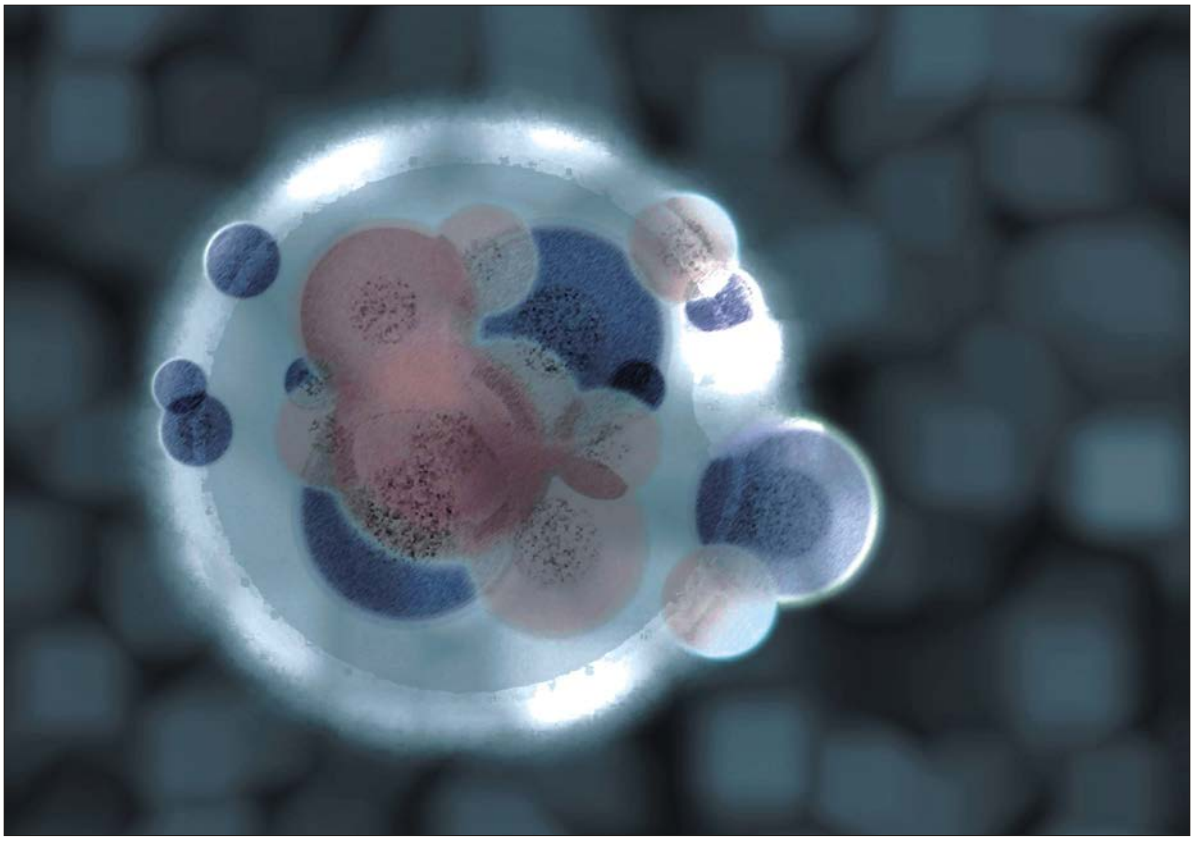

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The Effect of Low Electromagnetic Field in the Cerebellar Layers of Mice

[1,2]Y. Rosli and [1]P.J. Teoh
[1]Department of Biomedical Science, Faculty of Allied Health Sciences,
Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz,
53300, Kuala Lumpur, Malaysia
[2]ARC Centre of Excellence in Vision Science, CVS, School of Biological Sciences,
Australian National University, Acton 0200, Canberra ACT, Australia

Abstract: The study on the effects of low electromagnetic fields (EMF) towards human health has been ongoing for over three decades but it is inconclusive still as to whether or not EMF is harmful to human health. This study was undertaken to look at the effects of EMF exposure on the morphology of the cerebellum of adult mice and offspring of pregnant mice which were exposed to EMF during their gestational period. The intensity of EMF used in this study was 1.2 mT and 0 mT for treatment group and control group, respectively. The adult mice were exposed to EMF 5 days consecutively, each exposure session lasting for 6 h. The prenatal exposure was done on gestation day 3, 6, 9, 12 and 15. Then, the thickness of Granular Layer (GL) and Molecular Layer (ML) were measured using an image analyzer. Statistical analysis showed that there was a significant decrease in the number of Purkinje Cells (PCS), a decrease in the thickness of both the GL and ML in the 1.2 mT adult treatment group ($p<0.001$). In the offspring exposed prenatally, the number of PCS were also significantly decreased ($p<0.001$), but significant thickening of the GL ($p<0.05$) was observed in the 1.2 mT treatment group. The results obtained in this study, suggest that the exposure to EMF can cause changes to the morphology of cerebellum of adult mice and also affects the development of mice cerebellum.

