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Behavioral Changes in Freshwater Crab, *Barytelphusa cunicularis* after Exposure to Low Frequency Electromagnetic Fields

J. Corte Rosaria and Encily R. Martin

Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University,
Aurangabad 431004, Maharashtra, India

Abstract: The effect of Low Frequency Electromagnetic Fields (LF-EMF) was studied on the behavior of freshwater crab, *Barytelphusa cunicularis.* It was found that crabs showed a total aggregation between 60-90 minutes A maximum aggregation and aggressiveness was found during the same time period. The crabs behavioral pattern observed were similar to those reported for serotonin. The feeding rate was found to be higher in LF-EMF induced crabs, however, in eyestalk ablated crabs, the feeding was voracious and non selective. When crabs were injected with the selective serotonin reuptake inhibitors (SSRI)drugs, viz. citralopam, fluxeotine and setralin a maximum aggregation and aggressiveness was found in the order of fluxeotine>setralin>citralopam. It was concluded that serotonin neurotransmitters are involved in aggregation and aggressive behavior in crabs.

Key words: *Barytelphusa cunicularis* % Low Frequency Electromagnetic Fields % Behavior

INTRODUCTION

Effects of electric and magnetic fields have been observed in the behavior of marine, freshwater and terrestrial animal species. There are many underwater direct current cables under various seas all over the world, which carry electrical currents. These electrical current induce static magnetic fields with intensities up to 3.5mT around cables on the sea bottom, where there are many invertebrates and vertebrates species. Research has established that exposure to magnetic field of 3.7mT could influence the survival rate and fitness of common benthic animals of the Baltic Sea [1]. Investigation were also carried out on the crustacean (*Crangon crangon, Rhithropanopeus harrisii and Saduria entomon);* the mussel (*Mytilus edulis*)and the flounder (*Planthichthys flesus*).Yano *et al*. [2] studied the migratory behavior of the chum salmon (*Oncorhynchus keta*). Munro *et al*. [3] investigated the effect of pulse remagnetization on the orientation of inexperienced, juvenile migrant birds, such as the Australian silver eye (*Zosteropsl lateralis*). Similarly, Lohman *et al*. [4] found hatching loggerhead sea turtle, *Caretta caretta* when exposed to magnetic fields found in three widely separated oceanic regions, swam in the direction that would help to keep them within the currents of the North Atlantic gyre and facilitate their migratory pathway.

Several studies have investigated the effect of radio frequency (RFR) on the cholinergic system because of its involvement in learning and wakefulness in animals. Testylier *et al*. [5] reported modification of the hippocampal cholinergic system in rats during and after exposure to low intensity radio frequency (RFR). Barteri *et al*. [6] studied the RFR exposure induced structural and biochemical changes in AChE, the enzyme involved in acetylcholine metabolism. Vorobyov *et al*. [7] reported that repeated exposure to low-level extremely low frequency-modulated RFR affected baseline and scopolamine-modified electro encephalogram (EEG) in freely moving rats. However, Crouzier *et al*. [8] found no significant change in acetylcholine-induced EEG effect in rats exposed for 24 hours to a 1.8 MHz Global system for mobile communication (GSM) signal at 1.2 and 9 W/cm^2. There are several studies on the inhibitory and excitatory neurotransmitters. A decrease in (- aminobutyric acid (GABA), an inhibitory transmitter, content in the cerebellum was reported by Mausset *et al*. [9] after exposure to RFR at 4 W/kg. The same researchers [10] also reported changes in affinity and concentration of-N-methyl- D- aspartic acid (NMDA) and GABA receptors in the rat brain after an acute exposure at 6 W/kg. Changes in GABA receptors has also been reported by Wang *et al*. [11] and reduced excitatory synaptic activity and number of excitatory synapses in cultured rat hippocampal

Corresponding Author: Encily R Martin, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, Maharashtra, India, E-mail: martin_encily@yahoo.com.

neurons have been reported by Xu *et al*. [12] after RFR exposure. Beason and Semm [13] reported changes in the amount of neuronal activity by brain cells of birds exposed to GSM signal. Both increase and decrease in firing were observed. Salford *et al*. [14] reported cellular damage and death in the brain of rat after acute exposure to GSM signals. Tsurita *et al*. [15] reported no significant morphological change in the cerebellum of rats exposed for 2-4 weeks to 1439-MHz Time Division Mutiple Access (TDMA) field at 0.25 W/kg. More recently, Joubert *et al.* [16,17] found no apoptosis in rat cortical neurons exposed to GSM signals *in vitro*.

