1 attomolar that did not differ from spring water. The specific dosage of 100 nM produced additional marked reduction in activity for planaria exposed to either morphine or naloxone while only 1 pM of morphine produced this effect. Conclusion: The results support the presence of at least two receptor subtypes that mediate the diminished activity effects elicited by morphine specifically and suggests that exposure to the specifically patterned magnetic field produces a behavioral suppression whose magnitude is similar to the "dose independent" effects from this opiate.

(E) Nishimura T, Tada H, Fukushima M. Correlation between the Lunar Phase and Tail-Lifting Behavior of Lizards (*Pogona vitticeps*) Exposed to an Extremely Low-Frequency Electromagnetic Field. *Animals (Basel)*. 9(5). pii: E208, 2019. (AS, CE, BE)

We previously showed that the agamid lizard *Pogona vitticeps* responded to an extremely low-frequency electromagnetic field (ELF-EMF; frequency: 6 and 8 Hz; peak magnetic field: 2.6 μ T; peak electric field: 10 V/m) with tail-lifting behavior. In addition, the tail-lifting response to ELF-EMF disappeared when the parietal eyes of the lizards were covered by small round aluminum caps. This result suggests that the parietal eye contributes to light-dependent magnetoreception. In the present study, we set up an ELF-EMF group to evaluate the long-term effect of the ELF-EMF on lizards' behavior and examine our hypothesis that exposure to ELF-EMFs increases the magnetic field sensitivity in lizards. We therefore include the lunar phase (full moon/new moon) and K index as environmental factors related to the geomagnetic field in the analysis. The number of tail lifts per individual per day was the response variable while calendar month, daily mean temperature, daily mean humidity, daily mean atmospheric pressure, full moon, new moon, and K index were the explanatory variables. We analyzed an ELF-EMF group and a control group separately. In a multiple linear regression analysis, the independent determinants associated with the number of tail lifts were the full moon, the temperature, February, March, April, and May in the ELF-EMF group and March, April, May, and June in the control group. The *P. vitticeps* in the ELF-EMF group responded to the full moon whereas those in the control group did not. In addition, in the ELF-EMF group, the number of tail lifts was higher on days when the K index was higher ($P = 0.07$) in the first period whereas there was no such tendency in either period in the control group. **There is the possibility that the exposure to ELF-EMFs may increase magnetic-field sensitivity in lizards.**

Ohayon MM, Stolc V, Freund FT, Milesi C, Sullivan SS. The potential for impact of man-made super low and extremely low frequency electromagnetic fields on sleep. *Sleep Med Rev*. 47:28-38, 2019. (Review)

An ever-growing number of electromagnetic (EM) emission sources elicits health concerns, particularly stemming from the ubiquitous low to extremely low frequency fields from power lines and appliances, and the radiofrequency fields emitted from telecommunication devices. In this article we review the state of knowledge regarding possible impacts of electromagnetic fields on melatonin secretion and on sleep structure and the electroencephalogram of humans. Most of the studies on the effects of melatonin on humans have been conducted in the presence of EM fields, focusing on the effects of occupational or residential exposures. While some of the earlier studies indicated that EM fields may have a suppressive effect on melatonin, the results cannot be generalized because of the large variability in exposure conditions and other factors that may influence melatonin. For instance, exposure to radiofrequency EM fields on sleep architecture show little or no effect. However, a number of studies show that pulsating radiofrequency electromagnetic fields, such as those emitted from cellular phones, can alter brain physiology, increasing the electroencephalogram power in selective bands when administered immediately prior to or during sleep. Additional

research is necessary that would include older populations and evaluate the interactions of EM fields in different frequency ranges to examine their effects on sleep in humans.

(E) Ozdemir E, Demirkazik A, Gursoy S, Taskiran AS, Kilinc O, Arslan G. Effects of extremely low frequency electromagnetic fields on morphine analgesia and tolerance in rats. Gen Physiol Biophys. 2017 Aug 24. doi: 10.4149/gpb_2017008. [Epub ahead of print] (AS, CE, BE)

Several studies have demonstrated that the electromagnetic fields produce analgesic activity. The aim of this study was to investigate the effects of extremely low frequency (ELF) electromagnetic fields (EMF) on morphine analgesia and tolerance in rats. In the study, 78 adult male Wistar albino rats (approximately 240 ± 12 g) were used. The application of 50 Hz magnetic field, each day the same times for 30 minutes for 15 days, and a total of four times every 15 minute intervals. To constitute morphine tolerance, high dose of morphine (50 mg/kg) were administered for 3 days in rats and tolerance was evaluated on day 4. Prior to analgesia tests, the effective dose (5 mg/kg) of morphine was injected into rats. In the statistical analyzes of the data, analysis of variance (two-way ANOVA) was used and the multiple comparison determined by Tukey tests. The maximum analgesic effect of the 5 mT magnetic field was determined on 7 days. Administration of morphine (5 mg/kg) in rats exposed to a magnetic field, the analgesic effect was significantly higher compared to the morphine group ($p < 0.05$). Morphine tolerant animals exposed to a magnetic field, the analgesic effect was found significantly higher than morphine tolerant group rats ($p < 0.05$). Analgesia test data demonstrated that application of ELF-EMFs to rats increases the morphine analgesia and reduces morphine tolerance.

(E) Ozdemir E, Demirkazik A, Taskiran AS, Arslan G. Effects of 5-HT₁ and 5-HT₂ Receptor Agonists on Electromagnetic Field-Induced Analgesia in Rats. Bioelectromagnetics. 2019 May 31. doi: 10.1002/bem.22196. [Epub ahead of print] (AS, CE, BE)

Much evidence demonstrates the antinociceptive effect of magnetic fields (MFs). However, the analgesic action mechanism of the electromagnetic field (EMF) is not exactly understood. The aim of the present study was to investigate the effects of 5-HT₁ and 5-HT₂ receptor agonists (serotonin HCl and 2,5-dimethoxy-4-iodoamphetamine [DOI] hydrochloride) on EMF-induced analgesia. In total, 66 adult male Wistar albino rats with an average body mass of 225 ± 13 g were used in this study. The animals were subjected to repeated exposures of alternating 50 Hz and 5 mT EMF for 2 h a day for 15 days. Prior to analgesia tests, serotonin HCl (5-HT₁ agonist) 4 mg/kg, WAY 100635 (5-HT₁ antagonist) 0.04 mg/kg, DOI hydrochloride (5-HT₂ receptor agonist) 4 mg/kg, and SB 204741 (5-HT₂ antagonist) 0.5 mg/kg doses were injected into rats. For statistical analysis of the data, analysis of variance was used and multiple comparisons were determined by Tukey's test. Administration of serotonin HCl MF (5 mT)-exposed rats produced a significant increase in percent maximal possible effect (% MPE) as compared with EMF group ($P < 0.05$). On the contrary, injection of WAY 100635 to MF-exposed rats produced a significant decrease in analgesic activity ($P < 0.05$). Similarly, the administration of DOI hydrochloride significantly increased % MPE values as compared with the EMF group while SB 204741 reduced it ($P < 0.05$). In conclusion, our results suggested that serotonin 5-HT₁ and 5-HT₂

receptors play an important role in EMF-induced analgesia; however, further research studies are necessary to understand the mechanism.

(E) Özgün A, Marote A, Behie LA, Salgado A, Garipcan B. Extremely low frequency magnetic field induces human neuronal differentiation through NMDA receptor activation. J Neural Transm (Vienna). 2019 Jul 17. doi: 10.1007/s00702-019-02045-5. (CS, CE, CC, MC)

Magnetic fields with different frequency and intensity parameters exhibit a wide range of effects on different biological models. Extremely low frequency magnetic field (ELF MF) exposure is known to augment or even initiate neuronal differentiation in several in vitro and in vivo models. This effect holds potential for clinical translation into treatment of neurodegenerative conditions such as autism, Parkinson's disease and dementia by promoting neurogenesis, non-invasively. However, the lack of information on underlying mechanisms hinders further investigation into this phenomenon. Here, we examine involvement of glutamatergic Ca²⁺ channel, N-methyl-D-aspartate (NMDA) receptors in the process of human neuronal differentiation under ELF MF exposure. We show that human neural progenitor cells (hNPCs) differentiate more efficiently under ELF MF exposure in vitro, as demonstrated by the abundance of neuronal markers. Furthermore, they exhibit higher intracellular Ca²⁺ levels as evidenced by c-fos expression and more elongated mature neurites. We were able to neutralize these effects by blocking NMDA receptors with memantine. As a result, we hypothesize that the effects of ELF MF exposure on neuronal differentiation originate from the effects on NMDA receptors, which sequentially triggers Ca²⁺-dependent cascades that lead to differentiation. Our findings identify NMDA receptors as a new key player in this field that will aid further research in the pursuit of effect mechanisms of ELF MFs.

