# Exposure to Electromagnetic Field during Gestation Adversely Affects the Electrophysiological Properties of Purkinje Cells in Rat Offspring

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## ABSTRACT

**Background:** Prenatal adverse effects of radiofrequency electromagnetic fields (RF-EMF) exposure on nervous system are an issue of major concern.

**Objective:** Thus, in this study we evaluated the membrane current flow properties of Purkinje neurons after maternal exposure to 900 MHz pulsed RF-EMF.

**Material and Methods:** In this experimental study, during all days of pregnancy, rats in the EMF-exposed group were exposed to 900 MHz pulsed-EMF radiation for 6 h per day. The effects of RF-EMF exposure on the electrophysiological properties of the Purkinje cerebellum neurons from male pups were evaluated by whole-cell patch clamp recordings in current and voltage clamp modes. In voltage-clamp experiments, the holding potential was -60 mV, and a depolarizing voltage step (1000 ms duration) was applied from -60 to +50 mV in 10 mV increments at 2s intervals.

**Results:** The exposure group demonstrated reduced spontaneous firing associated with upward and rightward shift in I/V curve compared to the control rats. Moreover, the peak amplitude of the current for the exposure pups also revealed a significant decrement. The reversal potential was +40 mV and +20 mV for the control and RF-EMF groups, respectively and showed significant differences between the two groups.

**Conclusion:** The decrease in ion's conductance could be attributed to the observed decrease in the voltage onset of the inward current, peak amplitude and voltage shift.

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## Keywords

Electrophysiological Properties; Cell Phone; Purkinje Cells; Prenatal Injuries

## Introduction

Over the past decades, the exponential growth of wireless communication has caused great global concerns regarding the health effects of electromagnetic radiations [1,2]. According to the International Telecommunication Union, the number of cell phone subscriptions in 2008 was approximately 7 billion [3] and more than 80% of people were exposed to radiofrequency electromagnetic fields (RF-EMF). The adverse effects of EMF exposure from mobile phones

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have received considerable critical attention. Exposure to EMF has been shown to be related to the impairment of cognitive function [4-6] and working memory performance in human [7]. However, some studies show that exposure to RF-EMF at specific intensity levels can be linked to the induction of some potential and beneficial effects. It has previously been shown that the visual reaction time of students significantly decreased after exposure to radiofrequency radiation emitted by a mobile phone [8]. Furthermore, it has been reported that occupational exposure of radar workers to radar radiation decreased their reaction time [9].

Several studies have shown the negative effects of prenatal exposure to EMF on CNS development [10,11]. Moreover, it is revealed that prenatal exposure to EMF affects the development of cerebellum Purkinje cells [12]. Thus, a number of studies reported no obvious embryo-toxicity or teratogenicity for EMF exposure in the fetus [13,14]. This concept has also been challenged by Klose et al.'s studies demonstrating that long-term EMF exposure at early age even with relatively high SAR values does not have any impact on learning skills and behavior function [15]. Recent studies from our laboratory showed that prenatal exposure to EMF might cause functional changes to CA1 hippocampal and purkinje cells firing [5,6]. However, our study revealed altered electrophysiological properties in the EMF exposed fetus, which could not be related to behavioral changes. It has been shown that cerebellum is susceptible to EMF exposure, particularly in prenatal period [16]. Despite the importance of this issue, the electrophysiological effect of EMF is poorly understood, and there is much less information on the effects of EMF on the membrane current of the cells.

Based on our previous reports demonstrating an important role of intrinsic neuronal excitability in the EMF exposed fetus, we hypothesized that EMF might also alter the membrane current of the Purkinje cells. Therefore, this study aimed to investigate the current properties of the purkinje cells following exposure to 900 MHz EMF during the prenatal period, using the experimentally recorded current trace under voltage clamp.

## Material and Methods

### Experimental Procedures

In this experimental study, primiparous Wistar female rats, weighing 200–220 g, were used. Pairs of females were placed with single male rats in the late afternoon. The day 0 of pregnancy was confirmed by existence of vaginal smear or plug. Pregnant rats were randomly divided into the control and (N=10) mobile phone exposure groups (N=10). The experiments were conducted in accordance with the animal care using guidelines approved by the Institutional Ethics Committee at the Neuroscience Research Center of Kerman University of Medical Sciences.