Key words: EMF, electromagnetic field, cerebellum, purkinje cells

INTRODUCTION

Rapid advances in the electromagnetic field (EMF) technologies and communications has greatly increased the human populations' exposure to EMFs. For over three decades, ongoing debates on the adverse effects of EMF that gravitates around the low frequency region (<300 Hz) are still going strong (Jauchem, 1997; Salford *et al*., 2007) and have taken on a new impetus and motivated numerous studies reporting on biological effects and interactions with cells (Cameron *et al*., 1993; Delgado *et al*., 1982; Eccles, 1970; Huuskonen *et al*., 1998; Jeong, 2005; Mang *et al*., 1993; Martin, 1988, 1992; Mugnaini and Forstronen, 1967; Quesada and Genis-Galvez, 1983; Ubeda *et al*., 1994). There have been contradictory reports of acute effects of EMF, for example upon the cardiovascular (Hocking *et al*., 1994; Jauchem and Frei, 1994; Korpinen and Partanen, 1993; Korpinen and Partanen, 1996), reproductive and nervous system (Adey *et al*., 2000; Grafstrom *et al*., 2008; Salford *et al*., 2003, 2007). Despite all these reports, we are yet to have a conclusive answer to the main question-whether or not EMF is harmful to the human health.

The morphology of the cerebellar neurons and their intracortical connections has been studied intensively in the recent years. There are four main types of neurons in the cerebellar cortex; purkinje cells, granule cells, golgi cells and the stellate/basket cells. The purkinje and the golgi cells develop from the ventricular germinal layer whereas the other two arise from the external granular layer (Cajal, 1988). The normal complex structure and functioning of the CNS are related to the prenatal and postnatal developments of major neurons such as the Purkinje cells. Exposure to EMF during gestational stages has been shown to cause the morphological alteration to the offspring of mice and it is speculated that EMF may trigger alterations in CNS morphology too. Recent study by Ragbetli *et al*. (2007) have shown that purkinje cell development can be used to evaluate the effect of diclofenac sodium on the development of rat cerebellum; a reduced number of Purkinje cells may contribute to abnormal CNS development (Kudo *et al*., 2003; Sotelo, 2004). In the present study, our main objectives were to evaluate the effect of low frequency EMF exposure on the developing mice cerebellum as well as adult mice.

Corresponding Author: Y. Rosli, ARC CoE Vision Science Research School Biological Sciences,Australian National University, Canberra ACT 0200 Australia Tel: 61 02 6125-6799 Fax: 61-02 6125-5808

MATERIALS AND METHODS

Animals: All procedures in this study were carried out according to the practices approved by the Animal Ethics Committee, Universiti Kebangsaan Malaysia (FSKB/BIOMED/2008/YANTI/12-AUGUST/231-SEPT-2008-JUNE-2011). The study on the developing mice included sixteen female *Mus musculus* mice weighing between 120-140 g, obtained from the UKM Faculty of Medicine Animal House and were mated overnight in separate standard plastic cages. The presence of the vaginal plug in female mice designated as gestational day 0 of pregnancy. Pregnant mice were house separately in standard plastic cages on sawdust bedding in well ventilated at 24±2°C, under a 12/12 h light/dark cycle and fed *ad libitum*. They were separated randomly into a negative control group (8 animals) and 1.2 mT EMF exposed group (8 animals). In a parallel study, a batch of sixteen female adult *Mus musculus* mice were also divided into two groups, the negative control (8 animals) and 1.2 mT (8 animals) EMF exposed groups; housed in a similar setup to the pregnant mice groups.