(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) Pedersen C, Poulsen AH, Rod NH, Frei P, Hansen J, Grell K, Raaschou-Nielsen O, Schüz J, Johansen C. Occupational exposure to extremely low-frequency magnetic fields and risk for central nervous system disease: an update of a

Danish cohort study among utility workers. Int Arch Occup Environ Health. 2017 Apr 20. doi: 10.1007/s00420-017-1224-0. [Epub ahead of print] (HU, CE, ND)

PURPOSE: Evidence of whether exposure to extremely low-frequency magnetic fields (ELF-MF) is related to central nervous system diseases is inconsistent. This study updates a previous study of the incidence of such diseases in a large cohort of Danish utility workers by almost doubling the period of follow-up. **METHODS:** We investigated the risks for dementia, motor neurone disease, Parkinson disease, multiple sclerosis and epilepsy among 32,006 men employed at the 99 utility companies that supplied Denmark with electricity during the period 1900-1993. Cases were identified in the Danish National Patient Registry and the cohort was followed during 1982-2010. Exposure was estimated from a job-exposure matrix based on company records of job title and area of work and cohort members were allocated to one of three categories (<0.1 , $0.1-0.99$ and ≥ 1.0 μT). **RESULTS:** For dementia, multiple sclerosis and epilepsy the incidence rate ratios (IRR) were close to unity, but higher for motor neurone disease [IRR 1.24, 95% confidence interval (CI) 0.86-1.79] and lower for Parkinson disease (IRR 0.81, 95% CI 0.67-0.97) among workers exposed to ≥ 0.1 μT compared with the Danish population. For the highest level of exposure (≥ 1.0 μT), IRRs of 1.44, 1.78, 1.40 and 1.34 were observed for dementia, motor neurone disease, multiple sclerosis and epilepsy, respectively. **CONCLUSIONS:** We observed elevated risks of dementia, motor neurone disease, multiple sclerosis and epilepsy and lower risks of Parkinson disease in relation to exposure to ELF-MF in a large cohort of utility employees.

(E) Pelletier SJ, Lagacé M, St-Amour I, Arsenault D, Cisbani G, Chabrat A, Fecteau S, Lévesque M, Cicchetti F. The morphological and molecular changes of brain cells exposed to direct current electric field stimulation. Int J Neuropsychopharmacol. 2014 Dec 7. pii: pyu090. [Epub ahead of print] (CE, AE,MC, CC, EF)

BACKGROUND: The application of low-intensity direct current electric fields (DCEFs) has been experimentally used in the clinic to treat a number of brain disorders, predominantly using transcranial direct current stimulation (tDCS) approaches. However, the cellular and molecular changes induced by such treatment remain largely unknown. **METHODS:** Here, we tested various intensities of DCEFs (0, 25, 50 and 100 V/m) in a well-controlled in vitro environment in order to investigate the responses of neurons, microglia and astrocytes to this type of stimulation. This included morphological assessments of the cells, viability as well as shape and fiber outgrowth relative to the orientation of the DCEF. We also undertook ELISA assays and western immunoblotting to identify which molecular pathways were affected by DCEFs. **RESULTS:** In response to DCEF, neurons developed an elongated cell body shape with neurite outgrowth that was associated with a significant increase in GAP-43. Fetal midbrain dopaminergic explants grown in a collagen gel matrix also showed a reorientation of their neurites towards the cathode. BV2 microglial cells adopted distinct morphological changes with an increase in COX-2 expression but these were dependent on whether they had already been activated with lipopolysaccharide (LPS). Finally, astrocytes displayed elongated cell bodies with cellular filopodia that were oriented perpendicular to the DCEF. **CONCLUSION:** we show that cells of

the central nervous system can respond to DCEF's both in terms of their morphological shape and molecular expression of certain proteins and this in turn can help us to begin understand the mechanisms underlying the clinical benefits of DCEF.

(NE) Percherancier Y, Goudeau B, Charlet de Sauvage R, de Gannes FP, Haro E, Hurtier A, Sojic N, Lagroye I, Arbault S, Veyret B. Effects of 50 Hz magnetic fields on gap junctional intercellular communication in NIH3T3 cells. Bioelectromagnetics. 2015 Apr 3. doi: 10.1002/bem.21908. [Epub ahead of print](CS, AE, FC)

The present study focused on gap junctional intercellular communication (GJIC) as a target for biological effects of extremely low-frequency (ELF) magnetic field (MF) exposure. Fluorescence recovery after photobleaching microscopy (FRAP) was used to visualize diffusion of a fluorescent dye between NIH3T3 fibroblasts through gap junctions. The direct effect of 24 h exposure to 50 Hz MF at 0.4 or 1 mT on GJIC function was assessed in one series of experiments. The potential synergism of MF with an inhibitor of GJIC, phorbol ester (TPA), was studied in another series by observing FRAP when NIH3T3 cells were incubated with TPA for 1 h following 24 h exposure to MF. In contrast to other reports of ELF-MF effects on GJIC, under our experimental conditions we observed neither direct inhibition of GJIC nor synergism with TPA-induced inhibition from 50 Hz MF exposures.

(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) Prasad A, Teh DBL, Blasiak A, Chai C, Wu Y, Gharibani PM, Yang IH, Phan TT, Lim KL, Yang H, Liu X, Ali AH. Static Magnetic Field Stimulation Enhances Oligodendrocyte Differentiation and Secretion of Neurotrophic Factors. Sci Rep. 7(1):6743, 2017. (CS, CE, CC, FC)

The cellular-level effects of low/high frequency oscillating magnetic field on excitable cells such as neurons are well established. In contrast, the effects of a homogeneous, static magnetic field (SMF) on Central Nervous System (CNS) glial cells are less investigated. Here, we have developed an in vitro SMF stimulation set-up to investigate the genomic effects of SMF exposure on oligodendrocyte differentiation and neurotrophic factors secretion. Human oligodendrocytes precursor cells (OPCs) were stimulated with moderate intensity SMF (0.3 T) for a period of two weeks (two hours/day). The differential gene expression of cell activity marker (c-fos), early OPC (Olig1, Olig2, Sox10), and mature oligodendrocyte markers (CNP, MBP) were quantified. The enhanced myelination capacity of the SMF stimulated oligodendrocytes was validated in a dorsal root ganglion microfluidics chamber platform. Additionally, the effects of SMF on the gene expression and secretion of neurotrophic factors- BDNF and NT3 was quantified. We also report that SMF stimulation increases the intracellular calcium influx in OPCs as well as the gene expression of L-type channel subunits- CaV1.2 and CaV1.3. Our findings emphasize the ability of glial cells such as OPCs to positively respond to moderate intensity SMF stimulation by exhibiting enhanced differentiation, functionality as well as neurotrophic factor release.

(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) Reale M, Kamal MA, Patruno A, Costantini E, D'Angelo C, Pesce M, Greig NH. Neuronal Cellular Responses to Extremely Low Frequency Electromagnetic Field Exposure: Implications Regarding Oxidative Stress and Neurodegeneration. PLoS One. 2014 Aug 15; 9(8):e104973. doi: 10.1371/journal.pone.0104973. eCollection 2014. (CS, AE, OX, ND)

Neurodegenerative diseases comprise both hereditary and sporadic conditions characterized by an identifying progressive nervous system dysfunction and distinctive neuropathophysiology. The majority are of non-familial etiology and hence environmental factors and lifestyle play key roles in their pathogenesis. The extensive use of and ever increasing worldwide demand for electricity has stimulated societal and scientific interest on the environmental exposure to low

frequency electromagnetic fields (EMFs) on human health. Epidemiological studies suggest a positive association between 50/60-Hz power transmission fields and leukemia or lymphoma development. Consequent to the association between EMFs and induction of oxidative stress, concerns relating to development of neurodegenerative diseases, such as Alzheimer disease (AD), have been voiced as the brain consumes the greatest fraction of oxygen and is particularly vulnerable to oxidative stress. Exposure to extremely low frequency (ELF)-EMFs are reported to alter animal behavior and modulate biological variables, including gene expression, regulation of cell survival, promotion of cellular differentiation, and changes in cerebral blood flow in aged AD transgenic mice. Alterations in inflammatory responses have also been reported, but how these actions impact human health remains unknown. We hence evaluated the effects of an electromagnetic wave (magnetic field intensity 1mT; frequency, 50-Hz) on a well-characterized immortalized neuronal cell model, human SH-SY5Y cells. ELF-EMF exposure elevated the expression of NOS and O₂⁻, which were countered by compensatory changes in antioxidant catalase (CAT) activity and enzymatic kinetic parameters related to CYP-450 and CAT activity. Actions of ELF-EMFs on cytokine gene expression were additionally evaluated and found rapidly modified. Confronted with co-exposure to H₂O₂-induced oxidative stress, ELF-EMF proved not as well counteracted and resulted in a decline in CAT activity and a rise in O₂⁻ levels. Together these studies support the further evaluation of ELF-EMF exposure in cellular and in vivo preclinical models to define mechanisms potentially impacted in humans.

