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Research Report

# 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat

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ABSTRACT

The effects of electromagnetic fields (EMFs) emitted by mobile phones on humans hold special interest due to their use in close proximity to the brain. The current study investigated the number of pyramidal cells in the cornu ammonis (CA) of the 16-week-old female rat hippocampus following postnatal exposure to a 900 megahertz (MHz) EMF. In this study were three groups of 6 rats: control (Cont), sham exposed (Sham), and EMF exposed (EMF). EMF group rats were exposed to 900 MHz EMF (1 h/day for 28 days) in an exposure tube. Sham group was placed in the exposure tube but not exposed to EMF (1 h/day for 28 days). Cont group was not placed into the exposure tube nor were they exposed to EMF during the study period. In EMF group rats, the specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). All of the rats were sacrificed at the end of the experiment and the number of pyramidal cells in the CA was estimated using the optical fractionator technique. Histopathological evaluations were made on sections of the CA region of the hippocampus. Results showed that postnatal EMF exposure caused a significant decrease of the pyramidal cell number in the CA of the EMF group ($P<0.05$). Additionally, cell loss can be seen in the CA region of EMF group even at qualitative observation. These results may encourage researchers to evaluate the chronic effects of 900 MHz EMF on teenagers' brains.



## 1. Introduction

Mobile phones have been available since the end of the 1980s and have become common in the general population in recent years. In several countries, more than 80% of the population uses mobile phones today (Feychting et al., 2005). This worldwide expansion of the use of mobile phones has made electromagnetic field (EMF) exposure ubiquitous in modern

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society. Therefore, the possible side effects of EMFs emitted by cellular phones on human meningeal tissue, brain, and nervous system have aroused considerable interest among both the scientific community and the general public (Dubreuil et al., 2002; Mausset-Bonnefont et al., 2004; Hietanen, 2006; Lahkola et al., 2008). Although numerous studies have been carried out in the epidemiology, cellular biology, and toxicology research fields, the potential adverse effects of EMF exposure on the human central nervous system (CNS) are still controversial (Hietanen, 2006).

The effects of EMFs emitted by mobile phones on the CNS have become a particular focus of concern owing to the fact that they are used in close proximity to the brain (Mausset et al., 2001; Dubreuil et al., 2002, 2003; Odaci et al., 2008). According to EMF studies conducted on animal models during the prenatal and postnatal periods, the cellular responses to various forms of radiation, including ionizing and UV-radiation, are manifested as reversible or irreversible structural or functional changes (Koyu et al., 2005) on the CNS (Odaci et al., 2008). In vitro studies examined cell proliferation, DNA damage, gene expression, protein synthesis, embryonic development, and cancer promotion, while in vivo studies examined DNA in brain cells, blood–brain barriers, neuroendocrine, electro-neurophysiology, and CNS tumorigenesis as the ongoing responses commonly manifested in animal models (Lai and Singh, 1995, 1996; Lin, 1997; Xu et al., 2006; Arns et al., 2007). Some of these experiments were focused on hippocampal cell morphology (Xu et al., 2006; Odaci et al., 2008) due to the fact that the hippocampus is the part of the brain that controls important behavioral and cognitive functions, including spatial learning and working memory (Eichenbaum et al., 1992; McEwen, 1994; Lemaire et al., 2000; Eyre et al., 2003).

Studies reported that female humans including children and adolescents use regular mobile phone more than male humans and they also tend to talk more by mobile phone than male humans (Söderqvist et al., 2007; 2008). Therefore, it has been claimed that the female gender is more exposed to EMFs than the male gender. The current study investigates the effect of postnatal exposure to a 900 megahertz (MHz) EMF on the number of pyramidal cells in the cornu ammonis (CA) of the hippocampus of 16-week-old female rats.

## 2. Results

### 2.1. *Weight of female rats*

At the 16th week, there was no significant difference between EMF and Cont, and also between the weights of EMF and Sham groups ($P>0.05$).

