

The chronic effect of pulsed 1800 MHz electromagnetic radiation on amino acid neurotransmitters in three different areas of juvenile and young adult rat brain

Nawal A Ahmed¹, Nasr M Radwan¹, Heba S Aboul Ezz¹,
Yasser A Khadrawy²  and Noha A Salama¹

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Abstract

The extensive use of mobile phones worldwide has raised increasing concerns about the effects of electromagnetic radiation (EMR) on the brain due to the proximity of the mobile phone to the head and the appearance of several adverse neurological effects after mobile phone use. It has been hypothesized that the EMR-induced neurological effects may be mediated by amino acid neurotransmitters. Thus, the present study investigated the effect of EMR (frequency 1800 MHz, specific absorption rate 0.843 W/kg, power density 0.02 mW/cm², modulated at 217 Hz) on the concentrations of amino acid neurotransmitters (glutamic acid, aspartic acid, gamma aminobutyric acid, glycine, taurine, and the amide glutamine) in the hippocampus, striatum, and hypothalamus of juvenile and young adult rats. The juvenile and young adult animals were each divided into two groups: control rats and rats exposed to EMR 1 h daily for 1, 2, and 4 months. A subgroup of rats were exposed daily to EMR for 4 months and then left without exposure for 1 month to study the recovery from EMR exposure. Amino acid neurotransmitters were measured in the hippocampus, striatum, and hypothalamus using high-performance liquid chromatography. Exposure to EMR induced significant changes in amino acid neurotransmitters in the studied brain areas of juvenile and young adult rats, being more prominent in juvenile animals. It could be concluded that the alterations in amino acid neurotransmitters induced by EMR exposure of juvenile and young adult rats may underlie many of the neurological effects reported after EMR exposure including cognitive and memory impairment and sleep disorders. Some of these effects may persist for some time after stopping exposure.

Keywords

Electromagnetic radiation, amino acid neurotransmitters, hippocampus, striatum, hypothalamus, rat

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Introduction

Radiofrequency electromagnetic radiation (RF-EMR) is a form of energy between 10 kHz and 300 GHz in the electromagnetic spectrum. The rapid development and widespread use of mobile phone systems have raised concerns about the negative health effects of electromagnetic exposure on the human organism and, in particular, the brain (Hermann and Hossmann, 1997; Thomée et al., 2011). The proximity of the mobile phone device to the head permits 75% of the energy produced by the mobile phone to penetrate the head for a depth of several centimeters (Weinberger and Richter, 2002).

Human studies on the effect of EMR on child development and behavioral problems suffer from conflicting results and methodological limitations.

¹Department of Zoology, Faculty of Science, Cairo University, Giza, Egypt

²Medical Division, Department of Medical Physiology, National Research Center, Giza, Egypt

Corresponding author:

Yasser A Khadrawy, Medical Division, Department of Medical Physiology, National Research Center, El-Behouth Street, Giza 12622, Egypt.

Email: yaserask@yahoo.com

Concerns about the potential susceptibility of children to mobile phone radiation were first raised by the Independent Expert Group on Mobile Phones (2000) on the grounds that children have a developing nervous system and a longer lifetime of exposure than adults. Moreover, their brain tissue may absorb and conduct RF-EMR energy more than adults because of its higher water content and ionic concentration. There are multiple studies showing that children absorb more microwave (MW) radiation than adults because children have different physiognomy (Christ et al., 2010; de Salles et al., 2006; Gandhi et al., 1996; Wiart et al., 2008). Kuster (2009) clarified that the absorption of RF by the central nervous system (CNS) is higher (approximately 2×) in children because the MW source is closer, and the skin and bone are thinner.

Exposure to different frequencies of nonionizing EMR induced changes in blood–brain barrier (Nittby et al., 2009; Sirav and Seyhan, 2011), morphology (Narayanan et al., 2009), electroencephalogram (Leung et al., 2011; Mohammed et al., 2011; Lustenberger et al., 2013), and energy metabolism (Kwon et al., 2011). Different brain activities such as sleep (Munezawa et al., 2011), attention (Edelstyn and Oldershaw, 2002), learning and memory (Fragopoulou et al., 2010; Narayanan et al., 2009), and cognitive performance (Nittby et al., 2009) have also been reported to be affected by EMR emitted from mobile phone. In addition, frequent users of mobile phone suffer from stress, sleep disturbances, and symptoms of depression (Thomée et al., 2011).

