

## Electromagnetic Field and $\Gamma$ Aminobutyric Acid a Receptors Interaction in the Fear Response

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### ABSTRACT

Freezing behavior is a fearful reaction to specific stimuli, most commonly observed by animals. One of the neurotransmission system most consistently linked with fear response is the GABAergic system, in which GABA through GABA<sub>A</sub> receptor can influence fear response. The aim of the present investigation was to examine the effect of IP injection of muscimol (GABA<sub>A</sub> agonist) and bicuculline (GABA<sub>A</sub> antagonist) with and without EMF exposure on the frequency of fear response. Fifty adult male rats weighing 180-200 g were used. Animals were divided in ten groups, of which five groups were exposed to ELF-EMF for 30 days at 8 h day<sup>-1</sup> in a solenoid, creating an electromagnetic field of 500  $\mu$ T by a 50Hz electrical current created. Animals were then treated with various doses of muscimol and bicuculline before being exposed to electrical shock. Each animal were received 100 electrical shocks every session. After shock induction, the fear response was determined by monitoring the reaction of shocked animals to a normal rat which was placed in the electroshock chamber. Data were analyzed by Mann-Whitney test and  $p < 0.05$  was considered as significant level. It was shown that injection of muscimol at 0.5 mg kg<sup>-1</sup> in group with and without EMF exposure, significantly decreased the frequency of fear response but muscimol at 2 mg kg<sup>-1</sup> only in group exposed to EMF significantly decreased frequency of fear response. Bicuculline significantly decreased frequency of fear response in group without EMF exposure, but it significantly increased the frequency of fear response in group with EMF exposure. Results indicated that interaction of GABA<sub>A</sub> receptors and EMF exposure possibly involved in the modulation of fear response.

**Keywords:** Fear Response, EMF, Muscimol, Bicuculline, Freezing Behavior, Significantly Decreased, Injection of Muscimol, Electroshock Chamber, Decreased Frequency, Electrical Shock

### 1. INTRODUCTION

Along with technological advances and extensive use of electrical devices, exposure to extremely low frequency (50 Hz) Electromagnetic Field (ELF-EMF) has extensively been increased and seems to have a significant effect on public and occupational health (Bracken *et al.*, 1995; Forgacs *et al.*, 2004). 50 Hz electromagnetic field is generated by power lines, transformers and electrical devices which can be found

in our living environment. This has raised some concerns about the effects of ELF-EMF on human health (Forgacs *et al.*, 2004).

It has been shown that there is disruption in the activity of serotonergic, dopaminergic and GABAergic system in social fear animal models (Li *et al.*, 2001). Subcutaneous administration of phenibut (GABA<sub>A</sub> agonist) led to reduction in the probability of passive defensive (freezing) reactions in rabbits (Zyablitseva and Pavlova, 2008). Intra dorsal periaqueductal gray injection

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of muscimol produces an expected inhibitory effect on the anxiety-related responses and freezing whereas these injections into the inferior colliculus produce quite opposite (Reimer *et al.*, 2008).

Bicuculline microinjection in the medial hypothalamus causes panic-like responses in laboratory animals and the elaborated escape behavior organized in both dorsomedial and ventromedial hypothalamic nuclei followed by significant innate-fear-induced antinociception (Freitas *et al.*, 2009). Intracortical application of bicuculline attenuates the freezing behavior induced by contextual fear conditioning (Matsumoto *et al.*, 2005). Pentylentetrazol (GABA<sub>A</sub> antagonist) at dose of 30 mg kg<sup>-1</sup> significantly decreases freezing reaction (Zienowicz *et al.*, 2007).

Several studies indicate the effect of electromagnetic field on fear response. It has been shown that acquisition of conditioned fear was strongly inhibited in animals exposed to complex magnetic fields (Whissell *et al.*, 2009). Prenatal exposure to physiologically-patterned magnetic fields within a specific “window” of intensities that overlap with values found in many human habitats may produce long-term changes in fear behaviors (St-Pierre and Persinger, 2008). One of the neurochemical system most consistently linked with fear is the GABAergic system. GABA levels significantly differ in fear-conditioned compared to non-fear-conditioned animals, supporting the role of GABA in fear (Rea *et al.*, 2009). But there are a few studies on the interaction of electromagnetic field and GABA<sub>A</sub> receptor modulation. The aim of the present study was to investigate the interaction of electromagnetic field (as environmental factor) and modulation of GABA<sub>A</sub> receptor by IP injection of muscimol (GABA<sub>A</sub> agonist) and bicuculline (GABA<sub>A</sub> antagonist) on the fear behavior.