### 2.2. *Histopathological observations*

Sections of the control (Cont), sham exposed (Sham), and EMF exposed (EMF) group were examined at the 16th week (Fig. 1). The morphology of the pyramidal cells was normal in both the Cont (Fig. 1A) and Sham (Fig. 1B) groups of rats. However, there were substantial cell losses seen in the pyramidal cell layer of hippocampus of EMF group in comparison with other groups (Fig. 1C). A previous study reported damaged neurons ("dark cells") in rat brains after EMF exposure (Salford et al., 2003). A semi-quantitative analysis of dark cell counts in our sections showed that the numbers of dark cells were increased in comparison with the control and sham groups (Fig. 2).

### 2.3. *Pyramidal cell numbers in the cornu ammonis*

Pyramidal cell numbers were estimated in the three groups. In the control, the total number was about 595,000 (mean). This number is close to, but slightly lower than the number of about 650,000–700,000 reported in previous studies (Schmitz and Hof, 2005). This minor difference in absolute numbers is likely due to the use of paraffin sections with a bimodal particle distribution in the z-axis of tissue sections (see below). The total number of pyramidal cells in the EMF group was significantly lower than those of Cont and Sham groups ($P<0.05$; Table 1). However, there was no significant difference between the Cont and Sham groups' total number of pyramidal cells ($P>0.05$). In Table 1, the coefficient of variation (CV) and the coefficient of error (CE) of the estimated number of pyramidal cells for the Cont, Sham, and EMF groups are shown. The CE and CV values were within acceptable ranges as described in earlier studies (Gundersen and Jensen, 1987; West et al., 1991).

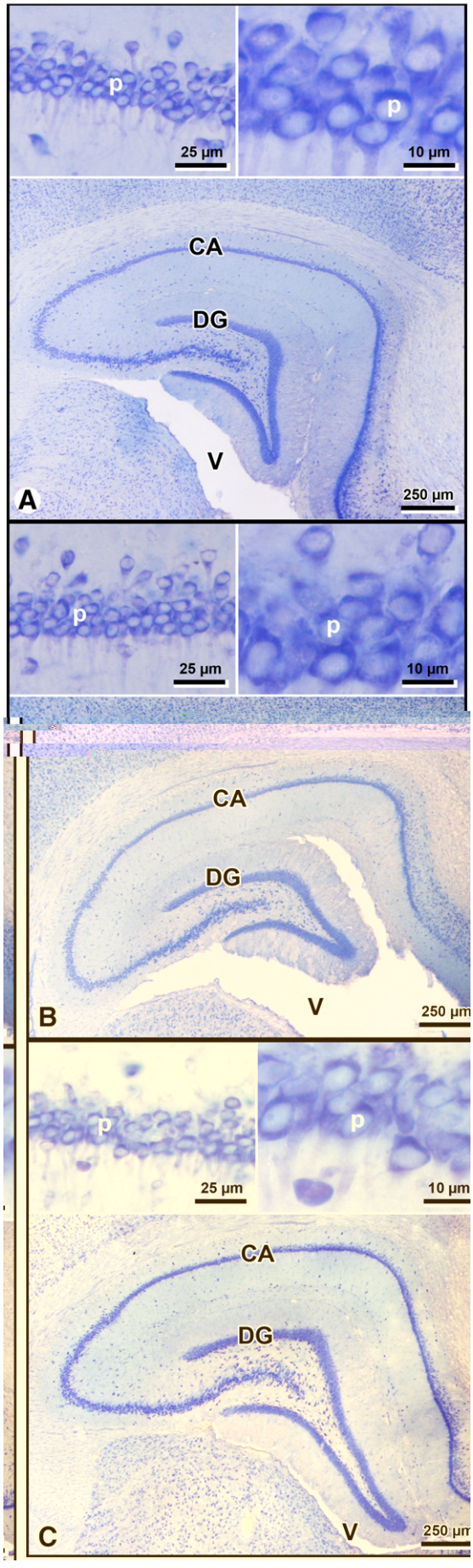
### 2.4. *Particle distribution in the z-axis axis of the section*

Analysis of the particle distribution in the z-axis showed that relatively more particles in the sections were located near the upper surface of the section. The core of the section had relatively fewer particles in comparison with the upper part of the section (Fig. 3). This result is consistent with a previous study on the distribution of particles in the z-axis of paraffin sections, and in particular with sections cut with a large or small knife angle (Baryshnikova et al., 2006), and may explain the small difference in absolute estimates of pyramidal cell numbers.