Amino acid neurotransmitters are among the most widely distributed neurotransmitters in the CNS. They include the excitatory amino acid neurotransmitters (glutamate and aspartate; Farso et al., 2009) and the inhibitory amino acid neurotransmitters (gamma aminobutyric acid (GABA), glycine, and taurine; Saransaari and Oja, 2010). Glutamate is the main excitatory transmitter in the CNS. It constitutes 50–60% of all neurotransmitters in the brain and is found in more than 80% of all neurons (Gao and Bao, 2011). The remaining 40–50% is GABAergic (Winfield et al., 1980, 1981). GABA is a major inhibitory amino acid in the brain (Zhang et al., 2004). Aspartate is also a major excitatory neurotransmitter and can activate neurons in the place of glutamate (Blaylock, 1997; Choi, 1988).

Glutamate has been shown to play important roles in many brain functions, including neurodevelopment (Luján et al., 2005), learning (Stanton, 1996), and the

stress response and anxiety disorders (Cortese and Phan, 2005; Meldrum, 2000). The balance of glutamatergic and GABAergic neurotransmission is crucial to the proper functioning of the CNS (Linden and Schoepp, 2006; Yildiz-Yesiloglu et al., 2006).

Mason et al. (1997) recorded significant increases in the concentrations of aspartic acid, serine, and glycine in the hypothalamus and caudate nucleus in response to the thermal stress produced by RFR exposure (5.02 GHz, 10 µs pulse width, 1000 pps), with no changes in the concentrations of glutamic acid and glutamine. Mausset et al. (2001) reported that RF exposure (900 MHz, specific absorption rate (SAR) of 4 W/kg) emitted with a pulsed mode (global system for mobile communications (GSM)) affected not only the distribution of GABA but also most likely its local concentration.

Kuznetsov et al. (1991) reported that irradiation of the whole body with 800 mHz MWs at a modulation frequency of 16 Hz readily decreased the GABA receptor concentration. Mausset-Bonnefont et al. (2004) reported a decrease in the amount of *N*-methyl-D-aspartate (NMDA) receptors at the postsynaptic membrane after exposure of rat head to 900-MHz pulsed MWs. Moreover, EMR-induced alterations in amino acid neurotransmitters have been reported after exposure to continuous 900-MHz radiofrequency radiation (RFR) (Ahmed et al., 2007; Khadrawy et al., 2008, 2009; Radwan et al., 2007; Noor et al., 2011).

However, limited literature deals with the effect of chronic exposure to pulsed EMR on amino acid neurotransmitters in different areas of young adult and juvenile rat brain. The appearance of adverse neurological effects after mobile phone use and the involvement of amino acid neurotransmitters in the normal functioning of the brain suggest that the EMR-induced neurological effects may be mediated by alterations in amino acid neurotransmitters. Thus, the aim of the present study was to investigate the effect of pulsed EMR (frequency 1800 MHz modulated at 217 Hz, SAR 0.843 W/g, power density 0.02 mW/cm²) on amino acid neurotransmitters in the hippocampus, striatum, and hypothalamus of both young adult and juvenile rats.

Materials and methods

Experimental animals

The experimental animals used in this study were juvenile (1-month-old and weighing 100 g) and young

adult (3-month-old and weighing 200–250 g) male Wistar albino rats. The animals were obtained from a fixed local supplier (Animal house of the National Research Centre). They were maintained on stock diet and kept under fixed appropriate conditions of housing. They were kept at a room temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $60 \pm 5\%$.

Chemicals

Absolute ethyl alcohol, analytical-grade lithium carbonate, and dansyl chloride were obtained from Sigma-aldrich (St Louis, Missouri, USA), while high-performance liquid chromatography (HPLC)-grade acetonitrile was supplied by Fisher (UK). Free amino acids for standards were purchased from BDH (England). HPLC-grade methanol (BDH), glacial acetic acid (Fisher), and triethylamine (Sigma-Aldrich, St Louis, Missouri, USA) were also used.

Electromagnetic exposure setup

The EMR exposure system and method of exposure were described in detail by Khadrawy et al. (2009). The electromagnetic field (EMF) was generated by a RF source that simulated the actual sources encountered in mobile communications such as stations, handsets, and so on. This source consisted of an RF signal generator (Aeroflex Company, Model: 2025, UK) with an RF output level up to 13 dBm and a frequency range of 0–2.5 GHz; two monopole antennas set in parallel with the help of a T junction, each being designed so that the reflection coefficient at its input is not more than -12 dBm and fed by a coaxial line through a BNC connector; a power amplifier (Stealth Microwave, Model: SM 0520-36, SSB Technologies, Inc., USA) to supply a power intensity of 0.02 mW/cm^2 at the rat location, which is equal to the average power radiated from an actual mobile communication source; and a field survey meter (Narda, EMR200, frequency from 0 to 4 GHz, Germany) to measure the power intensity of EMFs at different distances from the generator.