### Exposure to RF-EMF

A GSM cell phone simulator was used for EMF exposures. In the EMF-exposed group, the cell phone simulator and plexiglas cage were placed inside a standard Faraday cage. The simulator was set in exposure (talk) mode and powered through a stabilized power supply so that the antenna power supply as well as the field intensity were constant. The control group underwent the same conditions as the EMF group did (900 MHz Pulse-EMF exposure) on the cage but without irradiation. In all days of pregnancy period, the rats in the EMF group were exposed to 900 MHz pulsed-EMF exposure for 6 h per day (8:00 AM to 2:00 PM). For obtaining the maximum radiation level, the plexiglas cage was placed 40 cm away from the EMF source. The temperature was measured before, during and after the exposure by a standard rectal probe. The male pups were weaned from their mothers on day 23, than, all experiments were performed on

the postnatal days 30-35.

### Whole-cell Patch Clamp Recording

The effects of EMF exposure on the electrophysiological properties of the Purkinje cerebellum neurons were evaluated by Whole-cell patch clamp recordings in voltage and current clamp modes.

The animals were decapitated under Diethyl Ether anesthesia and their brains were removed quickly and placed in ice-cold artificial cerebro-spinal fluid (aCSF) containing 124.0 mMNaCl, 25 mM$NaHCO_3$, 10 mMd-glucose, 4.4 mMKCl, 2 mM$MgCl_2$, 1.25 mM$KH_2PO_4$ and 2 mM$CaCl_2$, which was bubbled with 95% $O_2$-5% $CO_2$. The parasagittal slices of the cerebellar vermis in 300 µm thicknesses were cut by vibroslicer. After one hour, the incubated slices were continuously superfused at 1–2 ml/min with a CSF. Whole-cell patch clamp recordings in voltage clamp mode from the Purkinje neurons were made using Multi-clamp 700 B amplifiers; the signals were digitized by Digidata 1320 A/D converter. Electrophysiological recordings were sampled at 10 kHz and filtered at 5 kHz, respectively as described previously [17]. The patch pipettes had a resistance of 3-6 MΩ when filled with internal solution containing (in mm) 125 potassium gluconate, 10 KCl, 10 HEPES, 1 $MgCl_2$, 2 $Na_2ATP$ and 0.4 $Na_2GTP$.

The purkinje neurons were visualized with a 60x water immersion objective using Nomarski-type differential interference contrast imaging with infrared illumination. After the establishment of a Giga seal, brief suction was applied to break through the cell membrane for the whole-cell configuration. Cells with a seal <1 GΩ before rupture of the membrane were discarded and the test seal function was constantly monitored throughout the recording to ensure that the seal was stable. Before entrance to voltage clamp mode, the spontaneous activity of all cells for 5 min was recorded in the current clamp mode. Cells without spontaneous activity or those in silent mode were discarded. All recordings were obtained in the presence of Kynurenic Acid (1 mM), a selective blocker of inotropic glutamate receptors and picrotoxin (100 µM), a known GABAA blocker [18]. In voltage-clamp experiments, the holding potential was -60mV, and a depolarizing voltage step (1000 ms duration) was applied from -60 mV to +50 mV in 10 mV increments at 2s intervals [19].

### Statistical Analysis

All data were first assessed to determine the normality using a Kolmogorov-Smirnov test. The results were found to be normally distributed expressed as mean±SEM and analyzed using an unpaired Student's t-test. $P<0.05$ was considered statistically significant.

## Results

### Effects of Prenatal Exposure to Mobile Phone on Reproductive Parameters

There were no significant changes in the body temperature of the male offspring as well as mothers exposed to EMF compared to the control group (Table 1). Moreover, as shown in Table 1, no pregnancy period changes were revealed in the control and mobile phone groups.

### Effects of Prenatal EMF Exposure on Spontaneous Firing

The frequency of the action potentials is the reciprocal of inter spike interval. Therefore, we studied the spontaneous firing of Purkinje neurons by evaluating ISIs. As shown in Figure 1, there was a significant difference between the two groups; the Purkinje neurons from the exposure group showed reduced spontaneous firing (Control: 47.9 ±2.06 versus Mobile phone exposed rats: 36.4± 1.89; $P<0.01$).

