DOI: 10.5567/pharmacologia.2014.267.271

Whole-Body Exposures to LTP-Promoting Magnetic Fields Facilitates Inhibitory Learning: Comparisons with Oral Alanine and Arginine Consumption

[1]Lucas W.E. Tessaro, [1]Quoc Hao Mach, [2]Jeffrey F. Rocca, [2]Lauren R. Morrison, [1]Ryan Y. Burke and [1,2]Michael A. Persinger

[1]Behavioural Neuroscience Program,

[2]Department of Psychology, Laurentian University, Sudbury, Ontario, P3E 2C6, Canada

ABSTRACT

Background: Pharmacological phenomena are based upon spatial interactions of molecules and biological targets. L-arginine and L-alanine are both naturally occurring amino acids and play a role in neurotransmission. In addition, specific electromagnetic fields can mimic the neurophysiological firing patterns of the hippocampus. This study was conducted to assess possible synergisms between classical neurotransmission and applied magnetic fields using inhibitory operant behaviour as an indicator of pharmacological efficacy. **Materials and Methods:** Rats were administered L-arginine or L-alanine through their water supplies for two weeks prior to operant training. Upon successful training, animals were tested by the differential reinforcement of low rates of responding (DRL)-6 sec task. During the DRL task, animals were exposed to either sham or LTP magnetic field (300 nT) conditions. **Results:** The group that received the treatment of L-alanine without the field increased its ability to inhibit behaviour by 10%, which was roughly equivalent to the group that received the magnetic field treatment alone. The L-arginine treated group did not differ from controls. When the L-alanine and magnetic field treatments were combined, the facilitation effects were eliminated. **Conclusion:** These results are an indication that the L-alanine and electromagnetic field applications act through the same biochemical pathways or receptor effects, as suggested by the absence of synergism.

Key words: LTP magnetic fields, alanine, arginine, DRL schedules, operant learning, rats, inhibitory learning

Pharmacologia 5 (7): 267-271, 2014

INTRODUCTION

The bases of pharmacological phenomena assume interactions between the three-dimensional space and configurations of both the agent and target molecules. On the other hand, the non-particle effects from applied magnetic fields are assumed to be related to their similarity to physiological temporal patterns (Martin *et al*., 2004). Although traditional arguments assume there are no "real" physical components (Binhi and Rubin, 2007) to weak magnetic fields that can exceed the required thermodynamic energies (Cifra *et al*., 2011) has emphasized that the "kT" problem is primarily for systems near equilibrium whereas living systems usually are not.

The assumption of Martin *et al*. (2004) that the influence of EMFs may be determined by their temporal structure was supported by Hu *et al*. (2010). Only whole body exposures to a particular 3-dimensiononal rotating, physiologically-patterned magnetic field prevented or reduced growth of tumours from injected melanoma cells. Many investigations during the last 15 years have demonstrated repeatedly the emergence of biological effects from EMF exposures with specific spatial-temporal configurations (Shahidain *et al*., 2001; Port *et al*., 2003; St-Pierre *et al*., 2008; Santini *et al*., 2009; Martinez-Samano *et al*., 2010).

Both facilitation and inhibitory effects from EMFs at the level of the cell have been thoroughly reviewed by (Santini *et al*., 2009). The conclusion was that, mechanisms involve biochemical pathways as numerous as the fields employed. Investigations into the differences between physiologically-patterned EMFs and those which could be considered "artificial" (sine waves) have demonstrated that intensities required for effects are lower in the former as compared to the latter (McKay *et al*., 2000). Lagace *et al*. (2009) has demonstrated that patterns of magnetic fields based on the Long-term Potentiation (LTP) process were most effective in regions ofthe brain in which LTP is

Corresponding Author: Michael A. Persinger, Behavioural Neuroscience Program, Laurentian University, Sudbury, Ontario, P3E 2C6, Canada Tel: 01-705-6754824 Fax: 01-705-6713884

endogenous. Magnetic fields are expected to penetrate diffusely throughout the entire brain. However the development of subsequent LTP in a given region is expected to be greatest in those regions whose natural spatial-temporal patterns are most congruent to the applied field. This concept is homologous to the action of hormones on specific target tissues even though all tissues within the body receive the same hormonal stimuli.