EMF exposure

At the beginning of the experiment, the RF signal generator output was connected to the power amplifier the output of which was in turn connected to the T junction, to feed concurrently the two monopole antennas. Each monopole antenna was placed at the center of a circular equally radiated container, made

of an opaque acrylic substance called presidex, pointing downward. The length of the monopole was set so that the antenna was resonant at the operating frequency. The antenna had an omnidirectional pattern in the azimuth plane through which the rats were to be uniformly distributed. Before placing the rats in the container, an intensity field meter was used to measure the EMF distribution at the different locations of the circular container, especially near the expected locations of the rat heads. The antenna was removed and the rats (16) were placed in the sectors (16) of the container with their heads toward the antenna. The container was designed in such a way that the animals were not restrained. The animals were left for a period to adapt and direct themselves spontaneously toward the antenna as the air openings were concentrated in the cover of the container around the antenna. The antenna was then placed again at the center of the container, and the rats were exposed to the RF fields at a frequency of 1800 MHz and a power density of 0.02 mW/cm^2 for 1 h. The average SAR was 0.843 W/kg as calculated by the finite-difference time-domain (FDTD) method. A geometric/electric model was constructed for the animal's head from the stereotaxic atlas of Paxinos and Watson (2008). An ellipsoid model with the internal anatomical layers was used. The electric properties were assigned to each layer. The animal head model was then subjected to EMR in the form of a plane wave with the power density as that measured by the field survey meter through the experimental exposure process. The FDTD algorithm was then applied to calculate the electric field distribution everywhere inside the head model. The SAR was then calculated at the desired points as $(E)^2/2\sigma$, where E is the electric field value at the point and σ is the conductivity of the tissue at this point.

Experimental design

The young adult and juvenile animals were each divided into two groups of 28 rats: control rats and EMR-exposed rats. The EMR-exposed groups of young adult (20 weeks) and juvenile rats (10 weeks) were exposed to EMR at a frequency of 1800 MHz modulated at 217 Hz, a power density of 0.02 mW/cm^2 , and an average SAR of 0.843 W/kg for 1 h daily for 1 month, 2 months, or 4 months. A subgroup of rats were exposed daily to EMR for 4 months and then left without exposure for 1 month to study the withdrawal effect of the exposure. The control consisted

of a group of both young adult and juvenile animals that were sham exposed. They were placed at the same time in similar containers for 1 h under the same conditions away from the RF source. The rats were fasted overnight before being euthanized by sudden decapitation 1 h after the last exposure of each time segment with a group of the control animals. The number of animals euthanized after each time segment was seven for each of the control and EMR-exposed rats.

Handling of tissue samples

The brain of each animal was quickly transferred to an ice-cold petri dish where it was dissected to separate the hippocampus, striatum, and hypothalamus. The brain areas were weighed and frozen at -42°C until analyzed.

Determination of amino acid concentrations

The procedure used in the present study was based on the HPLC method described by Márquez et al. (1986) with some modifications for application to brain tissue (Radwan et al., 1999).

Tissue preparation

Each of the hippocampus, striatum, and hypothalamus was homogenized in 75% ethyl alcohol. The homogenates were then centrifuged at $15,777 \times g$ at 4°C in a high-speed cooling centrifuge (Type 3k-30, Sigma, Germany) for 30 min. The clear supernatant was evaporated to dryness and frozen. Dansyl derivatization was carried out according to the method of Tapuhi et al. (1981). Ten microliters of each dansylated sample was finally injected into the HPLC system.

Chromatography

The HPLC system consisted of a WellChrom Mini-star K-501 pump (Knauer, Germany), a column thermostat $5-85^{\circ}\text{C}$ with injector equipped with a 20- μL loop (Knauer, Germany), a Luna 5 μm C-18 reversed phase column (5 μm particle size, $150 \times 4.6 \text{ mm}^2$ internal diameter) from Phenomenex (Torrance, CA, USA), a WellChrom spectrophotometer K-2600 with variable wavelength (Knauer, Germany), and a chromatography workstation (Eurochrom 2000).