### Effects of Prenatal EMF Exposure on Membrane Current

In the next series of the experiment to find

**Table 1:** No significant differences were observed in dam's body temperature, litter size, Length of pregnancy, pup's body weight and mortality rate between control and Mobile phone exposure groups.

| Dam's body temperature | | | Litter size | Length of Pregnancy (days)* | Body weight (g) | Mortality rate |
|---|---|---|---|---|---|---|
| Group | First exposure | Last exposure | | | PND 23 | |
| Control | 37.5±0.3 | 37.6±0.4 | 10.9±1.2 | 21.2±0.1 | 29.8±1.3 | 0 |
| Mobile | 37.61±0.54 | 37.6±0.38 | 9.81±1.07 | 20.93±0.4 | 31.2±2.4 | 0 |

*Postnatal Day

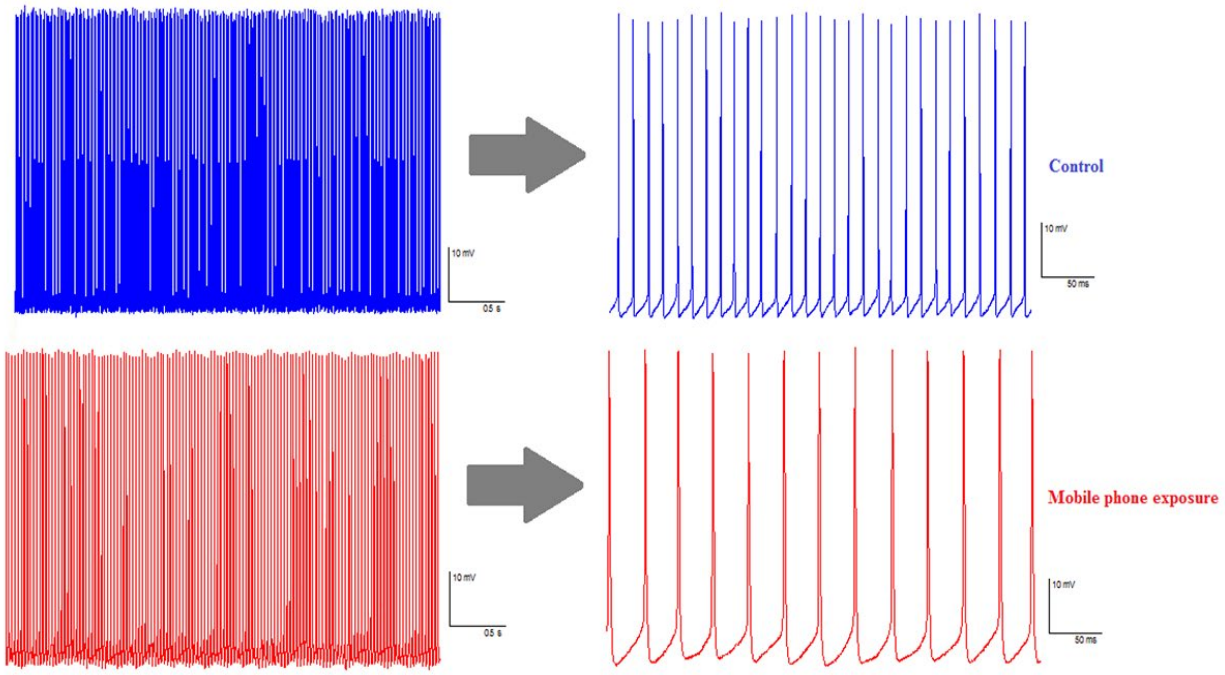


**Figure 1:** Whole cell patch clamp recordings revealed that prenatal EMF exposure affected the firing frequency of the purkinje cells of the rats' offspring slices.
Blue color traces: control; Red color traces: EMF

whether firing deficit is associated with changes of the membrane current, we evaluated the current flow properties in voltage clamp mode. There was a significant difference between voltage onsets of the inward current. The Purkinje neurons from the exposure group showed beginning of the inward current at voltage -39.01 ± 3.6 but this value was 58.6 ± 4.9 for the control pups (Figure 2). In addition, the peak amplitude of the current for exposure pups was -2220.9±274.07 and also did show a significant decrement response compared to the control group -2959.4±311.2 (Figure 3). As in Figure 4, the membrane current of the EMF group exhibited an upward and rightward shift. For the control group between about -50 and -20 mV, the current increased with increasing depolarization, but this value was -40 and -10 mV for the exposure group (Figure 4A). Moreover, the reversal potential