(NE) Reale M, D'Angelo C, Costantini E, Tata AM, Regen F, Hellmann-Regen J. Effect of Environmental Extremely Low-Frequency Electromagnetic Fields Exposure on Inflammatory Mediators and Serotonin Metabolism in a Human Neuroblastoma Cell Line. CNS Neurol Disord Drug Targets. 15(10):1203-1215, 2016. (CS, AE, CC)

Exposure to environmental extremely low-frequency electromagnetic fields (ELF-EMF) in everyday life is increasing and it is a matter of great debate whether exposure to ELF-EMF can be harmful to human health. The neuropathology and symptoms of neurodegenerative disease depends on factors other than genetic predispositions, such as environmental exposure to disease-related risk factors. Research focusing on a possible contribution of ELF-EMF to cell injury and to the development of neurodegenerative disorders is characterized by conflicting data from epidemiological and animal studies. Due to lack of a direct link between neurodegenerative processes and ELF-EMF exposure, our goal was to investigate if ELF-EMF exposure may represent a possible risk factor. In the present study, using neuronal-like SH-SY5Y neuroblastoma cells, we show that the balance between generation and elimination of reactive oxygen species, as well as the balance between pro- and anti-inflammatory cytokines linked to oxidative stress, was maintained ensuring that cells respond properly to ELF-EMF (50Hz /1mT). In SH-SY5Y-exposed cells we observed increased intracellular 5-hydroxyindoleacetic acid/5-hydroxytryptamine ratio reflecting the rate of transmitter synthesis, catabolism and release, while matrix metalloproteinases that play critical roles in neuronal cell death were not significantly altered. The results presented here indicate that changes caused by short (1h-3h) and sub-chronic (48 h) exposure to 50Hz/1mT ELF-EMF in SH-SY5Y cells are minor in comparison to the neuronal cell

damage expected to underlie neurodegeneration or cognitive impairment. Thus, these results are in accord with epidemiological studies that have provided little support for a link between ELF-EMFs and neurodegeneration.

Ritz T, Adem S, Schulten K. A model for photoreceptor-based magnetoreception in birds. *Biophys J.* 78(2):707-718, 2000. (Review)

A large variety of animals has the ability to sense the geomagnetic field and utilize it as a source of directional (compass) information. It is not known by which biophysical mechanism this magnetoreception is achieved. We investigate the possibility that magnetoreception involves radical-pair processes that are governed by anisotropic hyperfine coupling between (unpaired) electron and nuclear spins. We will show theoretically that fields of geomagnetic field strength and weaker can produce significantly different reaction yields for different alignments of the radical pairs with the magnetic field. As a model for a magnetic sensory organ we propose a system of radical pairs being 1) orientationally ordered in a molecular substrate and 2) exhibiting changes in the reaction yields that affect the visual transduction pathway. We evaluate three-dimensional visual modulation patterns that can arise from the influence of the geomagnetic field on radical-pair systems. The variations of these patterns with orientation and field strength can furnish the magnetic compass ability of birds with the same characteristics as observed in behavioral experiments. We propose that the recently discovered photoreceptor cryptochrome is part of the magnetoreception system and suggest further studies to prove or disprove this hypothesis.

(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. (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) Ross CL, Teli T, Harrison BS. Effect of electromagnetic field on cyclic adenosine monophosphate (cAMP) in a human mu-opioid receptor cell model. *Electromagn Biol Med.* 2015 Dec 29:1-8. [Epub ahead of print] (CS, AE, CC, MA)

During the cell communication process, endogenous and exogenous signaling affect normal as well as pathological developmental conditions. Exogenous influences such as extra-low-frequency electromagnetic field (EMF) have been shown to effect pain and inflammation by modulating G-protein receptors, down-regulating cyclooxygenase-2 activity, and affecting the calcium/calmodulin/nitric oxide pathway. Investigators have reported changes in opioid receptors and second messengers, such as cyclic adenosine monophosphate (cAMP), in opiate tolerance and dependence by showing how repeated exposure to morphine decreases adenylate cyclase activity causing cAMP to return to control levels in the tolerant state, and increase above control levels during withdrawal. Resonance responses to biological systems using exogenous EMF signals suggest that frequency response characteristics of the target can determine the EMF biological response. In our past research we found significant down regulation of inflammatory markers tumor necrosis factor alpha (TNF- α) and nuclear factor kappa B (NF κ B) using 5 Hz EMF frequency. In this study cAMP was stimulated in Chinese Hamster Ovary (CHO) cells transfected with human mu-opioid receptors, then exposed to 5 Hz EMF, and outcomes were compared with morphine treatment. Results showed a 23% greater inhibition of cAMP-treating cells with EMF than with morphine. In order to test our results for frequency specific effects, we ran identical experiments using 13 Hz EMF, which produced results similar to controls. This study suggests the use of EMF as a complementary or alternative treatment to morphine that could both reduce pain and enhance patient quality of life without the side-effects of opiates.

(E) Rostami A, Shahani M, Zarrindast MR, Semnanian S, Rahmati Roudsari M, Rezaei Tavirani M, Hasanzadeh H. Effects of 3 Hz and 60 Hz Extremely Low Frequency Electromagnetic Fields

on Anxiety-Like Behaviors, Memory Retention of Passive Avoidance and Electrophysiological Properties of Male Rats. J Lasers Med Sci. 7(2):120-125, 2016. (AS, CE, BE, EE, CC)

INTRODUCTION: The effects of electromagnetic fields on biological organisms have been a controversial and also interesting debate over the past few decades, despite the wide range of investigations, many aspects of extremely low frequency electromagnetic fields (ELF/EMFs) effects including mechanism of their interaction with live organisms and also their possible biological applications still remain ambiguous. In the present study, we investigated whether the exposures of ELF/EMF with frequencies of 3 Hz and 60 Hz can affect the memory, anxiety like behaviors, electrophysiological properties and brain's proteome in rats. **METHODS:** Male rats were exposed to 3 Hz and 60 Hz ELF/EMFs in a protocol consisting of 2 cycles of 2 h/day exposure for 4 days separated with a 2-day interval. Short term memory and anxiety like behaviors were assessed immediately, 1 and 2 weeks after the exposures. Effects of short-term exposure were also assessed using electrophysiological approach immediately after 2 hours exposure. **RESULTS:** Behavioral test revealed that immediately after the end of exposures, locomotor activity of both 3 Hz and 60 Hz exposed groups significantly decreased compared to sham group. This exposure protocol had no effect on anxiety like behavior during the 2 weeks after the treatment and also on short term memory. A significant reduction in firing rate of locus coeruleus (LC) was found after 2 hours of both 3 Hz and 60 Hz exposures. Proteome analysis also revealed global changes in whole brain proteome after treatment. **CONCLUSION:** Here, some evidence regarding the fact that such exposures can alter locomotor activity and neurons firing rate in male rats were presented.

(E) Sakhaie MH, Soleimani M, Pourheydar B, Majd Z, Atefimanesh P, Asl SS, Mehdizadeh M. Effects of Extremely Low-Frequency Electromagnetic Fields on Neurogenesis and Cognitive Behavior in an Experimental Model of Hippocampal Injury. Behav Neurol. 2017;2017:9194261. (AS, CE, BE, MC, MA, ND)

Exposure to extremely low-frequency electromagnetic fields may induce constant modulation in neuronal plasticity. In recent years, tremendous efforts have been made to design a suitable strategy for enhancing adult neurogenesis, which seems to be deterred due to brain senescence and several neurodegenerative diseases. In this study, we evaluated the effects of ELF-EMF on neurogenesis and memory, following treatment with trimethyltin chloride (TMT) as a neurotoxicant. The mice in all groups ($n = 56$) were injected with BrdU during the experiment for seven consecutive days to label newborn cells. Spatial memory was assessed by the Morris water maze (MWM) test. By the end of the experiment, neurogenesis and neuronal differentiation were assessed in the hippocampus, using immunohistochemistry and Western blot analysis. Based on the findings, exposure to ELF-EMF enhanced spatial learning and memory in the MWM test. ELF-EMF exposure significantly enhanced the number of BrdU+ and NeuN+ cells in the dentate gyrus of adult mice ($P < 0.001$ and $P < 0.05$, resp.). Western blot analysis revealed significant upregulation of NeuroD2 in ELF-EMF-exposed mice compared to the TMT-treated group ($P < 0.05$). These findings suggest that ELF-EMF might have clinical implications for the improvement of neurodegenerative processes and could help develop a novel therapeutic approach in regenerative medicine.

(E) Sakhnini L, Al Ali H, Al Qassab N, Al Arab E, Kamal A. Subacute exposure to 50-Hz electromagnetic fields affect prenatal and neonatal mice's motor coordination. J. Appl. Phys. 111(7):07B314, 2012. (AS, CE, BE, DE)

In this study, we investigate the possible effect of ELF-EMFs on motor performance in mice (prenatal and neonatal exposed mice). The mice performance is evaluated after 5 days of subacute exposure. Immature mice have been chosen for this study because the immature rodent brain still has the capacity to undergo proliferation, differentiation, and reorganization. Results from the rotarod experiments demonstrated a pronounced deficit in the learning abilities of the prenatal exposed groups, but no pronounced effect was observed for the neonatal exposed group.