Low EMF delivery: The low EMF was created by means of a solenoid coil, consisting of a rigid PVC tube and insulated copper wire of 0.8 mm diameter. The solenoid was supplied by adjustable DC power supply (Daedalon), EMF generated inside the coil was uniform and was measured using a digital Teslameter with axial and transverse probes (Daedalon); the difference between the theoretical and measured values was lower that 1%. The voltage (V) is kept constant by the DC current i; the related equation for the magnetic field is:

$$B = \mu_0 ni. \quad (1)$$

where, μ_0 is the permeability of free space and n = the number of turns (125).

In this study, the constant value of B for a fixed value of i (1.3 V) for all exposures was checked for each pre- and post-experiment. The animals were confined in the solenoid coil are ventilated via the mesh plastic cover, enclosing the PVC tube. The room temperature was kept at 24±2°C throughout the experiment.

In the experimental group the pregnant mice were exposed to EMF on gestation day 3, 6, 9, 12 and 15 for a period of 6 h. After delivery, the offspring were fed for 8 weeks. At the end of the eighth week, the animals were sacrificed using overdose of chloroform. The adult mice group, underwent 6 h of EMF exposure, starting at 9 am, daily for 5 consecutive days. The mice were sacrificed by chloroform overdose 6 h after the final EMF exposure. Mice of the control group were not exposed to low EMF.

Histology: After sacrifice, the mice were decapitated and cerebella were rapidly dissected from the calvaria. For convenience of comparison among each mouse, an equivalent area in the anterior lobe of each mouse was chosen and cut into about 1 cm lengths. One half of the cerebellum was fixed in 4% paraformaldehyde, processed and embedded in paraffin for sectioning. Serial sections of 5 μm thickness in a coronal plane were made and every fifth section through a set of consecutive sections were collected andstained with cresyl fast violet, Nissl and H and E.

Stereological analysis

Purkinje cell counts: Three slides (e.g., a, b and c), stained with H and E, were sampled from cerebellar sections in a systematic random manner and observed under x400 magnification using the light microscope (Olympus, Japan). Cell counts were performed by counting the number of purkinje cells with the widest profile of nucleus along a 1mm linear length of the cerebellar Purkinje cell layer (the viable purkinje cell density mm^{-1}) using an eyepiece micrometer. Purkinje cells with pyknotic characteristics were excluded from the count. Five areas on a slide were chosen and the purkinje cells of each field were calculated and mean calculated (a_1,...a_5) to represent the number purkinje cells observed per slide (Eq. 2). This was repeated for slides b and c, hence the average number of purkinje cells of each animal (A,B,..H) were calculated (Eq. 3). The average number of purkinje cells (N) in the experimental or control group was calculated by taking the mean of all animals of the same group (Eq. 4):

$$a=(a_1 + a_2 + a_3 + a_4 + a_5) \quad (2)$$

$$A=a+b+c \quad (3)$$

$$N_{Control} = \frac{(A+B+...H)}{8} \quad (4)$$

Cerebellar layer thickness measurement: The granular and molecular cerebellar layer thickness was measured using the Image analyzer (Leica, Jerman), attached to a camera (Pixelink PL4662, Canada). The thickness was measured using the Video Test-Master Morphology version 5 (VideoTest, Russia). 5 slides were randomly selected from each sample animal (a, b ... e) and five areas on each slide were chosen (e.g. a_1...a_5; Eq. 5) and the image of each area was grabbed by the frame grabber board via a PC and observed and measured at the x25 magnification. The thickness of the layer (T)

was estimated using the following formulas, eg. in the granular layer, where (A,+B....H) represents the sample animal in a selected study group.

$$a=(a_1 + a_2 + a_3 + a_4 + a_5) \quad (5)$$

$$A=a+b+c+d+e \quad (6)$$

$$T_{Granular} = \frac{(A+B+...H)}{8} \quad (7)$$

Special care in the measurements of the layer thickness was taken which may be biased to an unknown degree by a factor ignored in the tissue preparation, the shrinkage, which may be higher in younger tissue than in older ones. However, this factor may not have affected the results significantly, because we estimated the shrinkage parameter of each cerebellum by measuring the thickness of the lobular flank in fresh tissue and in Nissl stained sections. There was no significant difference in shrinkage of tissues between young and old mice. This has also been found in the human cerebellum by different methods (Andersen*et al*., 2003; Braendgaard *et al*., 1990).

Statistical analysis: In the offspring study groups, unpaired t-tests were carried out on the estimated number of Purkinje cells and estimated cerebellar layer thicknesses. Tukey post-hoc ANOVA was carried out on the adult mice study groups. The differences discussed in the text were significant at $p<0.001$ or $p<0.05$.