A recent study by Mach and Persinger (2009) demonstrated that an LTP-EMF significantly altered the processes associated with learning under whole-body conditions. Application of the field before training in a water maze severely interfered with the rats' acquisition and memory for the task. The magnitude of the interference was similar to saturation of the hippocampus by direct stimulation (Moser *et al*., 1998). Mach and Persinger (2009) concluded that rats with history of lithium/pilocarpine-induced seizures and subsequent brain damage, who typically cannot acquire a differential reinforcement of low rates of responding (DRL) schedule (Harrigan *et al*., 1990), improved significantly if they were exposed to the LTP-EMF during the task. It has also been shown that the DRL paradigm is an optimal measure for the processes associated with learning an inhibitory response and its associated behaviors (Persinger *et al*., 1976).

Specific forms of arginine have been shown to prevent the induction of LTP through the inhibition of nitric oxide synthase (Nowicky and Bindman, 1993), thereby suggesting the necessity for nitric oxide (NO) in LTP (Schuman and Madison, 1991; Santini *et al*., 2009). Whissell and Persinger (2007) had previously shown arginine delivered through the water supply could act as an NO donor, which is involved with the behavioral changes associated with exposed to weak theta-frequency EMFs. Alanine was chosen as it is the simplest amino acid that contains a methyl group which is known in general to enhance the efficacy of a compound.

The present study was designed to compare the effects of the LTP-EMF with two natural compounds (amino acids) known to affect LTP induction, alanine and arginine, delivered through the water supply. The study was thus conducted to investigate the possible interactions or synergisms between the applied, physiologically-patterned electromagnetic field and amino acids upon inhibitory behavior (Yun *et al*., 2002).

MATERIALS AND METHODS

Subjects: A total of 36 male Wistar albino rats were obtained from Charles River (Quebec). The rats were 90 days of age at the beginning of the experiment. Experimental protocol was submitted and approved by the Animal Care Committee of Laurentian University.

Training procedure: Rats were maintained in single plastic cages with corncob bedding within a temperature a light (12:12) controlled setting. Each rat was randomly assigned to one of six groups. There were given *ad libitum* availability to either tap water or 1 g L^{-1} of L-arginine or 1 g L^{-1} of L-alanine during the experiment. The rats were partially deprived of food for about a week until their body weights stabilized at 90% of their free feed weight. Fluids were refreshed once every 3 or 4 days.

After 90% body weight had been obtained, each rat was lever trained in a modified operant chamber programmed for CFR (continuous reinforcement, one response for every reward) for between one to 3 days until it achieved a criterion of 50 responses in one of the 30 min session. The rewards were Noyes food pellets. On each side of the 26×26×26 cm Plexiglas cage a steel nail (21 cm long) wrapped with wire was attached so that the magnetic field generated between the nails was maximum within the center of the chamber (Mach and Persinger, 2009).

Magnetic field exposures: Half the numbers of rats from each chemical condition was randomly assigned to the magnetic field or sham field group. The magnetic field was generated by converting a column of 225 numbers between 127 and 256 (positive polarity) to between 0 and + 5 V as reported by Mach and Persinger (2009). Specialized software converted the numbers through a digital to analogue converter to the appropriate current. The point durations, the time voltage of a particular value generated through the circuit, was 1 msec. The pattern was one 5 msec pulse followed 150 msec later by four more 5 msec pulses each separated by 10 msec after Rose *et al*. (1988) who applied the same pattern as electric current to hippocampal slices to evoke LTP. This pattern, which was 270 msec in duration, was presented once every 4 sec (total cycle 4.4 sec when the computer's port-time latency for each point is included) for the duration of the session. The strength of the field within the exposure area ranged between 70 and 425 nT (0.7 to 4.34 mG). The median level was 300 nT (3 mG).

Operant schedule: Each rat was tested by one of several experimenters for 30 min day^{-1} for 6 successive days. Due to the numbers of rats and limited time for testing, the experiment was completed in 6 separate blocks with the same number of rats from each of the six conditions in each block. The DRL 6 sec schedule was programmed by a computer that also recorded the total numbers of responses per daily 30 min session as well as the numbers of rewards (Noyes food pellets). The numbers of responses within successive IRT (inter-response time), 1 sec bins between 0 and 30 sec were also recorded by computer. IRTs greater than 30 sec were recorded within the 30-31 sec bin.