(E) Sales PM, de Andrade LM, Pitcher MR, Rola FH, Gondim FA. Levodopa enhances immobility induced by spinal cord electromagnetic stimulation in rats. Neurosci Lett. 633:196-201, 2016. (AS, CE, BE)

BACKGROUND: The repetitive ElectroMagnetic Stimulation (rEMS) is an innocuous method applied to modulate neurocircuits in real-time to study the physiology of the central nervous system and treat neuropsychiatric conditions. Preliminary data suggest that spinal rEMS induces behavioral changes in awake rats. However, the mechanisms behind this phenomenon remain largely unknown. METHODS: Twenty-five male Wistar rats were divided into five subgroups of five animals each: one subgroup was drug-free, two subgroups received Levodopa+Benserazide 250+25mg/kg for two or seven days, and the remaining two subgroups received Haloperidol 0.1 or 0.3mg/kg for two days. The animals were restrained during sham rEMS (day 1) followed by real rEMS of the cervicothoracic region at a different day (day 2 or 7, depending on subgroup). Four behavioral parameters were quantified: Walking, Climbing, Grooming, and Cornering. RESULTS: rEMS reduced Walking and increased Cornering duration when applied over the cervicothoracic region of drug-free animals. A pretreatment with Levodopa+Benserazide for two or seven days induced an additional decrease in Walking after rEMS. This reduction was maximum after the treatment for seven days and associated with extinction of Climbing and increase in Cornering. A pretreatment with Haloperidol 0.1mg/kg reduced Grooming after rEMS, but did not prevent the reduction in Walking. CONCLUSIONS: Cervicothoracic rEMS induced complex immobility responses that are in part modulated by dopaminergic pathways in rats. Further studies are necessary to determine the specific mechanisms involved.

(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) Salunke BP, Umathe SN, Chavan JG. Experimental evidence for involvement of nitric oxide in low frequency magnetic field induced obsessive compulsive disorder-like behavior. Pharmacol Biochem Behav. 2014 Apr 26. pii: S0091-3057(14)00115-4. doi: 10.1016/j.pbb.2014.04.007. [Epub ahead of print] (AS, CE, BE, OX)

It is well documented that extremely low frequency magnetic field (ELF MF) produced effects on the function of nervous system in humans and laboratory animals. Dopaminergic and serotonergic pathways have been implicated in obsessive compulsive disorder (OCD). Recently involvement of nitric oxide (NO) in OCD-like behavior is suggested. Hence, the present study was carried out to understand the involvement of dopamine, serotonin and NO in ELF MF induced OCD-like behavior. Swiss albino mice were exposed to ELF MF (50Hz, 10G) for 8h/day for 7, 30, 60, 90 and 120days by subjecting them to Helmholtz coils. OCD-like behavior was assessed in terms of marble burying behavior (MBB) test. Results revealed that ELF MF induced time dependant MBB, on 7th, 30th, 60th, 90th, and 120th exposure day. Further, levels of dopamine, serotonin and NO after 120days of ELF MF exposure were determined in regions of the brain. The neurohumoral studies revealed that exposure to ELF MF increased NO levels in cortex, hippocampus and hypothalamus, and levels of dopamine and serotonin remain unaffected. As OCD-like behavior after ELF MF exposure was associated with higher levels of NO with no significant change in serotonin and dopamine, the effect of such exposure was studied in groups concurrently treated with NO modulators, NO precursor, L-ARG (400mg/kg) or NOS inhibitor, L-NAME (15.0mg/kg) or 7-NI (10.0mg/kg). These treatments revealed that NO precursor exacerbated and NOS inhibitors attenuated ELF MF induced OCD-like behavior with corresponding changes in the levels of NO.

(E) Samiee F, Samiee K. Effect of extremely low frequency electromagnetic field on brain histopathology of Caspian Sea Cyprinus carpio. Electromagn Biol Med. 36(1):31-38, 2017. (AS, AE, MC)

There is limited research on the effect of electromagnetic field on aquatic organisms, especially freshwater fish species. This study was conducted to evaluate the effect of extremely low frequency electromagnetic field (ELF-EMF) (50 Hz) exposure on brain histopathology of *Cyprinus carpio*, one of the important species of Caspian Sea with significant economic value. A total of 200 healthy fish were used in this study. They were classified randomly in two groups: sham-exposed group and experimental group, which were exposed to five different magnetic field intensities (0.1, 1, 3, 5, and 7 mT) at two different exposure times (0.5 and 1 h). Histologic results indicate that exposure of *C. carpio* to artificial ELF-EMF caused severe histopathological changes in the brain at field intensities ≥ 3 mT leading to brain necrosis. Field intensity and duration of exposure were key parameters in induction of lesion in the brain. Further studies are needed to elucidate exact mechanism of EMF exposure on the brain.

(E) Savić T, Janać B, Todorović D, Prolić Z. The embryonic and post-embryonic development in two *Drosophila* species exposed to the static magnetic field of 60 mT. *Electromagn Biol Med.* 30(2):108-114, 2011. (AS, CE, DE)

In this study, a static magnetic field influence on development and viability in two different species, *Drosophila melanogaster* and *Drosophila hydei*, was investigated. Both species completed development (egg-adult), in and out of the static magnetic field induced by double horseshoe magnet. Treated vials with eggs were placed in the gap between magnetic poles (47 mm) and exposed to the average magnetic induction of 60 mT, while control ones were kept far enough from magnetic field source. We found that exposure to the static magnetic field reduced development time in both species, but statistical significance was found only for *D. hydei*. Furthermore, we found that the average viability of both *Drosophila* species exposed to the magnetic field was significantly weaker compared to control ones. These results indicate that 60 mT static magnetic field could be considered as a potential stressor, influencing on different levels the embryonic and post-embryonic development of individuals.

(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) Shafiei SA, Firoozabadi SM, Tabatabaie KR, Ghabaee M. Investigation of EEG changes during exposure to extremely low-frequency magnetic field to conduct brain signals. Neurol Sci. 2014 May 27. [Epub ahead of print] (HU, AE, EE)

There are evidences that confirm the effect of magnetic fields (MFs) on brain signals and some psychological disorders such as headache, migraine and depression. The aim of the present study was to investigate changes in EEG power spectrum due to localized exposure in different parts of the brain by extremely low-frequency magnetic fields (ELF-MFs) to extract some protocols for treatment of some psychological disorders. In addition, regular effects were investigated by increasing intensity of ELF-MF. Therefore, EEG relative power spectrum was evaluated at T4, T3, F3, F4, and Cz points, when all the points were exposed to MFs with 45, 17, 10, 5, and 3 Hz frequencies, separately. Intensity of MF was 0, 100, 240, or 360 μ T in four sessions. Significant changes were observed in different EEG bands caused by locally exposing to ELF-MF in different points of brain ($P < 0.05$). Some exposure to MFs decreased alpha band of frontal and central areas in closed-eyes state. Based on the findings in this study, some protocols can be designed using a combination of various MFs exposures to conduct the brain signals that is necessary to evaluate clinically.

(E) Shahbazi-Gahrouei D, Shiri L, Alaei H, Naghdi N. The effect of continuous ELF-MFs on the level of 5-HIAA in the raphe nucleus of the rat. J Radiat Res. 2016 Jan 24. pii: rrv093. [Epub ahead of print] (AS, CE, CC, MA)

The aim of this study was to investigate the effect of continuous extremely low frequency magnetic fields (ELF-MFs) with a frequency of 10 Hz and an intensity of 690-720 μ T on the level of 5-hydroxyindolacetic acid (5-HIAA) in adult male Wistar rats. A total of 24 adult Wistar male rats were used, and after exposure with an ELF-MF for 15 successive days, all rats in each test were anesthetized with chloral hydrate. Then, they were placed in a stereotaxic frame for surgery and a microdialysis process. Dialysate samples were analyzed to measure the amount of 5-HIAA by high performance liquid chromatography (HPLC) using electrochemical detection. Results showed that ELF-MF exposure for 15 days, 1 h daily, was not effective in altering the level of 5-HIAA. However, ELF-MF exposure for 15 days, 3 h daily, decreased the level of the 5-HIAA in the raphe nucleus. It can be concluded that ELF-MFs affect the serotonergic system and may be used to treat nervous system diseases. This study is an initial step towards helping cure depression using ELF-MFs.

(E) Shen JF, Chao YL, Du L. Effects of static magnetic fields on the voltage-gated potassium channel currents in trigeminal root ganglion neurons. Neurosci Lett. 415(2):164-168, 2007. (CS, AE, CC, FC)

To evaluate the effects of moderate-intensity static magnetic fields (SMF) on two types of voltage-gated potassium channel (VGPC) currents: $I(K,A)$ and $I(K,V)$, whole-cell patch-clamp experiments were conducted on acute dissociated rat trigeminal root ganglion (TRG) neurons. The results demonstrated that 125 mT SMF could influence the inactivation kinetics of these two VGPC currents by altering the inactivation rate and velocity. No significant change was observed in the activation properties. These findings supported the hypothesis that biological membrane would be deformed in moderate-

intensity SMF and the physiological characteristics of ion channels on the membrane would be influenced. The mechanism underlying the different effects of SMF on the I(K,A) and I(K,V) inactivation was also discussed.

(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.

(NE) Sorahan T, Mohammed N. Neurodegenerative disease and magnetic field exposure in UK electricity supply workers. Occup Med (Lond). 2014 Aug 7. pii: kqu105. [Epub ahead of print]. (CE, HU, ND)

BACKGROUND: Previous research has suggested a possible link between neurodegenerative

disease and exposure to extremely low-frequency electric and magnetic fields. AIMS: To investigate whether risks of Alzheimer's, motor neurone or Parkinson's disease are related to occupational exposure to magnetic fields. METHODS: The mortality experienced by a cohort of 73051 employees of the former Central Electricity Generating Board of England and Wales was investigated for the period 1973-2010. All employees were hired in the period 1952-82, were employed for at least 6 months and had some employment after 1 January 1973. Detailed calculations had been performed by others to enable an assessment to be made of exposures to magnetic fields. Poisson regression was used to calculate relative risks (rate ratios) of developing any of the three diseases under investigation for categories of lifetime, distant (lagged) and recent (lugged) exposure. RESULTS: No statistically significant trends were shown for risks of any of these diseases to increase with estimates of lifetime, recent or distant exposure to magnetic fields. CONCLUSIONS: There is no convincing evidence that UK electricity generation and transmission workers have suffered elevated risks from neurodegenerative diseases as a consequence of exposure to magnetic fields.