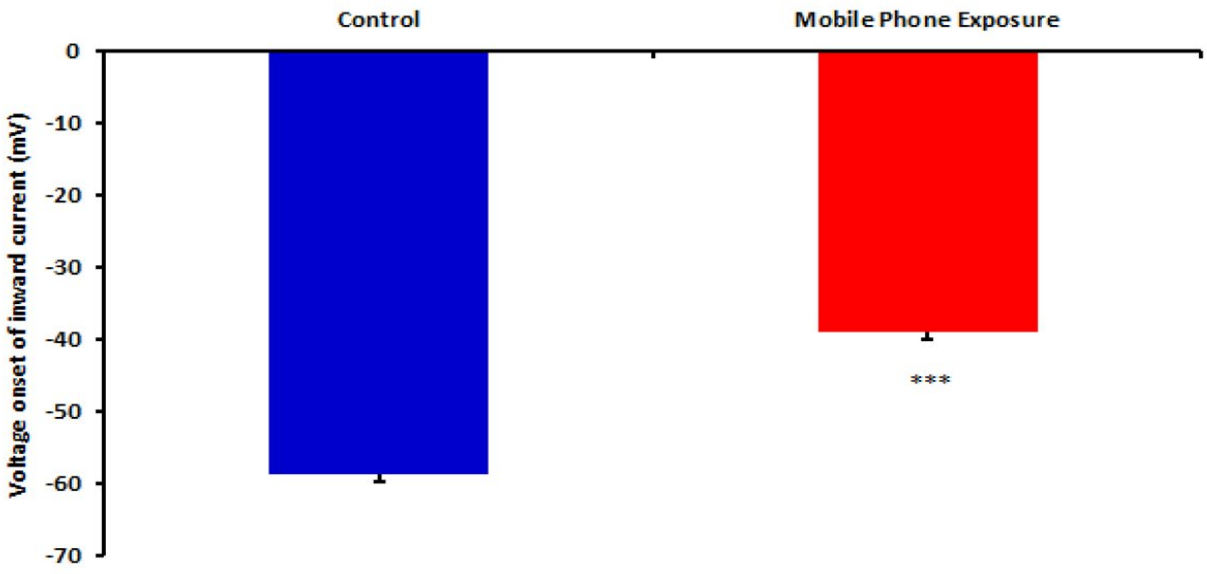


**Figure 2:** Prenatal EMF exposure decreased the voltage onset of inward current in the male pups. *** ($P < 0.01$) represents significant differences between prenatal EMF exposure group vs. control group

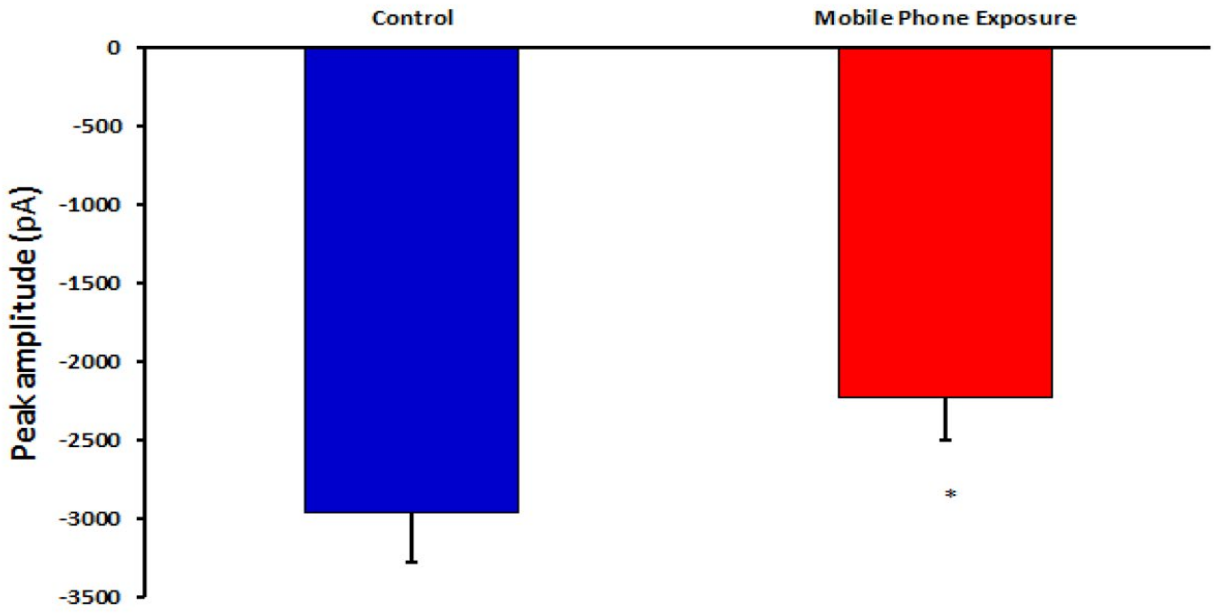


**Figure 3:** Peak amplitude of the current for the exposure pups showed a significant decrement response compared to the control group. * ($P < 0.05$)

was +40 mV and +20 mV for the control and EMF groups, respectively, and it showed significant differences between the two groups (Figure 4B).