The mobile phase consisted of 50/50 (V/V), methanol/water containing 0.6% glacial acetic acid, and 0.008% triethylamine. The flow rate was 1 mL/min, and the wavelength was 254 nm.

Data presentation and statistical analysis

Eurochrom 2000 software was used to calculate height ratio of each amino acid in sample or standard. The concentrations of the amino acids were expressed as micromoles per gram fresh tissue. Statistical comparisons between control and treated animals and the levels of significance were determined by Student's *t*-test, using SPSS (version 16). The significance was set at $p < 0.05$.

Results

The daily exposure of young adult rats to EMR (frequency 1800 MHz modulated at 217 Hz, SAR 0.843 W/kg, power density 0.02 mW/cm^2) for 1 month induced a significant decrease in the hippocampal level of glutamate and glutamine. The latter decreased significantly also after 4 months. This decrease continued after stopping EMR exposure for 1 month. In the hippocampus of juvenile rats, the daily EMR exposure induced a significant decrease in glutamate, aspartate, and glutamine after 1 month and a significant increase after 4 months of daily exposure. The significant increase in glutamate only continued after 1 month of stopping EMR exposure (Figure 1).

In the striatum of young adult rats, a significant decrease in glutamate and glutamine occurred after 2 months of daily EMR exposure. This was reversed to an increase after 4 months, being significant in the case of glutamate. After stopping exposure to EMR for 1 month, the striatal aspartate and glutamine decreased significantly. Contrary to the case in young adult animals, the daily exposure of juvenile rats to EMR for 2 and 4 months resulted in a significant increase in the striatal glutamate, aspartate, and glutamine. After 1 month of stopping the exposure, the striatal content of glutamate and glutamine increased significantly (Figure 1).

In the hypothalamus of young adult rats exposed to EMF for 1 month, a significant increase in glutamate and aspartate was recorded. The significantly increased level of glutamate was also recorded after 4 months. In the hypothalamus of juvenile rats, a significant decrease in glutamate and a significant increase in aspartate were recorded after 1 month of daily EMR exposure. After stopping EMR exposure, glutamate and glutamine showed a significant increase (Figure 1).

The effect of daily exposure to EMR (frequency 1800 MHz modulated at 217 Hz, SAR 0.843 W/kg, power density 0.02 mW/cm^2) on the levels GABA, glycine, and taurine is illustrated in Figure 2. In the