(E) Spasić S, Kesić S, Stojadinović G, Petković B, Todorović D. Effects of the static and ELF magnetic fields on the neuronal population activity in *Morimus funereus* (Coleoptera, Cerambycidae) antennal lobe revealed by wavelet analysis. *Comp Biochem Physiol A Mol Integr Physiol*. 2014 Nov 28. pii: S1095-6433(14)00249-9. doi: 10.1016/j.cbpa.2014.11.018. [Epub ahead of print] (AS, AE, EE)

To study the influence of a static magnetic field (SMF, 2mT) and extremely low frequency magnetic field (ELF MF, 50Hz, 2mT) on the neuronal population activity, the experiments were performed on adult longhorn beetles *Morimus funereus* (Coleoptera, Cerambycidae). Based on a wavelet analysis of the local field potentials (LFPs), our study showed for the first time that the effects of prolonged and repeated exposure to the ELF MF on the LFPs were irreversible within investigated time frame. The relative wavelet energy (RWE) of 4-8Hz frequency band was significantly increased after sine ELF MF (SnMF)/square ELF MF (SqMF) in comparison to the control value. The RWE of slower oscillations (1-2Hz) was significantly decreased after the repeated exposures to either SnMF or SqMF. The SqMF induced decreasing of the faster waves in the range of 64-128Hz. However, we did not prove with presented methods that exposure to the SMF for 5min produces any effects on the neuronal population activity. This study has proved the wavelet transform as a valuable tool for measuring the effects of SMF and ELF MF on the neuronal population activity in *M. funereus* antennal lobe.

(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, Bártová 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% methylanthranilate). 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) Sun ZC, Ge JL, Guo B, Guo J, Hao M, Wu YC, Lin YA, La T, Yao PT, Mei YA, Feng Y, Xue L. Extremely Low Frequency Electromagnetic Fields Facilitate Vesicle Endocytosis by Increasing Presynaptic Calcium Channel Expression at a Central Synapse. Sci Rep. 2016 Feb 18;6:21774. doi: 10.1038/srep21774. (AS, CE, FC)

Accumulating evidence suggests significant biological effects caused by extremely low frequency electromagnetic fields (ELF-EMF). Although exo-endocytosis plays crucial physical and biological roles in neuronal communication, studies on how ELF-EMF regulates this process are scarce. By directly measuring calcium currents and membrane capacitance at a large

mammalian central nervous synapse, the calyx of Held, we report for the first time that ELF-EMF critically affects synaptic transmission and plasticity. Exposure to ELF-EMF for 8 to 10 days dramatically increases the calcium influx upon stimulation and facilitates all forms of vesicle endocytosis, including slow and rapid endocytosis, endocytosis overshoot and bulk endocytosis, but does not affect the RRP size and exocytosis. Exposure to ELF-EMF also potentiates PTP, a form of short-term plasticity, increasing its peak amplitude without impacting its time course. We further investigated the underlying mechanisms and found that calcium channel expression, including the P/Q, N, and R subtypes, at the presynaptic nerve terminal was enhanced, accounting for the increased calcium influx upon stimulation. Thus, we conclude that exposure to ELF-EMF facilitates vesicle endocytosis and synaptic plasticity in a calcium-dependent manner by increasing calcium channel expression at the nerve terminal.

(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) Terraneo A, Leggio L, Saladini M, Ermani M, Bonci A, Gallimberti L. Transcranial magnetic stimulation of dorsolateral prefrontal cortex reduces cocaine use: A pilot study. Eur Neuropsychopharmacol. 2015 Nov 26. pii: S0924-977X(15)00361-2. doi: 10.1016/j.euroneuro.2015.11.011. [Epub ahead of print](HU, CE, BE, MA)

Recent animal studies demonstrate that compulsive cocaine seeking strongly reduces prelimbic frontal cortex activity, while optogenetic stimulation of this brain area significantly inhibits compulsive cocaine seeking, providing a strong rationale for applying brain stimulation to reduce cocaine consumption. Thus, we employed repetitive transcranial magnetic stimulation (rTMS), to test if dorsolateral prefrontal cortex (DLPFC) stimulation might prevent cocaine use in humans. Thirty-two cocaine-addicted patients were randomly assigned to either the experimental group (rTMS) on the left DLPFC, or to a control group (pharmacological agents) during a 29-day study (Stage 1). This was followed by a 63-day follow-up (Stage 2), during which all participants were offered rTMS treatment. Amongst the patients who completed Stage 1, 16 were in the rTMS group (100%) and 13 in the control group (81%). No significant adverse events were noted. During Stage 1, there were a significantly higher number of cocaine-free urine drug tests

in the rTMS group compared to control ($p=0.004$). Craving for cocaine was also significantly lower in the rTMS group compared to the controls ($p=0.038$). Out of 13 patients who completed Stage 1 in the control group, 10 patients received rTMS treatment during Stage 2 and showed significant improvement with favorable outcomes becoming comparable to those of the rTMS group. The present preliminary findings support the safety of rTMS in cocaine-addicted patients, and suggest its potential therapeutic role for rTMS-driven PFC stimulation in reducing cocaine use, providing a strong rationale for developing larger placebo-controlled studies

(E) Todorović D, Kalauzi A, Prolić Z, Jović M, Mutavdžić D. A method for detecting the effect of magnetic field on activity changes of neuronal populations of *Morimus funereus* (Coleoptera, Cerambycidae). Bioelectromagnetics. 28(3):238-241, 2007. (AC, AS, EE)

Modification of a new method for detecting changes in the activities of neuronal population and the nearest neuron is described. Preliminary measurements of the influence of a static magnetic field (2 mT) on neuronal population activity on eight individuals of an endangered insect species *Morimus funereus* are included. Five minutes exposure produced both excitatory (5/8) and inhibitory (3/8) effect on the activity of neuronal population of *M. funereus* antennal lobe. However, when the reversibility of induced effects was quantitatively analyzed, our results showed that they were prevalingly irreversible: (7/8) for the population, (6/8) for the nearest neuron.

(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. 89(1):44-50, 2013. (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 motorbehaviour 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.

(E) Todorović D, Prolić Z, Petković B, Kalauzi A. Effects of two different waveforms of ELF MFs on bioelectrical activity of antennal lobe neurons of *Morimus funereus* (Insecta, Coleoptera). Int J Radiat Biol. 2015 Jan 14:1-35. [Epub ahead of print](AS, AE, EE)

PURPOSE: External magnetic fields (MFs) interact with organisms at all levels, including the nervous system. Bioelectrical activity of antennal lobe neurons of adult *Morimus funereus* was analyzed under the influence of extremely low frequency MF (ELF MF, 50 Hz, 2 mT) of different characteristics (exposure duration and waveform). **MATERIAL AND METHODS:** Neuronal activity (background/neuronal population and those nearest to the recording electrode) in adult longhorn beetles was registered through several phases of exposure to the sine wave and square wave MFs for 5, 10 and 15 min. **RESULTS:** The sine wave MF, regardless of the exposure duration, did not change the reversibility factor of antennal lobe neuronal activity in adult *M. funereus*. In contrast, reversibility factors of the nearest neurons were significantly changed after the exposure to square wave MF for 10 and 15 min. **CONCLUSION:** *M. funereus* individuals are sensitive to both sine wave and square wave ELF MFs (50 Hz, 2 mT) of different duration, whereby their reactions depend on the characteristics of the applied MF and specificity of each individual.

(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.

(NE) van der Mark M, Vermeulen R, Nijssen PC, Mulleners WM, Sas AM, van Laar T, Kromhout H, Huss A. Extremely low-frequency magnetic field exposure, electrical shocks and risk of Parkinson's disease. Int Arch Occup Environ Health. 2014 Jun 18. [Epub ahead of print] (HU, ND)

PURPOSE: Previous studies did not provide strong evidence for an increased Parkinson's disease (PD) risk after exposure to extremely low-frequency magnetic fields (ELF-MF), but were limited in their scope to address other exposures related to the use of electricity such as

electrical shocks. We evaluated the associations of PD with exposure to ELF-MF, electrical shocks and having worked in "electrical occupations." **METHODS:** We conducted a hospital-based case-control study, including 444 PD patients and 876 age- and sex-matched controls. Occupational histories were collected in telephone interviews and were linked to job-exposure matrices on ELF-MF exposure and on electrical shocks. In addition, questions on use of household appliances involving ELF-MF exposure, experienced electrical shocks and potential confounders were asked. **RESULTS:** No association of PD risk with any of the evaluated exposures related to electricity was observed. We did, however, observe quite consistently reduced risk estimates across the majority of the exposure categories explored. Given the results of the previous studies and the absence of any postulated mechanism, this is unlikely to represent a true protective effect of ELF-MF or electrical shocks on the occurrence of PD. **CONCLUSIONS:** The results of this study suggest that no association exists between PD and exposure to ELF-MF, electrical shocks or having worked in "electrical occupations."