The experimental design was a three way analysis of variance with two between subject levels (amino acid in the water supply and magnetic field treatment) and one within subject level (days). These analyses were completed for the total numbers of responses and the proportion of correct responses (number of rewards divided by responses multiplied by 100). An additional four way analysis of variance with the additional within subject level (per day) for the numbers of responses within the first 10, 1 sec IRT bins (other bins contained too few responses) was completed.

Statistical analyses: All analyses involved PC SPSS-16. The basic experimental design was a three way analysis of variance with two between (alanine, arginine, or water; sham field vs. LTP patterned field) and one within (days) subject design. *Post hoc* analyses were primarily Tukey's, paired t-tests or combinations thereof. The dependent variables included total responses rewarded as well as IRTs. Omega-squared estimates were calculated to reflect the effect size.

RESULTS

Operant inhibitory learning as influenced by chemical and electromagnetic treatments: It has previously been shown that whole-body exposure to an LTP-EMF significantly enhances the learning of an operant inhibitory DRL task (Mach and Persinger, 2009) in brain damaged rats. In this expanded paradigm, the question was whether or not the externally applied LTP magnetic fields could interact with orally consumed amino acids. The operant task (DRL, differential reinforcement of low rates of responding) required the rats to learn to delay bar presses that would successfully lead to the reward (a food pellet). The proportions of rewarded responses to non-rewarded responses were then subjected to a three-way analysis of variance with two between (chemical treatment; magnetic field configuration) and one within (days) subject levels. The analysis demonstrated a statistically significant [$F(2, 26) = 5.02$, $p < .01$] interaction between the chemical treatments and magnetic field conditions which accounted for 28% of the variance. Subsequent *post hoc* analysis, as shown in Fig. 1, indicated that the primary source of this variance was the enhanced responses of the LTP-EMF group and the alanine-no field group, as compared with the water and sham field group. The main effects for chemical [$F(2, 26) = 2.60$, $p > .05$] and field [$F(1, 26) = 1.15$, $p > .05$] treatments were not significant statistically.

Enhanced learning rates resultant from electromagnetic stimulation: When the improvements of the two between-subjects variables

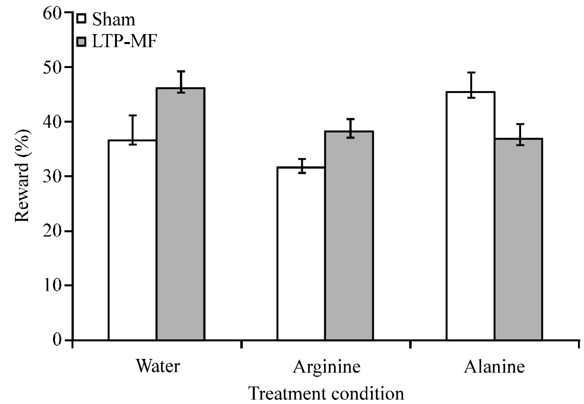

Fig. 1: Means and standard errors of the mean (bars) for percentages of responses rewarded during the DRL-6 sec schedule for rats consuming tap water or water containing arginine or alanine and then whole body exposed to the sham or LTP-patterned magnetic field

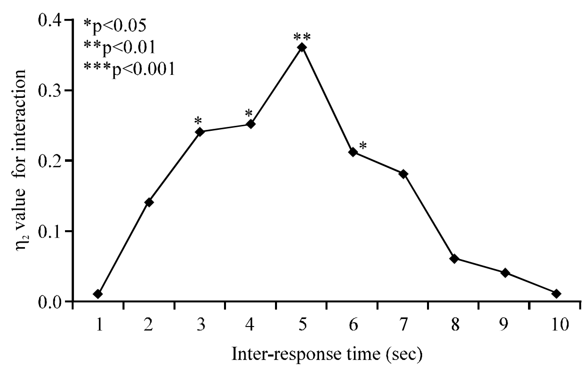

Fig. 2: Effective size of the amount of variance explained for each of the 1 secIRT (inter-response) bins. This illustrates that the frequency of responses were significantly different based on condition and field exposure at the 4 and 5 sec bins