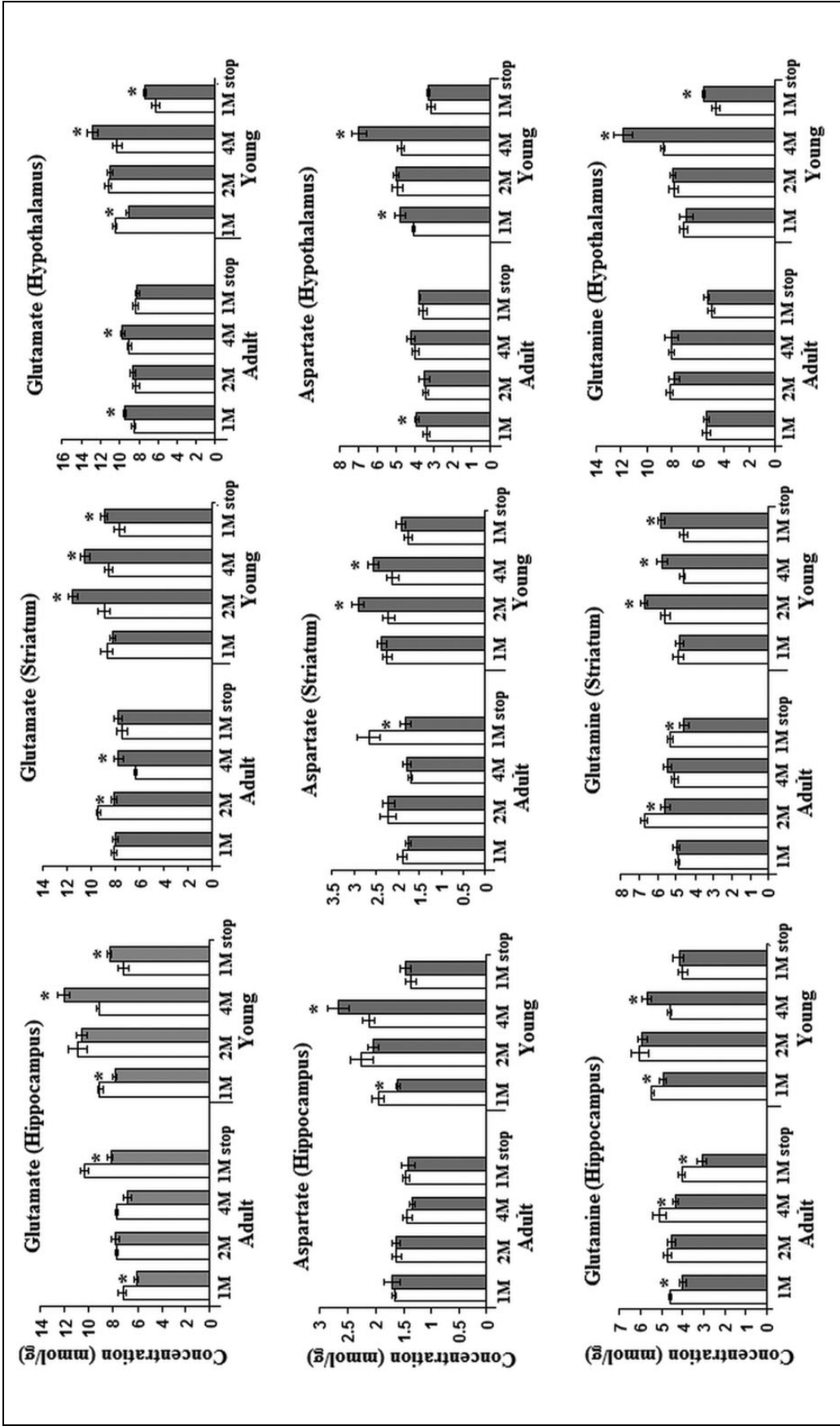


Figure 1. The effect of EMR (1800 MHz modulated at 217 Hz, power density 0.02 mW/cm², SAR 0.843 W/kg) on the levels of glutamate, aspartate, and glutamine acids in the hippocampus, striatum, and hypothalamus of the adult and young male albino rats. Empty bars indicate control and shaded bars indicate rats exposed to EMR. EMR: electromagnetic radiation; SAR: specific absorption rate.