(E) van Nierop LE, Slottje P, van Zandvoort MJE, de Vocht F, Kromhout 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) van Nierop LE, Slottje P, van Zandvoort M, Kromhout H. Simultaneous exposure to MRI-related static and low-frequency movement-induced time-varying magnetic fields affects neurocognitive performance: A double-blind randomized crossover study. Magn Reson Med. 2014 Sep 15. doi: 10.1002/mrm.25443. [Epub ahead of print] (AE, HU, BE)

PURPOSE: This experimental study aims to separate neurocognitive effects resulting from exposure to static magnetic stray fields (SMF) alone and the combination of SMF and low-frequency movement-induced time-varying magnetic fields (TVMF) using a 7 Tesla (T) MRI scanner in stand-by mode. METHODS: In a double-blind randomized crossover experiment, 36 healthy volunteers underwent four sessions, two exposed conditions, and two corresponding

sham conditions. The exposure conditions were in front of the scanner bore and consisted of 1.0 T SMF with or without 2.4 T/s TVMF, induced by standardized head movements before each of the five neurocognitive tasks. These specific tasks were selected because previous experiments showed negative effects of SMF + TVMF exposure on test performance. RESULTS: Exposure to SMF in combination with TVMF decreased verbal memory performance significantly and changed visual acuity. Similarly, attention and concentration were negatively affected with borderline significance. Exposure to SMF only did not have significant effects on the performance on any of the tasks. CONCLUSION: Neurocognitive effects were only observed when simultaneously exposed to SMF and TVMF from a 7 T MRI scanner. Therefore, exposure to TVMF seems essential in eliciting the neurocognitive effects in our present study and, presumably, previous experiments.

Vanderstraeten J, Burda H, Verschaeve L, De Brouwer C. Could Magnetic Fields Affect the Circadian Clock Function of Cryptochromes? Testing the Basic Premise of the Cryptochrome Hypothesis (ELF Magnetic Fields). Health Phys. 109(1):84-89, 2015. (opinion)

It has been suggested that weak 50/60 Hz [extremely low frequency (ELF)] magnetic fields (MF) could affect circadian biorhythms by disrupting the clock function of cryptochromes (the "cryptochrome hypothesis," currently under study). That hypothesis is based on the premise that weak (Earth strength) static magnetic fields affect the redox balance of cryptochromes, thus possibly their signaling state as well. An appropriate method for testing this postulate could be real time or short-term study of the circadian clock function of retinal cryptochromes under exposure to the static field intensities that elicit the largest redox changes (maximal "low field" and "high field" effects, respectively) compared to zero field. Positive results might encourage further study of the cryptochrome hypothesis itself. However, they would indicate the need for performing a similar study, this time comparing the effects of only slight intensity changes (low field range) in order to explore the possible role of the proximity of metal structures and furniture as a confounder under the cryptochrome hypothesis.

(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 gradients 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 CX, Hilburn IA, Wu DA, Mizuhara Y, Cousté CP, Abrahams JNH, Bernstein SE, Matani A, Shimojo S, Kirschvink JL. Transduction of the Geomagnetic Field as Evidenced from alpha-Band Activity in the Human Brain. eNeuro. 6(2). pii: ENEURO.0483-18.2019. (HU, AE, EE)

Magnetoreception, the perception of the geomagnetic field, is a sensory modality well-established across all major groups of vertebrates and some invertebrates, but its presence in humans has been tested rarely, yielding inconclusive results. We report here a strong, specific human brain response to ecologically-relevant rotations of Earth-strength magnetic fields. Following geomagnetic stimulation, a drop in amplitude of

electroencephalography (EEG) alpha-oscillations (8-13 Hz) occurred in a repeatable manner. Termed alpha-event-related desynchronization (alpha-ERD), such a response has been associated previously with sensory and cognitive processing of external stimuli including vision, auditory and somatosensory cues. Alpha-ERD in response to the geomagnetic field was triggered only by horizontal rotations when the static vertical magnetic field was directed downwards, as it is in the Northern Hemisphere; no brain responses were elicited by the same horizontal rotations when the static vertical component was directed upwards. This implicates a biological response tuned to the ecology of the local human population, rather than a generic physical effect. Biophysical tests showed that the neural response was sensitive to static components of the magnetic field. This rules out all forms of electrical induction (including artifacts from the electrodes) which are determined solely on dynamic components of the field. The neural response was also sensitive to the polarity of the magnetic field. This rules out free-radical "quantum compass" mechanisms like the cryptochrome hypothesis, which can detect only axial alignment. Ferromagnetism remains a viable biophysical mechanism for sensory transduction and provides a basis to start the behavioral exploration of human magnetoreception.

(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, ND, MA)

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) Wu CL, Fu TF, Chiang MH, Chang YW, Her JL, Wu T. Magnetoreception Regulates Male Courtship Activity in Drosophila. PLoS One. 2016 May 19;11(5):e0155942. (AS, CE, BE)

The possible neurological and biophysical effects of magnetic fields on animals is an area of active study. Here, we report that courtship activity of male *Drosophila* increases in a magnetic field and that this effect is regulated by the blue light-dependent photoreceptor cryptochrome (CRY). Naïve male flies exhibited significantly increased courtship activities when they were exposed to a ≥ 20 -Gauss static magnetic field, compared with their behavior in the natural environment (0 Gauss). CRY-deficient flies, *cryb* and *crym*, did not show an increased courtship index in a magnetic field. RNAi-mediated knockdown of *cry* in *cry*-GAL4-positive neurons disrupted the increased male

courtship activity in a magnetic field. Genetically expressing cry under the control of cry-GAL4 in the CRY-deficient flies restored the increase in male courtship index that occurred in a magnetic field. Interestingly, artificially activating cry-GAL4-expressing neurons, which include large ventral lateral neurons and small ventral lateral neurons, via expression of thermosensitive cation channel dTrpA1, also increased the male courtship index. This enhancement was abolished by the addition of the cry-GAL80 transgene. Our results highlight the phenomenon of increased male courtship activity caused by a magnetic field through CRY-dependent magnetic sensation in CRY expression neurons in Drosophila.

(E) Wyszowska J, Shepherd S, Sharkh S, Jackson CW, Newland PL. Exposure to extremely low frequency electromagnetic fields alters the behaviour, physiology and stress protein levels of desert locusts. Sci Rep. 2016 Nov 3;6:36413. doi: 10.1038/srep36413.(AS, AE, BE, CC, EE)

Electromagnetic fields (EMFs) are present throughout the modern world and are derived from many man-made sources including overhead transmission lines. The risks of extremely-low frequency (ELF) electromagnetic fields are particularly poorly understood especially at high field strengths as they are rarely encountered at ground level. Flying insects, however, can approach close to high field strength transmission lines prompting the question as to how these high levels of exposure affect behaviour and physiology. Here we utilise the accessible nervous system of the locust to ask how exposure to high levels of ELF EMF impact at multiple levels. We show that exposure to ELF EMFs above 4 mT leads to reduced walking. Moreover, intracellular recordings from an identified motor neuron, the fast extensor tibiae motor neuron, show increased spike latency and a broadening of its spike in exposed animals. In addition, hind leg kick force, produced by stimulating the extensor tibiae muscle, was reduced following exposure, while stress-protein levels (Hsp70) increased. Together these results suggest that ELF EMF exposure has the capacity to cause dramatic effects from behaviour to physiology and protein expression, and this study lays the foundation to explore the ecological significance of these effects in other flying insects.

(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) Yang G, Ren Z, Mei YA. Exposure to 50 Hz magnetic field modulates GABA_A currents in cerebellar granule neurons through an EP receptor-mediated PKC pathway. J Cell Mol Med. 2015 Jul 14. doi: 10.1111/jcmm.12626. [Epub ahead of print](CS, AE, FC, CC)

Previous work from both our lab and others have indicated that exposure to 50 Hz magnetic fields (ELF-MF) was able to modify ion channel functions. However, very few studies have investigated the effects of MF on γ -aminobutyric acid (GABA) type A receptors (GABA_A Rs) channel functioning, which are fundamental to overall neuronal excitability. Here, our major goal is to reveal the potential effects of ELF-MF on GABA_A Rs activity in rat cerebellar granule neurons (CGNs). Our results indicated that exposing CGNs to 1 mT ELF-MF for 60 min. significantly increased GABA_A R currents without modifying sensitivity to GABA. However, activation of PKA by db-cAMP failed to do so, but led to a slight decrease instead. On the other hand, PKC activation or inhibition by PMA or Bis and Docosahexaenoic acid (DHA) mimicked or eliminated the field-induced-increase of GABA_A R currents. Western blot analysis indicated that the intracellular levels of phosphorylated PKC (pPKC) were significantly elevated after 60 min. of ELF-MF exposure, which was subsequently blocked by application of DHA or EP1 receptor-specific (prostaglandin E receptor 1) antagonist (SC19220), but not by EP2-EP4 receptor-specific antagonists. SC19220 also significantly inhibited the ELF-MF-induced elevation on GABA_A R currents. Together, these data obviously demonstrated for the first time that neuronal GABA_A currents are significantly increased by ELF-MF exposure, and also suggest that these effects are mediated via an EP1 receptor-mediated PKC pathway. Future work will focus on a more comprehensive analysis of the physiological and/or pathological consequences of these effects.

(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.