Though most of the studies reported are from vertebrates and humans, no work has been reported in freshwater crustaceans. Thus in this context the present study was carried out to ascertain the behavioural changes to LF EMF and also the role serotonin inhibitor drugs to freshwater crabs, *Barytelphusa cunicularis*.

MATERIALS AND METHOD

The freshwater crab *Barytelphusa cunicularis* (West-Wood) were collected from the backwaters of Nathsagar Dam, Paithan, Aurangabad. The crabs were brought to the laboratory, they were cleaned with water three to four times for the first three hours. Thereafter, the crabs were transferred to plastic troughs for acclimation to laboratory conditions. The crabs were maintained at natural L:D cycle, at temperature 25±1°C and fed *ad libitum*. The water was changed daily with dechlorinated water.

Experimental Set up: Two exposure chambers were set up with a concentric coil connected (solenoid). The solenoid was wound in forward backward forward continuous layers of turns of 2mm diameter copper wire. It was driven by the 50 Hz power line with a load of 200W bulb. One of the exposure chamber was designated for control and other experimental. In control no current supply was given whereas, in experimental electric current supply was allowed to create an electromagnetic field. The crabs were exposed to short time course study for 24, 48, 72 and 96 hours. And another set of experiment was kept for long time course for 30, 60 and 90 days. For biochemical studies 10 crabs were placed in each set i.e control and experimental. The experimental sets for short and long time course were exposed to LF-EMF. For behavior studies 10 crabs were placed in each set i.e control and experimental. The experimental sets for short and long time course were exposed to LF-.EMF.The following behavioral studies were carried out.

Aggregation Behavior: The aggregation behavior of crabs was studied after exposure to LF-EMF in experimental and control set. The aggregation behavior of crabs were observed and recorded after 0, 15, 30, 45, 60, 75, 90, 120, 150 minutes of exposure. The result are expressed in percentage.

Feeding Behavior: The crabs were fed with fish food of known weight and the feeding behavior was observed in LF-EMF exposed and control sets. The percentage of food consumed after exposure to LF-EMF for 3 hours was recorded. Similarly, the feeding behavior after eyestalk ablation were studied.

Fecal Matter: In this experiment the total output of fecal matter of the crabs were recorded after 24 hours. After 24 hours the water was filtered and the fecal matter was collected and weighed. The fecal matter was expressed in percentage of weight.

Agressive Behavior: The aggressive behavior was observed after exposure to LF-EMF and the aggressive behavior was recorded for 0, 15,30,45,60 and 75 minutes. The results are expressed as percentage of aggressiveness.

Effect of Serotonin Inhibitor Drugs on Aggregation and Aggressive Behaviour: The aggregation behavior of crabs was studied after injecting with selective serotonin reuptake inhibitor (SSRI) drugs viz. setralin, citralopam and fluxeotine. The concentration injected was citalopham 27x 10^{-11} µg, setraline 5 x10^{-11} µg and fluxeotine 18x10^{-11} µg. In control crabs 1000µl of distilled water was injected. The percentage aggregation and aggressive behaviour to drugs were scored. The drugs were injected into the fourth walking leg of the crab and their behavior was observed for the first one hour. The drug injected crabs were kept separately in three different troughs.

Statistical Analysis: The data was subjected to statistical analysis, using the MS Office. The data was subjected to Students ‘t’ test, using MS Office software.

RESULTS

Aggregation Behavior: The freshwater crab, *B.cunicularis* showed a total aggregation and was peculiar in the sense that the maximum aggregation occurred near the/towards the source of the electric current supply. It was found that a 100% aggregation of crabs were observed, between 60 to 90 minutes.