## 3. Discussion

The rapid development of mobile communications contributes to the general argument and concern about the effects of EMF on human health. It also raises questions as to whether these effects are biological or non-biological, where the latter is an indirect effect following use of mobile phones rather than a direct effect of emissions (Söderqvist et al., 2008), and especially whether adverse effects of mobile phone use on the human brain and brain related tissues, such as cerebellum and hippocampus (Dubreuil et al., 2002; Mausset-Bonnefont et al., 2004), stem from the mobile phone's close proximity to the brain.

Usage of mobile phones by children and teenagers deserves a special concern because, with the relevance of mobile phone use among the general population in recent years, this group will experience a much higher cumulative exposure to EMF than previous generations (Söderqvist et al., 2007; 2008). In scientific research connected with EMF, another interesting question is whether children are more sensitive to EMF



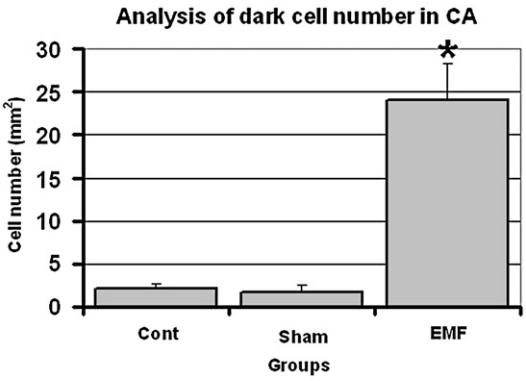




**Fig. 2 – Numerical density of dark ($mm^2$) cells in the sections of all groups. Dark cell density was significantly increased in comparison with both the control and sham groups, (Mean ± SEM), $^*p < 0.01$.**

exposure than adults. (Söderqvist et al., 2008). The main difference concerning the use of mobile phones between today's children and adults is that children start to use mobile phones at an early age and, therefore, will have a longer lifetime exposure (Schuz, 2005).

Although there are many concerns and debates among investigators, scientific grounds are not sufficient to prohibit or limit mobile phone use by children (Leitgeb, 2008). For example, while some researchers suggest that the use of mobile phones, for more than the 10-year latency period, may increase the risk of developing malignant brain tumors (Hardell et al., 2006, 2007), epidemiological studies do not support these findings (Hardell et al., 1999; Inskip et al., 2001; Auvinen et al., 2002; Lönn et al., 2005; Hardell et al., 2006; Schüz et al., 2006a,b; Lahkola et al., 2008). Therefore, a specific experimental study, such as ours, investigating the effect of postnatal exposure to EMF on the number of pyramidal cells in the CA region of the hippocampus of rats, may provide useful data on the possible toxic effects of mobile phone use on the CNS. We exposed female rats to 900 MHz EMF based on the fact that most mobile phones, in Europe, operate at a frequency of 900 MHz (Dubreuil et al., 2002, 2003; Koyu et al., 2005; Panagopoulos et al., 2007).

In the present study, 16-week-old female rats were used that may be comparable with the age of human teenagers. The intense use of mobile phones by teenagers is a serious concern as the biological and maturational processes of the brain are particularly vulnerable during the growth process. Recently, in

**Fig. 1 – Panoramic views of the Cont (A), Sham (B) and EMF (C) groups. There may not be easily seen a distinct difference among groups in the panoramic pictures of them. Difference between EMF and the Sham or Cont groups can be observed when density of pyramidal cell is compared between groups in the magnified views of group (the right pictures of A–C). As stated in the result section postnatal EMF exposure caused a significant decrease of the pyramidal cell number in the CA of the EMF group in comparison with the Sham or Cont groups. CA, cornu ammonis; DG, dentate gyrus; V, ventricle; p, pyramidal cell of cornu ammonis.**