(E) Yin C, Luo X, Duan Y, Duan W, Zhang H, He Y, Sun G, Sun X. Neuroprotective effects of lotus seedpod procyanidins on extremely low frequency electromagnetic field-induced neurotoxicity in primary cultured hippocampal neurons. Biomed Pharmacother. 82:628-639, 2016. (CS, AE, OX, MA)

The present study investigated the protective effects of lotus seedpod procyanidins (LSPCs) on extremely low frequency electromagnetic field (ELF-EMF)-induced neurotoxicity in primary cultured rat hippocampal neurons and the underlying molecular mechanism. The results of MTT, morphological observation, superoxide dismutase (SOD) and malondialdehyde (MDA) assays showed that compared with control, incubating neurons under ELF-EMF exposure significantly decreased cell viability and increased the number of apoptotic cells, whereas LSPCs evidently protected the hippocampal neurons against ELF-EMF-induced cell damage. Moreover, a certain concentration of LSPCs inhibited the elevation of intracellular reactive oxygen species (ROS) and Ca(2+) level, as well as prevented the disruption of mitochondrial membrane potential induced by ELF-EMF exposure. In addition, supplementation with LSPCs could alleviate DNA damage, block cell cycle arrest at S phase, and inhibit apoptosis and necrosis of hippocampal neurons under ELF-EMF exposure. Further study demonstrated that LSPCs up-regulated the activations of Bcl-2, Bcl-xl proteins and suppressed the expressions of Bad, Bax proteins caused by ELF-EMF exposure. In conclusion, these findings revealed that LSPCs protected against ELF-EMF-induced neurotoxicity through inhibiting oxidative stress and mitochondrial apoptotic pathway.

(E) Zeng Y, Shen Y , Hong L, Chen Y, Shi X, Zeng Q, Yu P. Effects of Single and Repeated Exposure to a 50-Hz 2-mT Electromagnetic Field on Primary Cultured Hippocampal Neurons. Neurosci Bull. 33(3):299-306, 2017. (CS, AE, CE, OX, FC)

The prevalence of domestic and industrial electrical appliances has raised concerns about the health risk of extremely low-frequency magnetic fields (ELF-MFs). At present, the effects of ELF-MFs on the central nervous system are still highly controversial, and few studies have investigated its effects on cultured neurons. Here, we evaluated the biological effects of different patterns of ELF-MF exposure on primary cultured

hippocampal neurons in terms of viability, apoptosis, genomic instability, and oxidative stress. The results showed that repeated exposure to 50-Hz 2-mT ELF-MF for 8 h per day after different times in culture decreased the viability and increased the production of intracellular reactive oxidative species in hippocampal neurons. The mechanism was potentially related to the up-regulation of Nox2 expression. Moreover, none of the repeated exposure patterns had significant effects on DNA damage, apoptosis, or autophagy, which suggested that ELF-MF exposure has no severe biological consequences in cultured hippocampal neurons.

(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 Al treatment groups (Al and MF+Al 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 Al treatment showed no significant effect on AD pathogenesis. ELF-MF+Al treatment induced no more damage than Al 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 Al overload.

(E) Zhang H, Dai Y, Cheng Y, He Y, Manyakara Z, Duan Y, Sun G, Sun X. Influence of extremely low frequency magnetic fields on Ca²⁺ signaling and double messenger system in mice hippocampus and reversal function of procyanidins extracted from lotus seedpod. Bioelectromagnetics. 38(6):436-446, 2017. (AS, CE, CC)

This research investigated the influence of extremely low frequency magnetic fields (ELF-MF; 50 Hz, 8 mT, 4 h per day, for 28 days) on calcium ion signaling and the double messenger system in the hippocampus of mice. Messengers that were studied included: G-protein, Ins(1,4,5)P₃ (IP₃), diacylglycerol (DAG), cAMP-dependent protein kinase (PKA), and Ca²⁺-dependent protein kinase C (PKC). The results showed that ELF-MF caused an increase in the levels of Gi protein, IP₃, DAG, PKA and PKC beta, calcium and calmodulin-dependent protein phosphatase calcineurin (PP2B), and intracellular Ca²⁺ content, and a decrease in calcium/calmodulin-dependent protein kinase II (CaMK II) and PKC alpha. In addition, ELF-MF exposure decreased the level of brain-derived neurotrophic factor (BDNF), which played a key role in hippocampal

neuronal cell death. However, oral administration of procyanidins from lotus seedpod (LSPCs) (especially 90 mg kg⁻¹) significantly recovered these changes, and nearly reached normal levels. All these showed that LSPCs may mediate calcium signal and double messenger system through Ca²⁺/CaMK II/CREB/BDNF and DG/PKC/MAPK signaling pathways to reverse the alteration caused by ELF-MF.

(NE) Zhang Y, Liu X, Zhang J, Li N. Short term effects of extremely low frequency electromagnetic fields exposure on Alzheimer's disease in rats. Int J Radiat Biol. 2014 Aug 13:1-35. [Epub ahead of print] (AS, CE, BE, ND)

Purpose: With the development and widespread use of electromagnetic field (EMF) technology, recent studies are focusing on the effects of EMF on human health. Recently, extremely low frequency electromagnetic fields (ELF-EMF) have been studied with great interest due to their possible effects on Alzheimer's disease (AD). The objective of the present study was to investigate the interaction between ELF-EMF exposure and memory impairment in rats. **Materials and methods:** Twenty healthy male Sprague Dawley (SD) rats were randomly divided into two groups (n=10). Animals were exposed to 100μT/50Hz ELF-EMF or subjected to sham exposure when 12 weeks old. After 12 weeks, Morris water maze (MWM) was used to test the changes in cognitive and memory ability. Amyloid-beta (Aβ) content in cortex, hippocampus and plasma were measured by ELISA assays. The morphology of neuron was detected by HE staining. **Results:** After exposure, the body weight of rats showed no difference compared with control group. The application of ELF-EMF did not induce any cognitive and memory impairment compared with sham exposure group. The determination of Aβ showed no significant change between two groups. And there was no histological change in ELF-EMF exposure group. **Conclusion:** The present study indicated that short term exposure of 100μT/50Hz ELF-EMF had no effects on cognition and memory of rats, and did not alter the expression of Aβ and the neuron morphology. However, more comprehensive studies are still required to elucidate the possible effects and underlying mechanisms of ELF-EMF exposure on living organisms.

(NE) Zhang Y, Liu X, Zhang J, Li N. Short-term effects of extremely low frequency electromagnetic fields exposure on Alzheimer's disease in rats. Int J Radiat Biol. 91(1):28-34, 2015. (AS, CE, BE, MC, CC)

PURPOSE: With the development and widespread use of electromagnetic field (EMF) technology, recent studies are focusing on the effects of EMF on human health. Recently, extremely low frequency electromagnetic fields (ELF-EMF) have been studied with great interest due to their possible effects on Alzheimer's disease (AD). The objective of the present study was to investigate the interaction between ELF-EMF exposure and memory impairment in rats. **MATERIALS AND METHODS:** Twenty healthy male Sprague Dawley (SD) rats were randomly divided into two groups (n = 10). Animals were exposed to 100 μT/50 Hz ELF-EMF or subjected to sham exposure when 12 weeks old. After 12 weeks, the Morris water maze (MWM) was used to test the changes in cognitive and memory ability. Amyloid-beta (Aβ) content in cortex, hippocampus and plasma were measured by ELISA assays. The morphology of neuron was detected by H&E staining. **RESULTS:** After exposure, the body weight of rats

showed no difference compared with the control group. The application of ELF-EMF did not induce any cognitive and memory impairment compared with the sham-exposure group. The determination of A β showed no significant change between the two groups, and there was no histological change in ELF-EMF exposure group. **CONCLUSION:** The present study indicated that short-term exposure of 100 μ T/50 Hz ELF-EMF had no effects on cognition and memory of rats, and did not alter the expression of A β and the neuron morphology. However, more comprehensive studies are still required to elucidate the possible effects and underlying mechanisms of ELF-EMF exposure on living organisms.

(E) Zhao QR, Lu JM, Yao JJ, Zhang ZY, Ling C, Mei YA. Neuritin reverses deficits in murine novel object associative recognition memory caused by exposure to extremely low-frequency (50 Hz) electromagnetic fields. Sci Rep. 2015 Jul 3;5:11768. doi: 10.1038/srep11768. (AS, CE, BE, MC)

Animal studies have shown that electromagnetic field exposure may interfere with the activity of brain cells, thereby generating behavioral and cognitive disturbances. However, the underlying mechanisms and possible preventions are still unknown. In this study, we used a mouse model to examine the effects of exposure to extremely low-frequency (50 Hz) electromagnetic fields (ELF MFs) on a recognition memory task and morphological changes of hippocampal neurons. The data showed that ELF MFs exposure (1 mT, 12 h/day) induced a time-dependent deficit in novel object associative recognition memory and also decreased hippocampal dendritic spine density. This effect was observed without corresponding changes in spontaneous locomotor activity and was transient, which has only been seen after exposing mice to ELF MFs for 7-10 days. The over-expression of hippocampal neuritin, an activity-dependent neurotrophic factor, using an adeno-associated virus (AAV) vector significantly increased the neuritin level and dendritic spine density. This increase was paralleled with ELF MFs exposure-induced deficits in recognition memory and reductions of dendritic spine density. Collectively, our study provides evidence for the association between ELF MFs exposure, impairment of recognition memory, and resulting changes in hippocampal dendritic spine density. Neuritin prevented this ELF MFs-exposure-induced effect by increasing the hippocampal spine density.