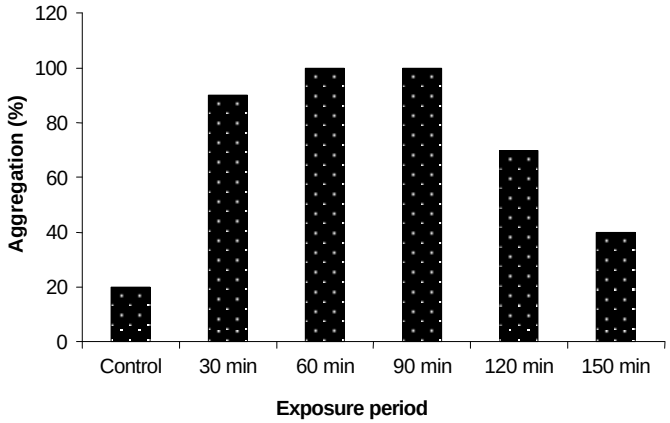

Fig. 1: Effect of LF-EMF on aggregation behavior

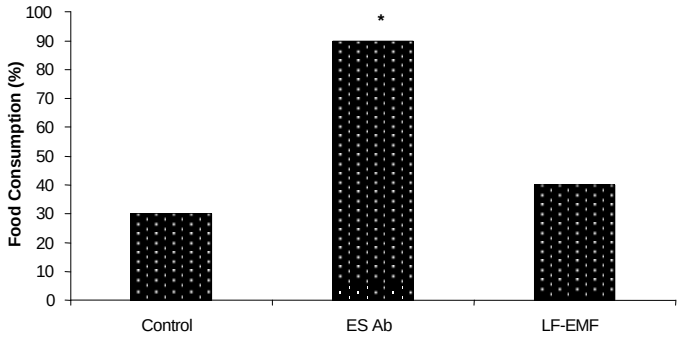

Fig. 2: Effect of LF-EMF on the feeding behavior

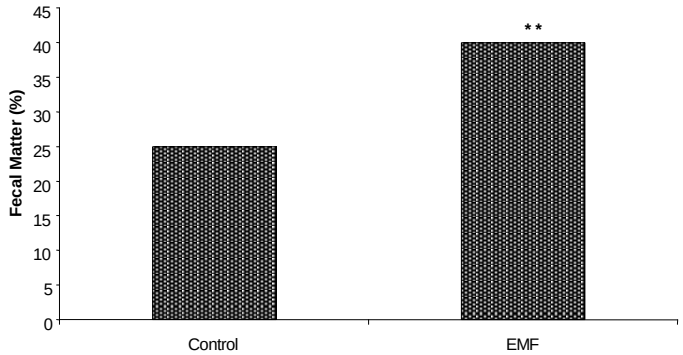

Fig. 3: Effect of LF-EMF on the fecal matter

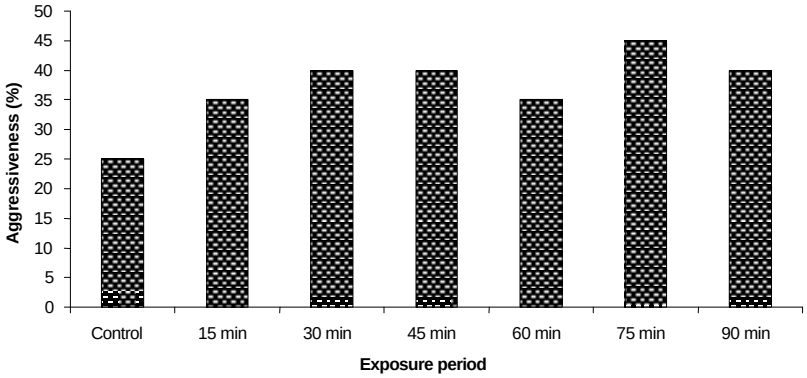

Fig. 4: Effect of LF-EMF on agressive behavior(%)