## 2. MATERIALS AND METHODS

### 2.1. Animals

All the procedures involving animal subjects were reviewed and approved by the Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University.

Fifty adult male Sprague-Dawley rats (180-200 gr) were used. The rats were acclimatized for 1 week before being used for the experiment. After preincubation at the same conditions, animals were randomly divided into 10 groups (n = 5): Sham 1 (received normal saline at the same volume of drugs); Sham 2 (exposed to EMF and received normal saline); Experimental 1 (received

muscimol at 0.5 mg kg<sup>-1</sup>); Experimental 2 (received muscimol at 2 mg kg<sup>-1</sup>); Experimental 3 (exposed to EMF and received muscimol at 0.5 mg kg<sup>-1</sup>); Experimental 4 (exposed to EMF and received muscimol at 2 mg kg<sup>-1</sup>); Experimental 5 (received bicuculline at 1 mg kg<sup>-1</sup>); Experimental 6 (received bicuculline at 3 mg kg<sup>-1</sup>); Experimental 7 (exposed to EMF and received bicuculline at 1 mg kg<sup>-1</sup>) and Experimental 8 (exposed to EMF and received bicuculline at 3 mg kg<sup>-1</sup>).

### 2.2. Electromagnetic Field Exposure System

Five groups of animals were exposed to ELF-EMF for 30 days at 8 h day<sup>-1</sup> in a solenoid. The solenoid created an electromagnetic field of 500 μT by a 50Hz electrical current. Humidity, temperature (23-24°C) and photoperiod (12 h dark: 12 h light) reflected natural conditions and were similar in all groups.

### 2.3. Drugs

Muscimol and bicuculline (purchased from Sigma Aldrich Co) were diluted in isotonic saline (0.9%) and injected Intraperitoneally (IP).

### 2.4. Behavioral Testing

Fear response was induced by application of 0.2 mA electrical current stimulation applied every 3 sec for 5 min, i.e., each animal was received 100 electrical shocks every session. After shock induction, the fear response was determined by monitoring the reaction of shocked animals to a normal rat which was placed in the electroshock chamber. The number of freezing behavior as the sign of fear response was appropriately recorded for 20 min.

### 2.5. Statistical Analysis

Data were analyzed using SPSS (version 18). Non parametric test Mann-Whitney was carried out to assess the effects of muscimol and bicuculline on fear response. Data were presented as Mean ± SEM. The significant value were considered p<0.05.

## 3. RESULTS

IP injection of muscimol at 0.5 mg kg<sup>-1</sup> significantly (p<0.05) decreased the frequency of fear response in comparison to that of sham 1; but muscimol at 2 mg kg<sup>-1</sup> did not (**Table 1**). IP injection of muscimol at both doses in animals exposed to EMF significantly (p<0.05) decreased frequency of fear response in comparison to that sham 2 (**Table 1**).

**Table 1.** Interaction of EMF and muscimul on frequency of fear response

Experiment type	Groups		Frequency of fear response
Without exposure to EMF	Sham 1		7.6±0.93
	Experimental 1	Muscimul 0.5 mg kg <sup>-1</sup>	2.2±0.8
With exposure to EMF	Experimental 2	Muscimul 2 mg kg <sup>-1</sup>	6.6±2.68 <sup>a</sup>
	Sham 2		5±0.12 <sup>a</sup>
	Experimental 3	Muscimul 0.5 mg kg <sup>-1</sup>	2.75±1.11 <sup>b</sup>
	Experimental 4	Muscimul 2 mg kg <sup>-1</sup>	2±1.15 <sup>b</sup>

Dissimilar characters showed significant level at p<0.05

**Table 2.** Interaction of EMF and Bicuculline on frequency of fear response

Experiment type	Groups		Frequency of fear response
Without exposure to EMF	Sham 1		7.6±0.93 <sup>a</sup>
	Experimental 5	Bicuculline 1 mg kg <sup>-1</sup>	3.8±1.91 <sup>b</sup>
	Experimental 6	Bicuculline 3 mg kg <sup>-1</sup>	9.6±1.12 <sup>a</sup>
With exposure to EMF	Sham 2		5±0.12 <sup>b</sup>
	Experimental 7	Bicuculline 1 mg kg <sup>-1</sup>	13±2.7 <sup>c</sup>
	Experimental 8	Bicuculline 3 mg kg <sup>-1</sup>	14.2±3.58 <sup>c</sup>