## Discussion

The prenatal adverse effects of EMF exposure on human nervous system are a subject of major concern. Thus, in this study we evaluated the membrane current flow properties of the Purkinje neurons after maternal exposure to 900 MHz Pulse-EMF. The results of this study showed that EMF emitted from mobile phones impaired the normal current flow of the cerebellar Purkinje neurons.

Prenatal exposure to 900 MHz EMF has been reported to cause a variety of abnormalities in the physiology and behavioral functions [12]. In our previous work, we observed that prenatal EMF exposure led to changes in the spontaneous activity of the neuronal excitability, AHP amplitude, spike frequency and half width in the Purkinje cells [5]. These results support our previous research in this area. Therefore, we hypothesized that EMF exposure would result in a shift in the membrane current properties, and that these changes would be associated with a decrease in the excitability of the Purkinje cells. It is interesting to note that in all cases of the EMF group, we observed a shift in the voltage onset of the inward current toward the positive voltage. The response to changes in the membrane potential with the gating is a hallmark of voltage-gated channels. S4 mutation usually led to changes in the activation property of these channels [3,20]. Another possible explanation for this is that the membrane depolarization decreases excitability via inactivation of $Na^+$ channels [21]. Thus, the observed decrease in the voltage onset of the inward current could be attributed to structural changes of voltage gated channels and/or membrane depolarization by any means in the EMF group. Moreover, we observed the predicted decrement in peak amplitude of the current. It is important to bear in mind that the ionic current is equal to that ion's conductance (g) multiplied by the driving force ($V_m$-E). It is possible to hypothesize that changes in driving force are less likely to occur; therefore, the decrease in the ion's conductance could be the cause of a decrement in the ionic current. However, it further supports the idea of structural changes and/or decre-

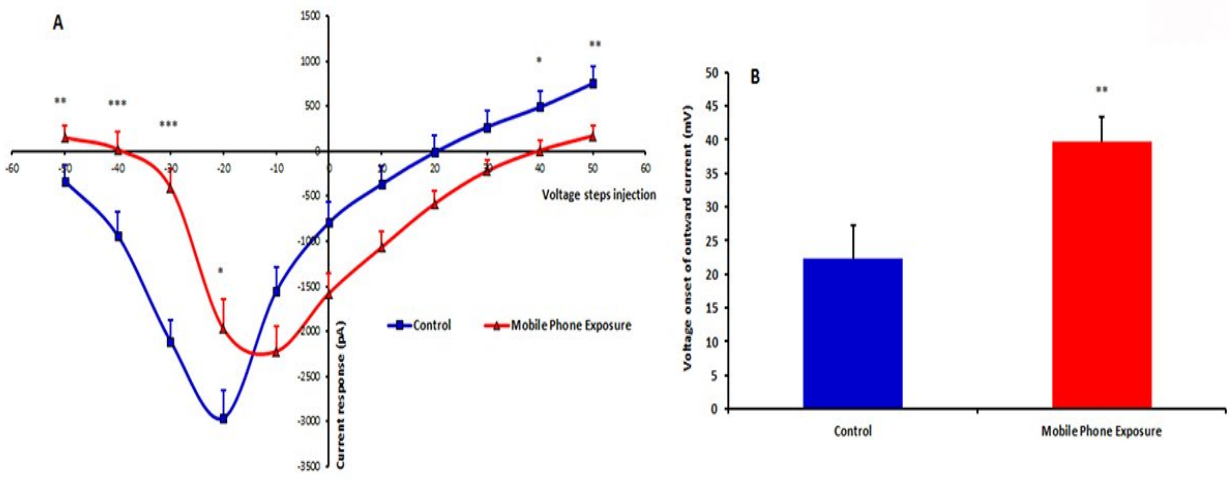


**Figure 4:** Effect of prenatal EMF exposure on voltage dependent currents. (A) the membrane current of the EMF group exhibited an upward and a rightward shift. (B) the reversal potential increased in the EMF group. * ($P < 0.05$), ** ($P < 0.01$) and *** ($P < 0.001$) represent significant differences between the prenatal EMF exposure group vs. the control group

ment of available voltage gated channels by EMF.

## Conclusion

Taken together, present results suggest that the decrease in ion's conductance could be an important reason for electrophysiological changes induced by EMF exposure.

## Acknowledgment

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## Conflict of Interest

None

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