were considered with respect to daily trials the learning rates of the magnetic field group was immediately apparent. This was supported by the significant interaction between the magnetic field group and days [$F(5,130) = 3.73$, $p < .01$] which accounted for 12% of the total variance. *Post hoc* analyses indicated that the primary source of this variance was those rats which received the LTP-EMF treatment (M=33%, SEM=3%) after 4 days compared to the sham-field group (M=45%, SEM=3%). The results of the analyses for the numbers of Inter-Response Time (IRTs) within specific one-second bin times for bin 1 to 10 for the strength of the interaction between field presence and chemical treatment are shown in Fig. 2. The effect sizes for the interactions were largest for the 4-5 sec bin. *Post hoc* analyses for bins 11 through 30 sec (the maximum) were completed in order to be thorough but no statistically significant effects were noted.

DISCUSSION

Previous experiments investigating the effect of the physiologically-patterned LTP magnetic fields had demonstrated significant improvements in the acquisition of an inhibitory task by animals with histories of epilepsy. It is perhaps unsurprising that this pattern of magnetic field exposure (imitating LTP when applied as current to hippocampal slices) increased rats' efficacies for this task. The results of the present study expand upon this previous work by indicating that this same LTP-EMF can promote the learning of an inhibitory behavior in normal rats. The effect is comparable to the administration of 1 g L^{-1} of L-alanine through the water supply.

L-arginine, a classic NO contributor, did not produce this improvement. Kato and Zorumski (1993) showed that NOS inhibitors facilitate the induction of LTP, and the administration of L-arginine overcame this enhancement of LTP induction. This amino acid was therefore selected partially as a negative control with the expectation of reduced LTP-efficacy and to assess the interactions with the induced LTP-field. L-alanine was selected on the bases of claims within the popular literature. According to some weight-lifters, alanine has been attributed to improved memory following chronic administration. Assuming approximately 50 cc of water consumption per day and a homogenous dispersion within tissue, the average rat consuming this concentration of alanine and arginine would contain micromole concentrations within brain tissue.

The largest proportion of the variance explained for the interaction between the compounds in the water supply and the magnetic field occurred in the 4-5 sec bin. The specific schedule for the operant task was a DRL-6, meaning that each rat was required to not respond for at least 6 sec. However the cycle for the LTP-field was once every 4.4 sec. This suggests that the LTP pattern as presented may have operated as a zeitgeber for neural circuits rather than a facilitator of LTP. Regardless, the applied magnetic field produced an effect that was comparable in magnitude to the effects of more than two weeks of consuming alanine in the water supply.

The results of this study offer further evidence to the efficacy and utilization of physiologically-patterned electromagnetic fields in pharmacological treatments. Given the work by Mach and Persinger (2009) who showed the improvement of DRL performance and shifts in inter-response times for seized rats and the present study's corroboration of this effect in normal rats, the application may be sufficient to expand to clinical conditions.

That the majority of variance fell within the 4-5 sec bin and the LTP cycle time of 4.4 sec could also suggest that various regions of the cerebrum respond differentially to the same field. Yun *et al*. (2002) found that the various layers of the entorhinal cortices and subsections of the hippocampal formation may also respond to specific, optimal parameters through which LTP induction occurs in these areas. These results indicated the L-alanine and electromagnetic field applications act through the same biochemical pathways or receptor effects, as suggested by the absence of synergism.

REFERENCES

Binhi, V.N. and A.B. Rubin, 2007. Magnetobiology: The kT paradox and possible solutions. Electromagn. Biol. Med., 26: 45-61.

Cifra, M., J.Z. Fields and A. Farhadi, 2011. Electromagnetic cellular interactions. Prog. Biophys. Mol. Biol., 105: 223-246.

Harrigan, T., O. Peredery and M.A. Persinger, 1990. Failure to acquire an inhibitory task following seizure-induced brain damage. Percept. Motor Skills, 70: 268-270.

Hu, J.H., L.S. St-Pierre, C.A. Buckner, R.M. Lafrenie and M.A. Persinger, 2010. Growth of injected melanoma cells is suppressed by whole body exposure to specific spatial-temporal configurations of weak intensity magnetic fields. Int. J. Radiat. Biol., 86: 79-88.