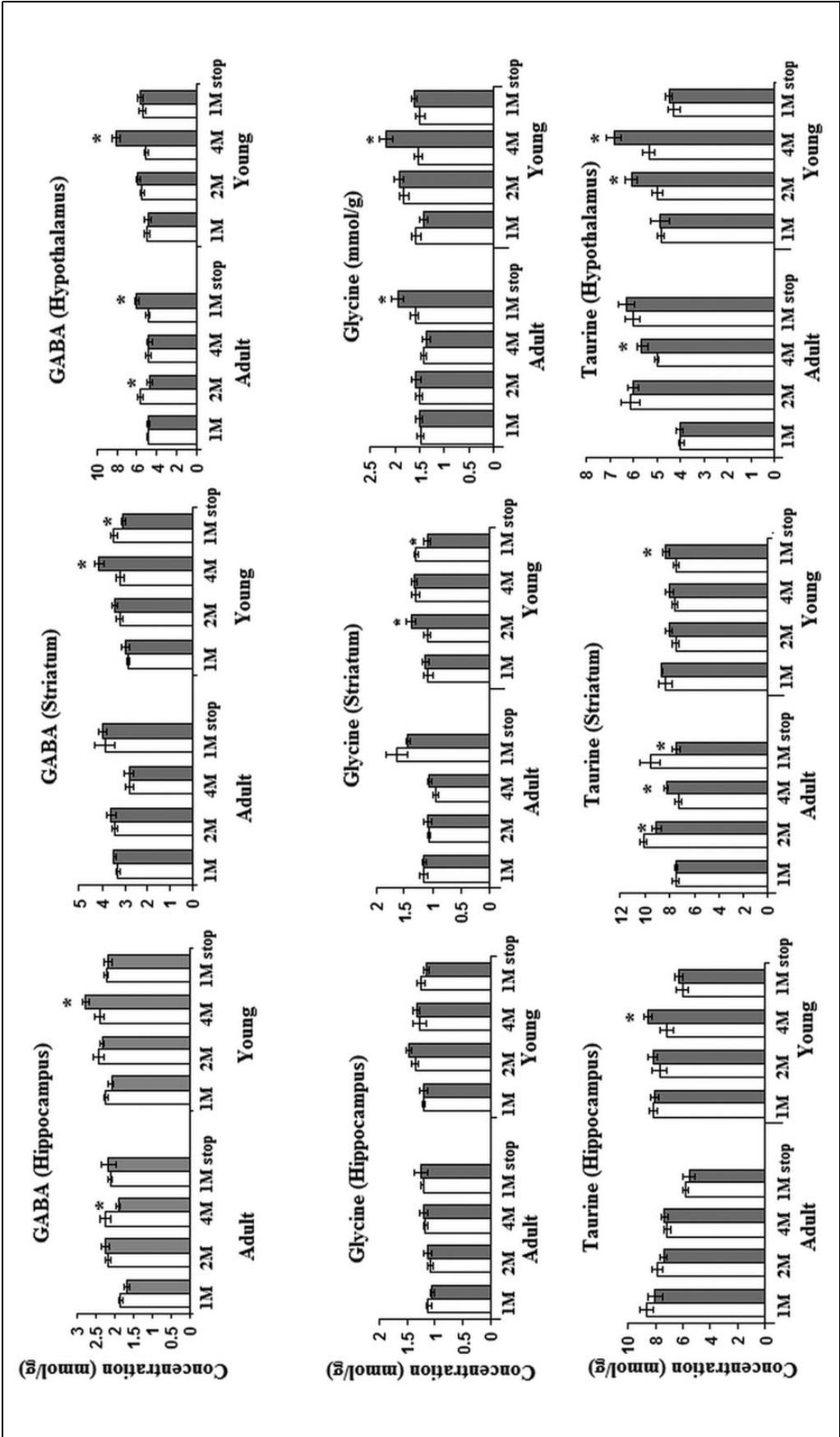


Figure 2. The effect of EMR (1800 MHz modulated at 217 Hz, power density 0.02 mW/cm², SAR 0.843 W/kg) on the levels of GABA, glycine, and taurine in the hippocampus, striatum, and hypothalamus of the adult and young male albino rats. Empty bars indicate control and shaded bars indicate rats exposed to EMR. EMR: electromagnetic radiation; SAR: specific absorption rate; GABA: gamma aminobutyric acid.

hippocampus of young adult rats, GABA showed a significant decrease after 4 months of daily EMR exposure. However, in juvenile rats GABA and taurine increased significantly after 4 months.

In the striatum of young adult rats, daily exposure to EMR induced a significant decrease in taurine levels after 2 months, followed by a significant increase after 4 months. However, stopping EMR for 1 month induced a significant decrease in taurine. In the striatum of juvenile rats glycine and GABA increased significantly after 2 and 4 months, respectively. However, after 1 month of stopping exposure to EMR, a significant decrease in the striatal GABA and glycine and a significant increase in taurine were recorded.

In the hypothalamus of young adult rats exposed daily to EMR, a significant decrease in the level of GABA was recorded after 2 months. However, taurine increased significantly after 4 months. After 1 month of stopping, GABA and glycine decreased significantly. In the hypothalamus of juvenile rats, the daily EMR exposure for 4 months resulted in a significant increase in GABA, glycine, and taurine. The latter also showed an early significant increase after 2 months (Figure 2).

Discussion

The present study showed that the exposure of young adult male rats to EMR induced significant decreases in hippocampal glutamate and glutamine levels after 1 month. Glutamine level also decreased significantly after 4 months of EMR exposure, while glutamate content showed a nonsignificant decrease.

The hippocampus controls behavioral and cognitive functions and is the main area responsible for learning and memory (Solati, 2011). It has been observed that the hippocampus is particularly vulnerable to MW exposure (Odaci et al., 2008; Megha et al., 2015a, 2015b). Several reports indicated that exposure to EMR produced deficits in learning, working memory, and cognitive function in rats and mice (Fragopoulou et al., 2010; Li et al., 2015; Tang et al., 2015). In addition, impaired cognitive performance has been reported in adult (Leung et al., 2011) and young (Abramson et al., 2009) humans exposed to GSM.

Glutamate, as an excitatory neurotransmitter, has a significant role in memory and learning. This effect takes places through NMDA receptors in the hippocampus, which are important in the regulation of synaptic plasticity as long-term potentiation (LTP),

short- and long-term memory, working memory, spatial and nonspatial learning, and cognitive processes (Jafari-Sabet, 2011; Stephens et al., 2011). In addition, Jing et al. (2000) suggested that glutamine could improve the learning ability and memory of rats.

Therefore, the decreases in hippocampal glutamate and glutamine content in the present study following daily exposure of young adult rats to EMR for 1 month may explain the impairment of memory and learning induced by EMR exposure.