RESULTS

Light microscopic studies on the cerebellum revealed significant differences between groups of adult mice exposed to the EMF compared to the control (Fig. 1A, C)

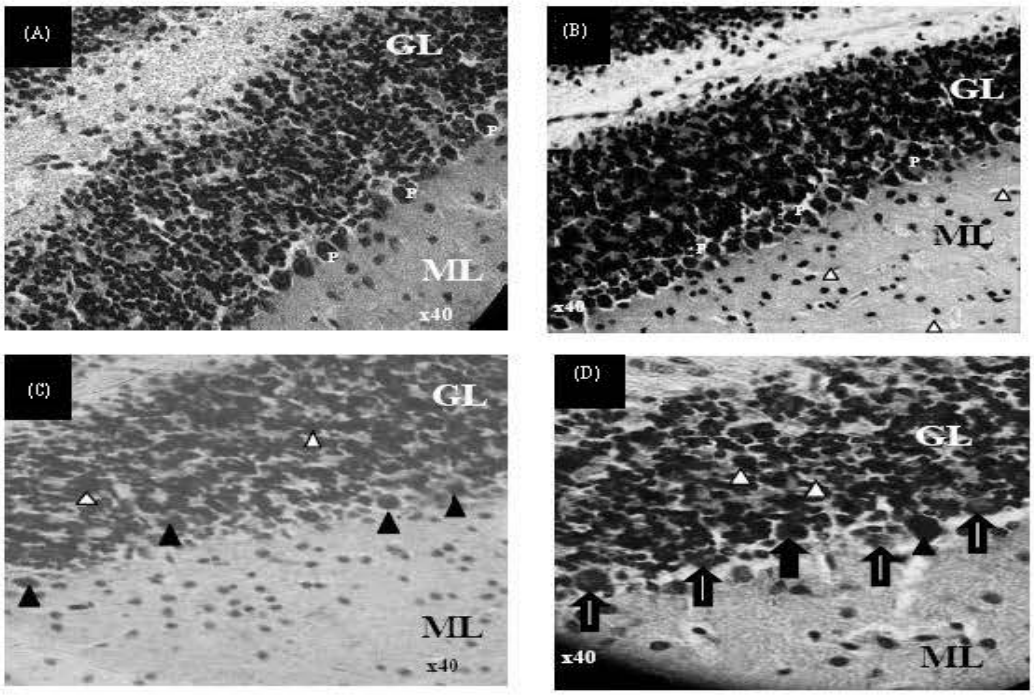

Fig. 1: Icrophotographs of the paraffin sections of cerebellar cortex of adult and offspring after EMF exposure. A and B: Typical features of control cerebella of (A) adult and (B) offspring shows the Granular Layer (GL), the Purkinje Cells (P) and the Molecular Layer (ML) and the granule cells are grouped in clusters. The presence of longitudinal zones is displayed in A whereas the transverse orientations the parallel fibres are more obvious in B (white arrowheads). (C): Cerebellar cortex of the adult experimental group. The Purkinje cells (black arrowheads) are widely separated, the demarcation between the GL and the ML is less maintained and the longitudinal zones of the ML are less often observed. The granule cells clusters (white arrowheads) are also further apart. (D): Cerebellar cortex of offspring experimental group. The Purkinje cells (black arrows) are not as sparse compared to C; but most shows different stages of apoptosis (black arrows with white streak). The separation of the GL and the ML is also less maintained and the granule cells clusters (white arrowheads) are also less compact compared to B

Table 1: The stereological analysis results

	Adult		Offspring	
Parameters	Control (n = 8)	1.2 m T (n = 8)	Control (n = 8)	1.2 mT (n = 8)
Purkinje cells (cells mm^{-1})	16.06±0.3	10.38±0.2**	16.29±0.6	11.97±0.2**
Granular layer (μm)	156.80±10.4	121.34±2.7*	115.83±3.5	16.67±6.0*
Molecular layer (μm)	194.94±7.9	160.80±6.6*	154.67±2.9	163.79±3.7

*Significance level of $p<0.05$, Significance level of $p<0.001$

and offspring that were exposed to low EMF during gestation period (Fig. 1B, D). The granular layer thickness remains almost the same but it consists of many pyknotic cells, between which there are large extracellular spaces with remains of the cytoplasmic structures that lacks organelles. The molecular layer is broader, crossed by orifices and vacuoles. There are fewer Purkinje cells, mostly with roundish soma and also many with degenerative signs: vacuolation, large cellular spaces, disorientation and the present of more pyknotic cells, shrunken nuclear wall with dark fragmented intracellular materials. The Purkinje layer is also misaligned and the demarcation between the ML and the GL is less observed.