**Table 1 – Mean total pyramidal cell numbers, mean disector number, section thickness, number of steps, CV and CE of stereological analysis for estimation of total neuron number in the CAs of the Cont, the Sham and the EMF groups of rats**

| | Cont group ($n$=6) | Sham group ($n$=6) | EMF group ($n$=6) |
|---|---|---|---|
| Total pyramidal cell number[a] | 595,209±25,111 | 600,053±25,651 * | 504,905±11,997 ** |
| Disector particle number | 1035 | 1038 | 893.3 |
| Section thickness (μm) | 21.75 | 21.25 | 21.4 |
| Number of steps for counting | 323 | 325 | 317 |
| Number of sampled sections | 15 | 15.3 | 14.5 |
| CE | 0.031 | 0.031 | 0.033 |
| CV | 0.042 | 0.041 | 0.023 |

[a] Values are mean±SD. CA, cornu ammonis; Cont, control group; Sham, sham exposed group; EMF, electromagnetic field exposed group; CE, coefficient of error; CV, coefficient of variation.
* $p<0.05$, Sham group vs EFM group.
** $p<0.05$ Cont group vs EFM group.

findings similar to ours, a study reported evidence of neuronal damage in the cortex, hippocampus, and basal ganglia in the brains of EMF-exposed rats. Interestingly, they also used 12–26-week-old rats. They showed scattered and grouped dark neurons, which were often shrunken and darkly stained, homogenized with loss of discernible internal cell structures. They also reported that the percentage of abnormal neurons is roughly thought to be maximally around 2%, but in some restricted areas they dominated the picture (Salford et al., 2003).

Although the previous histopathological observation clearly demonstrated neuronal damage in the cortex, hippocampus, and basal ganglia in the brains of exposed rats, these were semi-quantitative results (Salford et al., 2003). Our analyses showed that the total pyramidal cell number in the CA of the hippocampus of the EMF group was significantly lower than that of the Cont and Sham groups.

To our knowledge, this finding is the first quantitative report in the literature about EMF effects on the adult rat brain. Recently, we reported the effect of prenatal exposure to a 900 MHz EMF on the number of granule cells in the dentate gyrus (DG) of 4-week-old rats. Our previous results showed, for the first time, a cell loss in the DG due to prenatal EMF exposure, and cell loss can be seen as a result of chronic prenatal exposure to EMFs (Odaci et al., 2008).

In conclusion, we showed that exposure to 900 MHz EMF results in neuronal damage and cell loss in the CA region of the 16-week-old female rat hippocampus. Based on the literature relevant to our current study findings, it has been proposed that chronic exposure of EMF to postnatal 12–16-week-old female rats plays a critical role in the neuronal formation of the hippocampus. The findings of our current study may encourage researchers to evaluate the chronic effects of 900 MHz EMF on CNS of teenagers. Additional experimental studies are necessary to define the effects of EMF with different duration on CNS neurons.

## 4. Experimental procedures

### 4.1. *Animals and study protocol*

Eighteen Wistar Albino 12-week-old female rats, with initial weights of 270–300 g, were obtained from the Experiment Animals Research and Application Center of Afyon Kocatepe University (Afyon, Turkey). All experiments described in the present study were conducted according to institutional guidelines. The Animal Ethics Committee of Afyon Kocatepe University approved the protocol, and appropriate measures were taken to minimize pain or discomfort by our study group. Animals were maintained under a 12:12-h day/night cycle in a temperature-controlled animal room (22±1 °C) in our laboratory, with continuous free access to food and water ad libitum. Rats were randomly divided into three equal groups as described previously (Odaci et al., 2008): the control ($n$=6) (Cont), the sham exposed ($n$=6) (Sham), and EMF exposed ($n$=6) (EMF). In short for the EMF group, rats were exposed to 900 MHz EMF 1 h/day for 28 days in an exposure tube, the rats of Sham group, were placed into the exposure tube without exposure of EMF. The exposure period for the EMF and Sham groups was from 11:00–12:00 a.m. each day. No exposure was applied to the rats of Cont group, and they lived freely in their cages under the normal laboratory conditions without stress.