In the present study, the significant decreases in GABA and glutamine levels obtained after 4 months of daily EMR exposure in the hippocampus of young adult rats were accompanied by a nearly control-like level of glutamate. This may be explained by the shift of hippocampal GABA and glutamine toward glutamate to keep the latter amino acid in the normal range. It is recognized that GABA is converted principally to glutamate and succinic semialdehyde by GABA transaminase (Van den Berg, 1970). However, after stopping EMR exposure for 1 month, significant decreases in hippocampal glutamate and glutamine content occurred in young adult rats restoring GABA to nearly control level. It may also be postulated that the changes in neurotransmitter amino acids and subsequently their functions may last at least for 1 month after stopping exposure in young adult rats.

The present exposure of juvenile animals to EMR induced significant decreases in the hippocampal levels of the excitatory amino acids glutamate and aspartate after 1 month. This effect could be attributed to the damping effect of chronic exposure to MWs (2.4 W/kg GSM 1800 MHz) during maturation on development of dendrites and excitatory synapses of hippocampal neurons in culture (Ning et al., 2007).

It has been found that the excessive release of glutamate from presynaptic neurons or reduced uptake of glutamate from the synaptic cleft may lead to chronic depolarization of the postsynaptic membrane (Miguel-Hidalgo et al., 2002). The persistent depolarization of the neuronal cell membrane leads to excitotoxicity and necrosis of cortical and subcortical neurons (Dodd, 2002). Therefore, the present increases in the levels of the hippocampal excitatory amino acids of juvenile rats after 4 months of daily EMR exposure may lead to a state of hyperexcitability, and this may render the animals susceptible to excitotoxicity and neuronal death.

Taurine has been reported to modulate the ability of mitochondria to remove intracellular calcium during glutamate excitotoxicity, thus preventing neuronal

death (El Idrissi, 2008). In addition, Kamisaki et al. (1993) provided evidence that taurine plays an important role in neuromodulation with which it regulates the depolarization-evoked release of GABA by activating presynaptic GABA (B) receptor. Thus, the present increase in taurine level after 4 months of EMR exposure in the hippocampus of juvenile rats may represent a neuroprotective feedback against the EMR-induced increase in the excitatory amino acids (glutamate and aspartate). This could be mediated by facilitating the release of GABA. In addition, the present increase in glutamine concentration may provide another mechanism by which the brain attempts to reduce the excessive increase in excitatory amino acid levels due to EMR exposure.

Salford et al. (2003) provided evidence for neuronal damage caused by nonthermal MW exposure from GSM mobile phones in the cortex as well as the hippocampus and basal ganglia of exposed rats. Thus, the observed increase in glutamate level in the hippocampus of juvenile rats after stopping EMR exposure for 1 month, in the present study, may lead to a tendency toward excitotoxicity and neuronal damage in the hippocampus of juvenile rats. The study of Khadrawy et al. (2008) showed also degenerative changes in the hippocampus of both adult and young rats exposed to EMR.

Furthermore, the decrease in the present study in striatal glutamate and glutamine content of young adult rats after 2 months of daily EMR exposure may lead to disturbances in learning and memory functions as the striatum has a prominent role in learning and memory (Sadeh et al., 2010). However, the significant increase in striatal glutamate and glutamine content after 4 months may have an excitotoxic effect.

In the striatum of juvenile rats, in the present study, the exposure to EMR for 2 months resulted in significant increases in striatal glutamate, aspartate, and glutamine levels, while the GABA level was unchanged. These increases continued significantly after 4 months of exposure and persisted after stopping exposure in case of glutamate and glutamine and were accompanied by a significant increase in taurine level. However, significant decreases in striatal GABA and glycine levels were recorded after stopping exposure. From these results, it may be suggested that a state of hyperexcitability may develop in the striatum of juvenile rats after 2 months and continues throughout the adulthood stage and even after 1 month of stopping EMR exposure. This hyperexcitability is mediated by the increases in the excitatory

amino acids and decreases in the inhibitory amino acids in spite of the increased taurine level. In addition, the increase in glutamate in the striatum of juvenile rats may indicate that learning and memory were still affected after stopping exposure.

Several investigators observed that serum corticosterone and adrenocorticotrophic hormone (ACTH) levels increased significantly in rats exposed to 900 MHz and 1.439 GHz (Imaida et al., 1988a, 1988b; Prochnow et al. 2011; Ragy, 2015). These changes may mediate the stressor effect of EMF (Lai, 1992). In addition, it has been reported by Mann et al. (1998) that exposure of humans to pulsed high-frequency EMFs caused an alteration in the hypothalamic–pituitary–adrenal axis (HPA) with a slight, transient increase in serum cortisol level following the onset of field exposure which continued for 1 h.