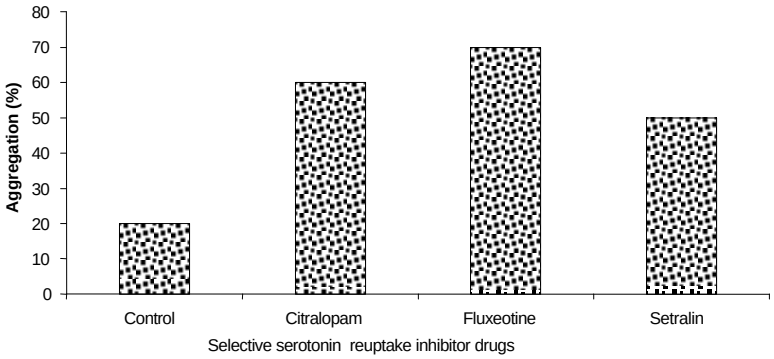

Fig. 5: Effect of SSRI drugs on the aggregation behavior

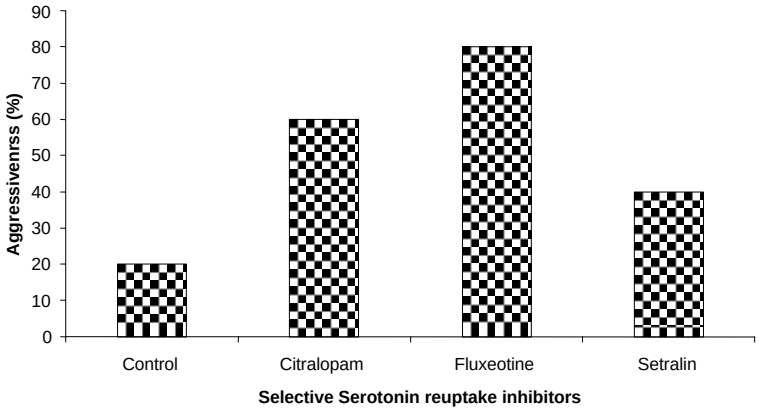

Fig. 6: Effect of Drugs on the aggressive behaviour

After this the crabs slowly segregated from each other and was found to act similar to control crabs. In control crabs such aggregation behavior was not seen. The percentage of aggregation is shown in fig.1.

Feeding Behavior: Among the feeding behavior it was found, that there was no significant ($P<0.05$) difference in the feeding behavior in LF-EMF exposed and non LF-EMF exposed crabs. However, when the crab's eyestalks were ablated a voracious feeding habit was observed ($P<0.05$). In eyestalk ablated crabs a 100% feeding was found during the first few hours. The percentage of feeding in crabs are shown in fig.2.

Fecal Matter: Amount of fecal matter was determined after exposure of crabs to LF-EMF and control crabs for 24 hours. It was found that the fecal matter excreted by EMF exposed crabs were higher than control crabs ($P< 0.05$). The amount of fecal matter is expressed in percentage and is depicted in fig.3.

Aggressive Behavior: A steady increase in aggressive behavior was observed in crabs exposed to LF-EMF. A high degree of aggressiveness was found after one hour of exposure.However, after one hour the aggressiveness became mild and showed less aggressiveness even after exposure to LF-EMF for a long time (Fig.4).

Effect of Drugs on Aggregation and Aggressive Behavior: The crabs were injected with SSRI drugs viz. setralin, citralopam and fluxeotine. It was found that maximum aggregation was found in the order of fluxeotine>setralin> citralopam. Similarly, the aggressive behavior was observed in drugs in the order of fluxeotine>setralin> citralopam (Figs.5,6).

DISCUSSION

Behavior is an overt response to any changes in environment and is often interlinked to intrinsic and extrinsic factors [18]. An increasing role of neurotransmitters in the behavior of invertebrate have been suggested and there is an overwhelming evidence to demonstrate that neurotransmitters play an important role(s) apart from neuronal circuitry. Behavior is subject of vast study and all aspects of behavior is not well established. It has been found that at every movement, an animal showed a varied behavioral response, for its own

survival and perhaps for evolutionary adaptation. The animals have a well developed system to receive most, if not all signaling molecules; such as, dopamine, nor-ephineprine, octopamine, serotonin and tyramine. They are believed to have a coherent hormonal control in a variety of behavioral context; such as, feeding and aggression [19-24]. In crustaceans the role of neurotransmitters in modulation of behavior has been proved beyond doubt.

The crabs, *B. cunicularis*, when exposed to LF-EMF, showed a total aggression and the animals moved towards the source of power supply. A total aggregation was found between 60-90 minutes, thereafter, the crabs showed segregation and the behavior was similar to that of control crabs. The initial response to LF-EMF may be due to some kind of changes in the metabolism, probably, at metabolic or in neuronal circuitry. When correlated with aggressive behavior, it showed the same time span, such that the animals shown for aggregation i.e. 60-90 minutes. When correlated to aggressive behavior the maximum aggressiveness was found during the same period. However, the aggressiveness was less than 50% Thus, a link between the two, aggression and aggressiveness may be possible. Under the influence of EMF the aggregation was linked to an increased serotonin level in crustaceans [25, 26]. The serotonin is involved in aggregation [22, 24, 27]. The feeding rate in *B. cunicularis* was found to be higher. Kravitz [27] reported that feeding and aggressiveness in crustaceans are related to serotonin level. Also in animals, serotonin plays a selective role in feeding [24]. In the present study, the aggregation and aggressiveness in crabs was seen after about 1hour indicating the possible role of serotonin. However, in eyestalk ablated crabs, the feeding was voracious and nonselective. Thus, it is concluded that in crabs, the eyestalk control the selective feeding behavior. The eyestalk ablation probably deprives the selective feeding associated with serotonin as observed in other animals [28]. However, more research is necessary to corroborate this finding in *B. cunicularis.*

In crustaceans biogenic amines serves as a circulating neurohormones. Injection of serotonin and octopamine causes in crabs, stable and stereological postures that resemble in those seen in dominant and subordinate lobsters [29]. Serotonin enhances the release of excitatory and inhibitory nerve terminals and causes muscle to undergo a long lasting voltage and calcium ion sensitive contracture to generate calcium ion action potentials. Serotonin increases the rate of firing of excitatory neurons to flexors muscles and inhibitory neurons to extensor muscles. Livingstone *et al*. [28], reported a higher concentration of serotonin and octopamine in two peripheral locations in the lobster nervous system and at lower concentration throughout the ventral nerve cord.