All rats were anaesthetized with urethane 1.25 g/kg intraperitoneally and were perfused with neutral formalin intracardially at the end of 16 weeks. After the brains were processed through graded alcohols and xylene, they were embedded in paraffin blocks. The hippocampus sections were taken using a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany) with disposable metal microtome blades (Type N35, Feather Company, Osaka, Japan) to obtain 30-μm-thick serial sections in a sagittal plane from the paraffin block of tissues. Each sampled section of brain hemisphere that included the hippocampus was collected on gelatin–formaldehyde mixture coated slides and stained with cresyl violet for stereological and histopathological evaluations.

### 4.2. *Exposure system and application of electromagnetic field*

Exposure system and application of electromagnetic field in this study was described previously (Koyu et al., 2005; Köylü et al., 2006; Yildiz et al., 2006; Odaci et al., 2008), in brief, a specially designated EMF exposure system, with a round plastic tube cage and a dipole exposure antenna was used for the exposure of female rats to EMF. An electromagnetic energy generator, which produces 900 MHz continuous modulated EMR (2 W peak output power and 1±04 mW/cm$^2$ power density), was manufactured at the Electromagnetic Compatibility Laboratory at Suleyman Demirel University (Koyu et al., 2005; Ozguner et al., 2005; Köylü et al., 2006; Yildiz et al., 2006) and used in this study. The specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). It should be noted that we only obtained the average value in the whole brain but not the