(E) Zhen J, Qian Y, Fu J, Su R, An H, Wang W, Zheng Y, Wang X. Deep Brain Magnetic Stimulation Promotes Neurogenesis and Restores Cholinergic Activity in a Transgenic Mouse Model of Alzheimer's Disease. Front Neural Circuits. 11:48, 2017. (AS, CE, MC, ND, MA)

Alzheimer's disease (AD) is characterized by progressive decline of memory and cognitive functions. Deep magnetic stimulation (DMS), a noninvasive and nonpharmacological brain stimulation, has been reported to alleviate stress-related cognitive impairment in neuropsychiatric disorders. Our previous study also discovered the preventive effect of DMS on cognitive decline in an AD mouse model. However, the underlying mechanism must be explored further. In this study, we investigated the effect of DMS on spatial learning and memory functions, neurogenesis in the dentate gyrus (DG), as well as expression and activity of the cholinergic system in a transgenic mouse

model of AD (5XFAD). Administration of DMS effectively improved performance in spatial learning and memory of 5XFAD mice. Furthermore, neurogenesis in the hippocampal DG of DMS-treated 5XFAD mice was clearly enhanced. In addition, DMS significantly raised the level of acetylcholine and prevented the increase in acetylcholinesterase activity as well as the decrease in acetyltransferase activity in the hippocampus of 5XFAD mice. These findings indicate that DMS may be a promising noninvasive tool for treatment and prevention of AD cognitive impairment by promoting neurogenesis and enhancing cholinergic system function.

(E) Zheng Y, Ma W, Dong L, Dou JR, Gao Y, Xue J. Influence of the on-line ELF-EMF stimulation on the electrophysiological properties of the rat hippocampal CA1 neurons in vitro. Rev Sci Instrum. 88(10):105106, 2017. (CS, AE, CC, EE)

The extremely low frequency electromagnetic fields (ELF-EMFs) have been shown to have an environmentally negative effect on humans' health; however, its treatment effect is beneficial for patients suffering from neurological disorders. Despite this success, the application of ELF-EMF has exceeded in the understanding of its internal mechanism. Recently, it was found that on-line magnetic stimulation may offer advantages over off-line magnetic exposure and has proven to be effective in activating the prefrontal cortex pyramidal neurons in vitro. Here, we perform computational simulations of the stimulation coils in COMSOL modeling to describe the uniformity of the distribution of the on-line magnetic field. Interestingly, the modeling data and actual measurements showed that the densities of the magnetic flux that was generated by the on-line stimulation coils were similar. The on-line magnetic stimulator induced sodium channel currents as well as field excitatory postsynaptic potentials of the rat hippocampal CA1 neurons and successfully demonstrated its extensive applications to activate neuronal tissue. These findings further raise the possibility that the instrument of on-line magnetic stimulation may be an effective alternative for studies in the field of bioelectromagnetics.

(E) Zheng Y, Dou JR, Gao Y, Dong L, Li G. Effects of 15 Hz square wave magnetic fields on the voltage-gated sodium and potassium channels in prefrontal cortex pyramidal neurons. Int J Radiat Biol. 93(4):449-455, 2017. (CS, AE, FC)

PURPOSE: Although magnetic fields have significant effects on neurons, little is known about the mechanisms behind their effects. The present study aimed to measure the effects of magnetic fields on ion channels in cortical pyramidal neurons. **MATERIALS AND METHODS:** Cortical pyramidal neurons of Kunming mice were isolated and then subjected to 15 Hz, 1 mT square wave (duty ratio 50%) magnetic fields stimulation. Sodium currents (I_{Na}), transient potassium currents (I_A) and delayed rectifier potassium currents (I_K) were recorded by whole-cell patch clamp method. **RESULTS:** We found that magnetic field exposure depressed channel current densities, and altered the activation kinetics of sodium and potassium channels. The inactivation properties of I_{Na} and I_A were also altered. **CONCLUSION:** Magnetic field exposure alters ion channel function in neurons. It is likely that the structures of sodium and potassium channels were influenced by the applied field. Sialic acid, which is an important component of the channels, could be the molecule responsible for the reported results.

(E) Zheng Y, Ma XX, Dong L, Gao Y, Tian L. Effects of single- and hybrid-frequency extremely low-frequency electromagnetic field stimulations on long-term potentiation in the hippocampal Schaffer collateral pathway. Int J Radiat Biol. 2019 May 29;1-21. (CS, AE, EE)

Purpose: To study the different effects of single- and hybrid-frequency magnetic fields on long-term potentiation (LTP) in synaptic plasticity. **Materials and methods:** Based on the online electromagnetic field stimulation system and field excitatory postsynaptic potentials (fEPSPs) recording system, we applied four different single- and hybrid-frequency magnetic fields with an intensity of 1 mT to the Schaffer collateral (CA1) pathway of rat hippocampal slices in vitro.

Results: The amplitude of fEPSPs decreased significantly under both single- and hybrid-frequency magnetic stimulation. Lower single-frequency magnetic stimulation on LTP had a greater regulating effect, while the regulating effect among four different hybrid-frequency extremely low-frequency electromagnetic fields (ELF-EMFs) stimulations on LTP showed no significant differences. **Conclusion:** Single-frequency magnetic stimulation produces more significant regulatory effects, and the lower the frequency, the more significant the regulatory effect. The effect of hybrid-frequency magnetic stimulation in each group was similar, and there was no significant difference between each group. The 15-Hz single-frequency magnetic stimulation group showed the most significant regulatory effect, but once it was mixed with other higher frequency magnetic stimulation, its regulation effect was significantly weakened.

(E) Zheng Y, Cheng J, Dong L, Ma X, Kong Q. Effects of exposure to extremely low frequency electromagnetic fields on hippocampal long-term potentiation in hippocampal CA1 region. Biochem Biophys Res Commun. 2019 Jul 31. pii: S0006-291X(19)31439-1. doi: 10.1016/j.bbrc.2019.07.085. (CS, AE, EE)

Exposure to environmental electromagnetic fields, especially to the extremely low-frequency (ELF < 300 Hz) electromagnetic fields (EMFs) might produce modulation effects on neuronal activity. Long-term changes in synaptic plasticity such as long-term potentiation (LTP) involved in learning and memory may have contributions to a number of neurological diseases. However, the modulation effects of ELF-EMFs on LTP are not yet fully understood. In our present study, we aimed to evaluate the effects of exposure to ELF-EMFs on LTP in hippocampal CA1 region in rats. Hippocampal slices were exposed to magnetic fields generated by sXcELF system with different frequencies (15, 50, and 100 Hz [Hz]), intensities (0.5, 1, and 2 mT [mT]), and duration (10 s [s], 20 s, 40 s, 60 s, and 5 min), then the baseline signal recordings for 20 min and the evoked field excitatory postsynaptic potentials (fEPSPs) were recorded. We found that the LTP amplitudes decreased after magnetic field exposure, and the LTP amplitudes decreased in proportion to exposure doses and durations, suggesting ELF-EMFs may have dose and duration-dependent inhibition effects. Among multiple exposure duration and doses combinations, upon 5 min magnetic field exposure, 15 Hz/2 mT maximally inhibited LTP. Under 15 Hz/2 mT ELF-EMFs, LTP amplitude decreases in proportion to the length of exposure durations within 5 min time frame. Our findings illustrated the potential effects of ELF-EMFs on synaptic plasticity and will lead to better understanding of the influence on learning and memory.

(E) Zuo H, Liu X, Wang D, Li Y, Xu X, Peng R, Song T. RKIP-Mediated NF- κ B Signaling is involved in ELF-MF-mediated improvement in AD rat. Int J Med Sci. 15(14):1658-1666, 2018. (AS, CE, C, BE, MA, ND)

In a previous study, we reported the positive effects of extremely low frequency electromagnetic field (ELF-MF) exposure on Alzheimer's disease (AD) rats; however, the underlying mechanism remains unclear. In addition, we found that Raf-1 kinase inhibitor protein (RKIP) was downregulated by microwave exposure in the rat hippocampus. Our hypothesis was that RKIP-mediated NF- κ B pathway signaling is involved in the effect of ELF-MF on the AD rat. In this study, D-galactose intraperitoneal (50 mg/kg/d for 42 d) and A β ₂₅₋₃₅ hippocampal (5 μ L/unilateral, bilateral, single-dose) injection were implemented to establish an AD rat model. Animals were exposed to 50 Hz and 400 μ T ELF-MF for 60 continuous days. The spatial memory ability of the rat was then tested using the Morris water maze. Protein expression and interaction were detected by western blotting and co-immunoprecipitation for RKIP-mediated NF- κ B pathway factors. The results showed that ELF-MF exposure partially improved the cognitive disorder, upregulated the levels of RKIP, TAK1, and the RKIP/TAK1 interaction, but downregulated p-IKK levels in AD rats. These results indicated that RKIP-mediated NF- κ B pathway signaling plays an important role in the ELF-MF exposure-mediated improvements in the AD rat. Our study suggested that ELF-MF exposure might have a potential therapeutic value for AD. Further in depth studies are required in the future.