There was a 22.3% of reduction in the GL thickness and a 17.5% ML reduction in thickness in the adult experimental group. Interestingly we report an increase in the cerebella layer thickness of the offspring experimental group (18% GL, 5.6% ML) compared to the controls. We also found, a reduction of 35.4% PCS per mm in the adult and 26.5% in the offspring experimental group. In both control groups, thegranular layers are of considerable thickness and compact, the Purkinje cells demarcated the granular and molecular layer clearly (Fig. 1A, B). In the adult treatment group, the granular layer was thinner and granule cells were dispersed, very sparse Purkinje cells bordered the cerebella layers (Fig. 1C). In the offspring, exposed to EMF during gestation however, the conditions were not as severe, although the granule cells were dispersed, but the thickness was maintained. The Purkinje cells were reduced compared to control (Fig. 1B) but occasionally displayed pyknotic pattern (black arrowheads, Fig. 1D). The results from the stereo analysis confirmed the reduced granular layer thickness in adult treated group and reduced numbers of Purkinje cells in both treatment groups compared to their respective controls were described in Table 1.

DISCUSSION

The cerebellum is one of the best characterized regions of the brain with respect to the development. It undergoes dramatic developmental changes during the first 3 weeks of postnatal life in the mouse (Goldowitz and Hamre, 1998). Duringthis period, neuroblasts proliferate in the EGL and differentiate into cerebellar granular cells that migrate toward the internal granular layer. This leads to a dramatic increase in the volume of the cerebellum (over 1,000-fold) and to the formation of the mature cerebellar structure, including deep fissures and folia. In contrast to the granular cells, PCS have already stopped proliferating at birth and then (P3-P28) extend their dendrites into the molecular cell layer, where they form synapses with parallel fibers and climbing fibers from granule cells and inferior olivary nucleus cells, respectively (Hatten and Heintz, 1995; Voogd and Glickstein, 1998).

The noxious effect of EMF on the cerebellar cortex is more apparent in the treatment group. Our study concurs with (Espinar *et al*., 1997), who reported histological changes in chick embryo exposed to static magnetic fields, he suggested that in the case where the exposure is low, some injured nerve cells can actually regenerate if the period between exposure and sacrifice in sufficient Similar effect was seen in our offspring experimental groupand when the period between exposure and sacrifice period is short, then the cells cannot overcome damage which is evident in an adult experimental group.

According to Smeyne *et al*. (1995), PCS have been identified as a key element in the topographical organisation of the cerebellum, as such increase of 18% in GL thickness despite the 26.5% reduction of PCS per mm of cerebellum is quite remarkable. Two possible factors which may have contributed to this effect:. the first, intermittent exposure on selected gestation days allows sufficient recovery time for the PCS and the granular cells.

Previous studies have also demonstrated that PCS can control the mitotic activity of granule cells (Herrup, 1983; Smeyne *et al*., 1995; Sonmez and Herrup, 1984), which leads to the second factor i.e., although the number of PCS are reduced, but the time course of which the actual cell death occurs is long (Dusart *et al*., 2006) as PCS do not undergo significant levels of natural cell death (Dusart *et al*., 2006; Norman *et al*., 1995). Therefore, the granule cells are not affected by the PCS undergoing apoptosis and continue to proliferate in the offspring experimental group. This study demonstrates, the impact of depleting number of PCS is to affect the size of granule cell populations. With a very short recovery time (the period between low EMF exposures), a reduction of 35.4% PCS per mm coincides with 22.6% reduction of GL thickness compared to the controls in the adult experimental group.

CONCLUSION

The present study shows that low EMF can induce irreversible destruction to the brain of adult mice, specifically the reduction of the purkinje cells with the sequel of thinning of the granular layer. The effect appears less remarkable in the offspring which were exposed to EMF during gestation period, as they were born with no obvious physical defects and the regeneration of the cerebellar granular layer in the developing mice masks the sinister Purkinje cells reduction. Further, studies are required to establish this alteration in the microenvironment of a neuron during its development can compromise its later maturation and also to establish the molecular mechanism of EMF action.

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