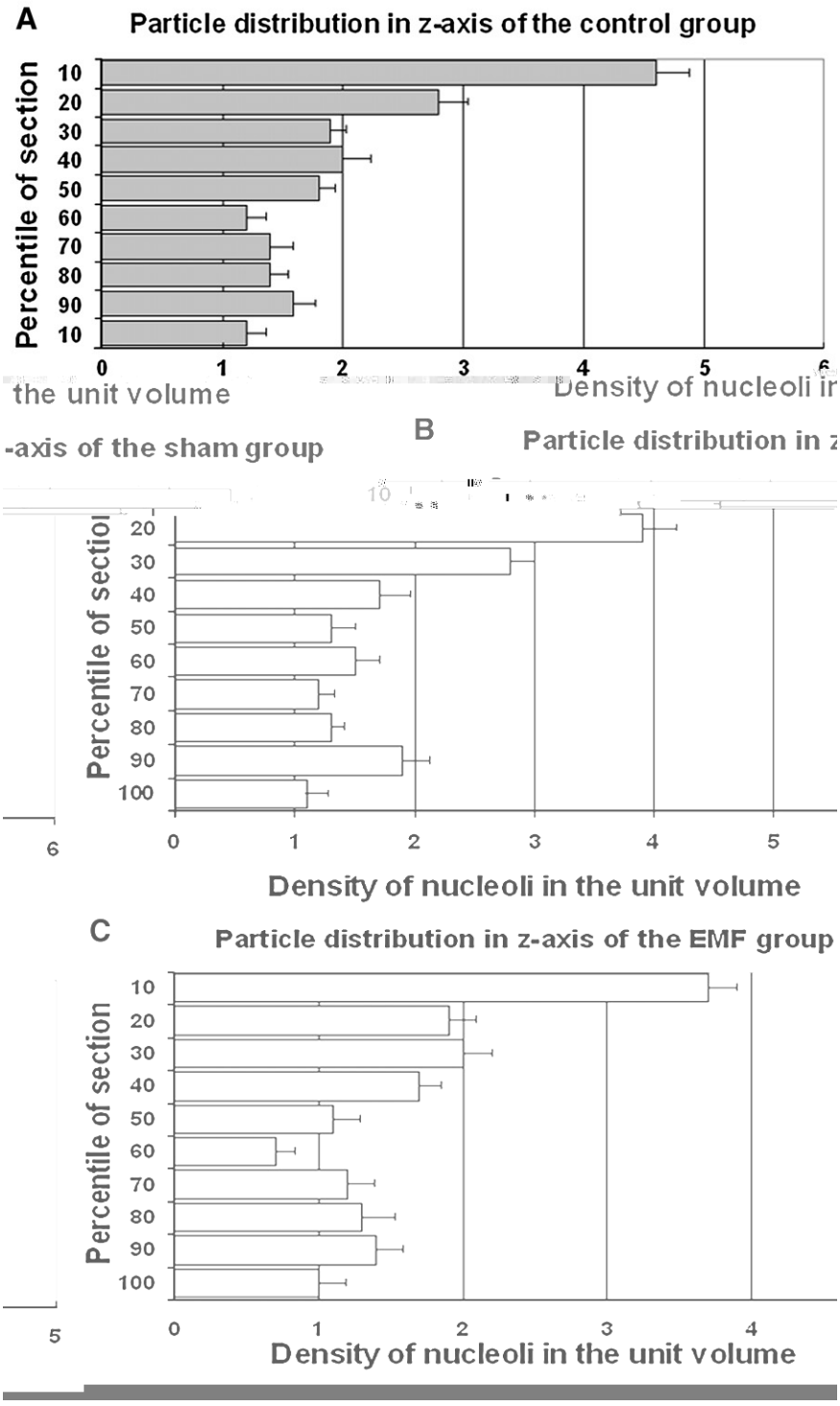



**Fig. 3 – Particle distribution in z-axis of the paraffin sections. The densities of nucleoli in the sections are seen. The top of the tissue section (adjacent to the coverslip) is the "19" percentile, the bottom of the section (adjacent to the glass slide) is the "109" percentile. All analyses showed that particles are more crowded in the upper part of sections, partly in the bottom, but not in the core of the sections. A, the control; B, the sham; C, EMF; (Mean ± SEM).**

SAR value in the area where we looked for morphological changes. Therefore, the brain average SAR value of 2 W/kg showed only the average value in the whole brain. The peak SAR value was obtained by means of model calculations, and the power density measurements were made using an EMF meter (Holaday Industry Inc., Adapazarı, Turkey). The EMF group of rats was positioned in close contact above the dipole antenna and exposed to the EMF (Koyu et al., 2005; Köylü et al., 2006; Ozguner et al., 2005; Yildiz et al., 2006; Odaci et al., 2008) that was positioned with a distance of 1 cm between

them and perpendicular to the dipole antenna inside the tube. Heads of rats were positioned in the direction of antenna. The long axis of the antenna was perpendicular to the long axis of the rats, since all rats were equally exposed to EMF between two ends of the antenna. The Sham group rats were positioned as the rats of EMF group without EMF exposure. The position of rats during EMF exposure in each day was changed so that exposure can cover the whole brain (Odaci et al., 2008).

### 4.3. *Stereological analysis system and stereological analyses*

Stereological analyses were done blind to treatment to obtain unbiased results. The total number of pyramidal cells in the CA of the hippocampus was estimated by means of the optical fractionator technique as explained previously (West et al., 1991; Hausdorf et al., 2008; Odaci et al., 2008). In brief, cell counting was done using a stereological workstation that is controlled by software (CAST; Olympus, Glostrup, Denmark). Analysis was carried out at a final magnification of ×5139 (i.e., by using a 63× Leica HCX Plan Apo objective; NA=1.40). Total number of pyramidal cells of hippocampus (*N*) was estimated via following formula:

$$N = \sum Q \cdot \frac{1}{\text{ssf}} \cdot \frac{1}{\text{asf}} \cdot \frac{1}{\text{tsf}}$$

where ∑Q represents the total disector number of pyramidal cells counted in all optically sampled fields of the hippocampus; ssf is section-sampling fraction (1/15); asf is area sampling fraction (100/2500), and tsf is thickness sampling fraction.