The contribution of glutamate to the regulation of the HPA has been shown by several studies (Wittmann et al., 2005; Zelena et al., 2005). Therefore, it could be concluded that the long-term exposure of young adult male rats to EMR, in the present study, may result in a stress-like state mediated by the increase in the hypothalamic excitatory amino acid concentrations after 1 month, the decrease in the GABA level after 2 months and the increase in glutamate level after 4 months. This probably stimulates the release of the stress hormone corticotropin-releasing hormone (CRH), which in turn results in the release of ACTH from the anterior pituitary and glucocorticoids from the adrenal cortex.

The increased hypothalamic GABA levels after 1 month of stopping EMF may be an attempt to terminate the stress response as GABA inhibits the release of CRH (Hillhouse and Milton, 1998) and ACTH (Keim and Shekhar, 1996) to avoid the development of chronic stress in young adult animals. Moreover, the present changes in the hypothalamic amino acid neurotransmitters could mediate the reported sleep disturbances induced by EMR Hung et al., 2007; (Mann and Röschke, 1996; Mohammed et al., 2013) since hypothalamic amino acids have been involved in the regulation of sleep (Ali et al., 1999; Azuma et al., 1996; Campbell et al., 2002).

Exposure of juvenile rats to EMR (frequency 1800 MHz modulated at 217 Hz, SAR 0.843 W/kg, power density 0.02 mW/cm²) induced a significant decrease in hypothalamic glutamate level after 1 month of EMR exposure accompanied by a significant increase in aspartate concentration. This was followed by a significant increase in taurine content after 2 months

and glutamate, aspartate, glutamine, glycine, taurine, and GABA after 4 months.

The increase in hypothalamic taurine level after 2 months of EMR exposure may contribute to the acceleration of development and onset of adulthood in juvenile animals as taurine could stimulate growth hormone and prolactin secretion in rats (Ikuyama et al., 1988). The increase in the excitatory amino acids induced after 4 months of exposure could be counteracted by the homeostatic mechanisms provided by the increase in glutamine level after 4 months and the neuroprotective role of taurine with the increase in GABA and glycine levels.

After stopping EMR exposure of juvenile animals for 1 month, the increase in the hypothalamic glutamate level continued and was accompanied by a significant increase in glutamine concentration and no significant changes in aspartate, taurine, GABA, and glycine. These increases in glutamate and glutamine levels suggest that a persistent change in the nervous system was still evident after 1 month of stopping exposure and may render juvenile rats susceptible to a state of increased excitation in adulthood.

It may be concluded that the alterations in amino acid neurotransmitters induced by the daily exposure of both young adult and juvenile rats to EMR (frequency 1800 MHz modulated at 217 Hz, SAR 0.843 W/kg, power density 0.02 mW/cm²) were more prominent in the striatum than in the other two areas and in juvenile than in young adult animals. Thus, it may be suggested, from the present data, that juvenile animals may be more vulnerable to the adverse effects of EMR than young adult animals. This is confirmed by the findings of Hyun and Oh (2006) that the brain-averaged SAR of a 3-week-old rat was about 3.19 times higher than that of a 10-week-old rat. The authors attributed this to the thinness of the skin and the skull and the smaller size of the brain of juvenile rats.

As the present exposure of rats to EMR was carried out through an antenna placed at the center of the container away from the body of animals, unlike the condition in human where the mobile phone is placed in contact with the ear, therefore the thermal effect of EMF is excluded.

It may be postulated from the present data, in both young adult and juvenile rats, that the disturbances in amino acid neurotransmitters in the hippocampus, striatum, and hypothalamus may participate in many of the adverse effects reported after EMR exposure, which include cognitive impairment, memory loss,

and sleep disorders which may persist for some time after stopping exposure.

Therefore, we recommend that the use of mobile phones by children should be restricted to prevent potential adverse effects on learning and cognitive functions including susceptibility to neurodegenerative diseases in adulthood. Older individuals should also limit the duration and frequency of mobile phone use to minimize potential adverse effects.

Authors' note

All experiments were carried out in accordance with research protocols established by the animal care committee of the National Research Center (Cairo, Egypt), which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Declaration of Conflicting Interests

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ORCID iD

Yasser A Khadrawy  <http://orcid.org/0000-0003-2562-535X>

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