Dissimilar characters showed significant level at p<0.05

IP injection of bicuculline at 1 mg kg<sup>-1</sup> significantly (p<0.05) decreased frequency of fear response in comparison to that of sham 1; but bicuculline 3 mg kg<sup>-1</sup> did not (**Table 2**). IP injection of bicuculline at both doses in animals exposed to EMF significantly (p<0.05) increased frequency of fear response in comparison to that of sham 2 (**Table 2**).

Sham 2 group exposed to EMF showed a significant (p<0.05) decrease in the frequency of fear response in comparison to that of sham 1 (**Table 1 and 2**).

#### 4. DISCUSSION

Some investigations show that EMF exposure can affect behaviors such as passivity, situational anxiety and fear response (Balassa *et al.*, 2009; St-Pierre and Persinger, 2008; Whissell *et al.*, 2009).

In the present study muscimul at 0.5 mg kg<sup>-1</sup> significantly decreased the frequency of fear response, but muscimul at 2 mg kg<sup>-1</sup> had not effect. Muscimol infusion impaired differential fear responding as the level of freezing to inhibitory conditioned stimulus was indiscriminable from that to excitatory conditioned stimulus (Lee and Choi, 2012). Muscimol injections into the Anterior Hypothalamic Nucleus (AHN) and Ventromedial Nucleus of the Hypothalamus (VMHDM) significantly reduced conditioned freezing whereas inhibition of GABA<sub>A</sub> transmission increased this

conditioned response in the AHN (Santos and Brandao, 2011). Furthermore the GABA<sub>A</sub> receptor agonists diazepam (3 and 6 mg kg<sup>-1</sup>) and chlordiazepoxide (10 mg kg<sup>-1</sup>) significantly reduced the expression of fear-potentiated startle post-training (Risbrough *et al.*, 2003).

In the present study bicuculline at 1 mg kg<sup>-1</sup> significantly decreased the frequency of fear response, but bicuculline 3 mg kg<sup>-1</sup> had not effect. Microinjections of the GABA<sub>A</sub> receptor antagonist bicuculline into the dorsal periaqueductal grey matter have been used to evoke explosive defensive reactions and it has been proposed that it is more logical to study these responses as physiologic demonstration of fear (Coimbra *et al.*, 2006). Local infusions of semicarbazide or bicuculline into inferior colliculus caused freezing and escape, respectively, in a dose-dependent manner (Borelli *et al.*, 2006).

IP administration of 2 mg kg<sup>-1</sup> midazolam (GABA<sub>A</sub> agonist) reduced conditioned freezing, whereas administration of 1 mg kg<sup>-1</sup> bicuculline only marginally potentiated conditioned freezing (Zhang and Cranney, 2008). Donatti and Leite-Panissi (2009) showed that muscimol injection into basolateral nuclei of amygdala decreased tonic immobility duration as fear mark, whereas bicuculline injection to that region increased it.

In the present study EMF led to diminished frequency of fear response. McKay (2000) reported that magnetic fields display evidence of forgetting, as inferred by their

marked attenuation of freezing behavior, during contextual extinction 24 h later. These results can be due to alters in the response of receptor, up regulation of receptor or neurotransmitter level alteration (Varani *et al.*, 2002). In the present study muscimol at both doses significantly decreased the frequency of fear response in animals were exposed to EMF and bicuculline at both doses significantly increased the frequency of fear response in animals were exposed to EMF. In the literature there was not any study related to interaction of EMF and GABAergic system on fear response; so it seems that clarification of possible mechanisms behind the observed effect needs further studies. According to present data it seems that co-administration of muscimol potentiated EMF-induced decrease in the frequency of fear, while bicuculline had opposite effect.

## 5. CONCLUSION

Our results indicated that:

- In animals that there was not exposure to EMF both of the muscimol and bicuculline in low dose decreased the frequency of fear response
- In animal were exposed to EMF, muscimol at both doses significantly decreased the frequency of fear response and bicuculline at both doses significantly increased it

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