Kato, K. and C.F. Zorumski, 1993. Nitric oxide inhibitors facilitate the induction of hippocampal long-term potentiation by modulating NMDA responses. J. Neurophysiol., 70: 1260-1263.

Lagace, N., L.S. St-Pierre and M.A. Persinger, 2009. Attenuation of epilepsy-induced brain damage in the temporal cortices of rats by exposure to LTP-patterned magnetic fields. Neurosci. Lett., 450: 147-151.

Mach, Q.C. and M.A. Persinger, 2009. Behavioral changes with brief exposures to weak magnetic fields patterned to stimulate long-term potentiation. Brain Res., 1261: 45-53.

Martin, L.J., S.A. Koren and M.A. Persinger, 2004. Thermal analgesic effects from weak, complex magnetic fields and pharmacological interactions. Pharmacol. Biochem. Behav., 78: 217-227.

Martinez-Samano, J., P.V. Torres-Duran, M.A. Juarez-Oropeza, D. Elias-Vinas and L. Verdugo-Diaz, 2010. Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: A preliminary report. Int. J. Radiat. Biol., 86: 1088-1094.

McKay, B.E., M.A. Persinger and S.A. Koren, 2000. Exposure to a theta-burst patterned magnetic field impairs memory acquisition and consolidation for contextual but not discrete conditioned fear in rats. Neurosci. Lett., 292: 99-102.

Moser, E.I., K.A. Krobert, M.B. Moser and R.G. Morris, 1998. Impaired spatial learning after saturation of long-term potentiation. Science, 281: 2038-2042.

Nowicky, A.V. and L.J. Bindman, 1993. The nitric oxide synthase inhibitor, N-monomethyl-L-arginine blocks induction of a long-term potentiation-like phenomenon in rat medial frontal cortical neurons *in vitro*. J. Neurophysiol., 70: 1255-1259.

Persinger, M.A., G.F. Lafreniere and H. Falter, 1976. Oral taurine effects on inhibitory behavior: Response transients to step-like schedule changes. Psychopharmacology, 49: 249-252.

Port, M., M. Abend, B. Romer and D. Van Beuningen, 2003. Influence of high-frequency electromagnetic fields on different modes of cell death and gene expression. Int. J. Radiat. Biol., 79: 701-708.

Rose, G.M., D.M. Diamond, K. Pang and T.V. Dunwiddie, 1988. Primed Burst Potentiation: Lasting Synaptic Plasticity Invoked by Physiologically Patterned Stimulation. In: Synaptic Plasticity in the Hippocampus, Haas, H.L. and G. Buzsaki (Eds.). Springer, Germany, pp: 96-98.

Santini, M.T., G. Rainaldi and P.L. Indovina, 2009. Cellular effects of Extremely Low Frequency (ELF) electromagnetic fields. Int. J. Radiat. Biol., 85: 294-313.

Schuman, E.M. and D.V. Madison, 1991. A requirement for the I ntercellular messenger nitric oxide in long-term potentiation. Science, 254: 1503-1506.

Shahidain, R., R.D. Mullins and J.E. Sisken, 2001. Calcium spiking activity and baseline calcium levels in ROS 17/2.8 cells exposed to extremely low frequency electromagnetic fields (ELF EMF). Int. J. Radiat. Biol., 77: 241-248.

St-Pierre, L.S., A. Mazzuchin and M.A. Persinger, 2008. Altered blood chemistry and hippocampal histomorphology in adult rats following prenatal exposure to physiologically-patterned, weak (50-500 nanoTesla range) magnetic fields. Int. J. Rad. Biol., 84: 325-335.

Whissell, P.D. and M.A. Persinger, 2007. Emerging synergisms between drugs and physiologically-patterned weak magnetic fields: Implications for neuropharmacology and the human population in the twenty-first century. Curr. Neuropharmacol., 5: 278-288.

Yun, S.H., I. Mook-Jung and M.W. Jung, 2002. Variation in effective stimulus patterns for induction of long-term potentiation across different layers of the entorhinal cortex. J. Neurosci., 22: R214-R218.