Serotonin effects the level of aggressiveness in lobsters with subordinates becoming more likely to initiate encounters and less likely to withdraw [30-34]. The latter submissive behavior is attributed to the octopamine and tyramine neurotransmitter. There are reports that, chemical communication may be mediated *via* amine metabolites, that are secreted in crustacean urine [35], sulphate metabolites of serotonin has been identified in crayfish lobsters, *H. americanus* urine [31] and concentration of this metabolite may trigger the signaling mechanism which indicates dominance status to conspecifics. In the present study, it is presumed that, metabolites of 5-HT may be secreted in the form of urine; however, the urine with metabolite in these animals, after exposure to LF-EMF was not recorded. But, with the available literature, the phenomenon cannot be ruled out. What is interesting is that the fecal matter, amount is higher in crabs exposed to LF-EMF. This increase is attributed to LF-EMF. It is known that under stress there is a higher release of digestive enzymes, due to which the digestion rate is higher and accordingly, the amount of fecal matter is higher [36, 37]. The present study does not conclusively, prove that, if any metabolites of neurotransmitters are excreted through the fecal matter, nor the metabolites were analyzed in crab urine. Thus, from the literature, wherein a strong support for the excretion is available, it is corroborated that, biogenic amines may be excreted by the *B.cunicularis.* Research in this area is currently underway in our laboratory.

The metabolic signaling mechanism is mediated by the hemolymph L- lactate by biogenic amines. This induces behavioral hypothermia in shorecrab, *C maenas*, after fighting and physiological mechanism are thereafter important in this species [38]. The amine serotonin hydroxytryptamine creatine sulphate complex [(5-HT)-CS] has been attributed to aggression in various animals including humans [20, 39]. The nature of association appears not to be so simple as it is believed.No correlation can be made in vertebrate and invertebrate. In vertebrates, low 5-HT or changes in amine neurons function that lowers the effectiveness of serotoninergic neurons generally co-relates with the amine levels of aggression [22, 39]. Whereas, in invertebrates the opposite is true [18, 40, 41].

The clawed decapod crabs, success is based largely on physical superiority [25, 42, 43]. Thus the resident population is bound by a system of dominant /

subordinate relationships based on initial agonistic behavior / encounter [26]. The fight occurs as a rule closely matching prediction of game theory (ie. Sequential assessment strategies), in which animals acquire information about an opponent's strength and fight abilities in a stepwise manner [44-47]. This kind of behavior is seen in normal crabs, not exposed to LF-EMF; however, the exposed crabs showed a lower response. This response may be due to the LF-EMF. The LF-EMF might be affecting the biogenic amines and thus, may be inhibiting the serotonin release or some other metabolite may be formed that may be inhibiting the aggressive behavior. Though, the aggressive behavior was higher than control, the aggressive behavior was less than 45%. Indicating, a mild increase, after the influence of LF-EMF. The complete mechanism of the behavioral regulation / control is not studied. Various workers have reported that in invertebrates the amine function, such as, that of serotonin are involved in swimming [48, 49] and feeding [50].

In the present study, the selective serotonin reuptake inhibitor (SSRI) was injected. The three anti depressant drugs, viz. citralopam, fluxeotine and setralin are commonly used anti depressant drugs for clinical purpose. The study confirmed that serotonin neurotransmitters are involved in the aggregation and aggressive behavior in freshwater crab *B. cunicularis*.

Thus, it is concluded that *B. cunicularis*, showed a total aggregation between 60-90 minutes, after exposure to LF-EMF and thereafter, the crabs showed segregation. When correlated aggregation and aggressive behavior was found during the same period. The observed behavior was similar to those reported for serotonin. The feeding rate in *B. cunicularis* was found to be higher in LF-EMF exposed crabs however, in eyestalk ablated crabs, the feeding was voracious and nonselective. When the crabs were injected with the selective serotonin reuptake inhibitor (SSRI) drugs, viz. citralopam, fluxeotine and setralin a maximum aggregation and aggressiveness was found in the order of fluxeotine>setralin>citralopam.

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