Prior to beginning the study, a pilot study was carried out to determine the sampling and counting schedule. On the basis of the pilot study, the first section in the series to be analyzed was chosen at random from the first 7 sections, and every successive 7th section was collected from the series, resulting in a section-sampling fraction (ssf) of 1/7. It has been shown that about 14–17 sections from each brain are adequate to estimate the total neuron number during the application of the optical fractionator method (Gundersen and Jensen, 1987; West et al., 1991; Schmitz and Hof, 2005; Tunc et al., 2007; Odaci et al., 2008). The counting frame size and step size are 596 $\mu m^2$ and 40,000 $\mu m^2$ for pyramidal cells in the CA respectively. This means that area sampling fraction (asf) is 596 $\mu m^2$/40,000 $\mu m^2$. Disector height was 10 μm and a 5-μm zone at the uppermost part of the section was excluded from the analysis at every step as the upper guard zone. Therefore, a thickness sampling fraction (tsf) of 10 μm/*t* was used, where *t* represents the mean section thickness (Table 1).

The nucleus of pyramidal cell was accepted as disector particle and counted if the largest nuclear profile of it came into focus within the unbiased virtual counting frames systematically and randomly spaced throughout the delineated regions. An expert on the type of cells in the hippocampus did cell counting in the CA region. The pyramidal neurons in the CA of hippocampus can be easily distinguished from other cell types like neuroglia in the pyramidal cell layer. Since the size of nuclei of pyramidal cells is larger than the nuclei of neuroglia and also the cytoplasm of neuron in this layer is stained darkly with cresyl violet, while neuroglia do not have such features. On the other hand, the percent of neuroglia in the pyramidal cell layer are low like 2–3%. The amount would not significantly affect our pyramidal cell counting. Estimated total numbers of neurons were calculated from the number of counted neurons and the sampling probability (Gundersen, 1986; West et al., 1991). During the counting of the pyramidal neurons of areas CA1 to CA3, these neurons were not considered to be different parts of the region because it is difficult to define exact boundaries between the hippocampal areas (Odaci et al., 2008).

By means of the coefficient of error (CE) and the coefficient of variation (CV), the efficiency of sampling and the convenient number of sampled cells for the estimation of total neuron number were checked as previously reported (Gundersen and Jensen, 1987). The CE of the sampling schedule of the hippocampus was validated from the pilot study. As stated previously, the CE should be ≤10% (Gundersen and Jensen, 1987; West et al., 1991). It was also possible to estimate the CV within the hippocampus in each group. This is valuable data to see whether the number of subjects in each group is sufficient. In Table 1, details of counting procedures, the mean CV for each group, and the mean CE for stereological estimation of neuron number and other stereological parameters are given.

Particle distribution in z-axis was analyzed as previously described (Baryshnikova et al., 2006; Hatton and von Bartheld, 1999). In brief, a stereological workstation that is controlled by software (CAST-GRID®-Computer Assisted Stereological Toolbox-Olympus, Copenhagen, Denmark) was used to control measure and record the data. The sections were viewed with an immersion oil objective (Obj. 63×/1.40) on a Leica microscope to identify the nucleoli of pyramidal cells in the hippocampus. The localization of each pyramidal cell nucleolus within the z-axis and within the view field was determined by using the microcator with a high resolution (Total magnification 3212). For the measurement of the position of each nucleolus, the thickness of the section was determined by focusing in one sweep from the upper to the lower section surface. The position of nucleoli in the z-axis of tissue sections was determined for pyramidal cells in paraffin sections. The thickness of each section was measured in each step of z-axis analysis. After completing the counting of particle distribution in z-axis, the whole section thickness in each analyzing point was divided to 100 than that particle localization in z-axis was determined as percentile of section (10% to 100%).

### 4.4. *Statistical analysis*

All data were expressed as means ± standard deviation (SD) for each group. Comparisons between the cell numbers of hippocampus were carried out using Kruskal–Wallis test. As stated previously when the variances in the population are not equal, nonparametric test must be used (Tunc et al., 2007). In this study variances were not homogeneous and compared groups were independent so we used Kruskal–Wallis one-way analysis of variance in lieu of parametric single-factor between-subjects analysis of variance. To compare neuron numbers between the groups we used one of nonparametric

test called the Mann–Whitney *U* test. Mean values were considered to be significantly different when $p<0.05$. All statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 13.0, SSPS Inc., Chicago, IL, USA).

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