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Variations in amino acid neurotransmitters in some brain areas of adult and young male albino rats due to exposure to mobile phone radiation

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Abstract. – *Background and Objectives:* **Mobile phone radiation and health concerns have been raised, especially following the enormous increase in the use of wireless mobile telephony throughout the world. The present study aims to investigate the effect of one hour daily exposure to electromagnetic radiation (EMR) with frequency of 900 Mz (SAR 1.165 w/kg, power density 0.02 mW/cm^2) on the levels of amino acid neurotransmitters in the midbrain, cerebellum and medulla of adult and young male albino rats.**

Materials and Methods: **Adult and young rats were divided into two main groups (treated and control). The treated group of both adult and young rats was exposed to EMR for 1 hour daily. The other group of both adult and young animals was served as control. The determination of amino acid levels was carried out after 1 hour, 1 month, 2 months and 4 months of EMR exposure as well as after stopping radiation.**

Results: **Data of the present study showed a significant increase in both excitatory and inhibitory amino acids in the cerebellum of adult and young rats and midbrain of adult animals after 1 hour of EMR exposure. In the midbrain of adult animals, there was a significant increase in glycine level after 1 month followed by significant increase in GABA after 4 months. Young rats showed significant decreases in the midbrain excitatory amino acids. In the medulla, the equilibrium ratio percent (ER%) calculations showed a state of neurochemical inhibition after 4 months in case of adult animals, whereas in young animals, the neurochemical inhibitory state was observed after 1 month of exposure due to significant decrease in glutamate and aspartate levels. This state was converted to excitation after 4 months due to the increase in glutamate level.**

Conclusion: **The present changes in amino acid concentrations may underlie the reported adverse effects of using mobile phones.**

Key Words:

Electromagnetic radiation, Amino acid neurotransmitters, Midbrain, Cerebellum, Medulla.

Introduction

Mobile phone radiation and health concerns have been raised, especially following the enormous increase in the use of wireless mobile telephony throughout the world. Mobile telephone antennae emit low level radiofrequency (RF) electromagnetic fields in the microwave range with wavelength frequency band starting from about 900 MHz[1].

Low-frequency magnetic field induces circulating currents within the human body. The strength of these currents depends on the intensity of the outside magnetic field. If sufficiently large, these currents could cause stimulation of nerves and muscles or affect other biological processes[2]. Due to the close proximity of the mobile phone device to the head, the human brain is exposed to relatively high specific absorption rates (SARs) compared to the rest of the body[3]. However, it has been reported that different brain regions could respond differently to radiofrequency radiation (RFR)[4].

It has been indicated that an electromagnetic field influences the biological functions of nerve cells and induces changes in neurotransmitter contents[5]. The efflux of calcium ions from brain tissue is an important neurochemical effect of RFR as calcium ion plays an important role in the functions of the nervous system such as the release of neurotransmitters[6].

Many hazardous effects on the nervous system have been described due to electromagnetic field (EMF) of digital mobile phone. EMF emitted from mobile phone could affect sleep[7], learning and memory[8,9], attention[10], cognitive performance[11], headache[12] and disturbances in blood brain barrier permeability[13,14]. Recently, disturbances in the hypothalamic[15], thalamic and striatal[16] amino acid neurotransmitters after short- and long-term exposure to electromagnetic radia-

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tion (EMR) were reported. Moreover, Khadrawy et al.[17] suggested that the changes in cortical amino acid neurotransmitters due to EMR exposure may underlie the EMR-induced changes in cortical excitability. However, the influence of mobile phones on heart rate and blood pressure is still problematic[18,19]. Furthermore, Balik et al.[20] indicated that there is no effect on redness of the eyes and vision disturbance, but some statistical evidences indicated that mobile phone may cause blurring of vision, secretion, inflammation and lacrimation of the eyes.

The present study aims to investigate the variations in amino acid neurotransmitters in the midbrain, cerebellum and medulla of adult and young male albino rats due to their exposure to electromagnetic radiation (EMR) at a frequency of 900 MHz, power density of 0.02 mW/cm^2 and SAR of 1.165 W/kg. The study also extended to investigate the status of amino acid neurotransmitters after stopping exposure to EMR.

Materials and Methods

Experimental Animals

The experimental animal used in this study is the male albino rat. Both young (one month old) and adult (four months old) animals were used and provided with food and water *ad libitum*. All experiments were carried out in accordance with research protocols established by the Animal Care Committee of the National Research Center, Egypt.

Electromagnetic Exposure Setup

The EMR exposure system and method of exposure were described previously by Khadrawy et al[17].

Experimental Design

Adult and young rats were divided into two main groups (treated and control). The 1st group of both adult and young rats was exposed to EMR (frequency 900 MHz, power density 0.02 mW/cm^2 and average SAR 1.165 W/kg) simultaneously for 1 hour daily. The 2nd group of both adult and young animals was placed at the same time in a similar container for 1 hour away from the RF source and served as control animals. A subgroup from each treated and control animals was sacrificed after 1 hour, 1 month, 2 months and 4 months of daily exposure. Another subgroup from the treated animals (adult and young) was left for 1 month without exposure (after 4 months of daily exposure) to study the withdrawal effect of the radiation and were then sacrificed with a group of the control animals. The number of treated and control rats were listed in the Tables of results between parentheses.

The animals were killed by sudden decapitation and brain areas were dissected out, weighed and kept frozen until analyzed.

Reagents and Chemicals

Absolute ethyl alcohol (Riedel, Darmstadt, Germany) was used for homogenization. Lithium carbonate (Merck, Darmstadt, Germany), dansyl chloride (Sigma, St. Louis, MO, USA) and HPLC grade acetonitrile (Hypersolv, BDH Chemicals, Ltd., Poole, Dorset, UK) were used for dansylation. Free amino acids and their dansyl derivatives were purchased from BDH Chemicals, Ltd. (Poole, Dorset,UK). HPLC grade methanol (Riedel, Darmstadt, Germany), synthesis grade triethylamine (Merck, Darmstadt, Germany) and deionized water were used to prepare the mobile phase.

Determination of Amino Acid Concentrations

The method applied in this study was based on HPLC method employed by Márquez et al.[21] with some modification for application to brain tissue[22]. Each brain area was homogenized in 3 ml ethyl alcohol (75%). Two other ml was used to rinse the homogenizer (Heidolph DIAX 900, Germany). The precipitated protein was removed by centrifugation at 12000 r.p.m. (21.036 g) for 30 minutes at 4ºC using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany). The clear supernatant was evaporated to dryness and stored at -80ºC.

Dansylation Reaction

Dansyl derivatization was carried out according to the method of Tapuhi et al.[23], using Dns-Cl in acetonitrile and a 40 mM lithium carbonate solution (pH. 9.5) as a reaction buffer.

Chromatography

The HPLC system consisted of a Wellchrom Mini-star K-501 pump (Knauer, Berlin, Germany), a column thermostat 5-85ºC with injector equipped with a 10 µl loop (Knauer, Berlin, Ger-

many), a luna 5µ C18 reversed phase column (5 µm particle size, 15 cm × 4.6 mm I.D.) from phenomenex, Torrance, CA, USA, a Wellchrom Spectrophotometer K-260b with flow cell (Knauer, Berlin, Germany) and a Chromatography workstation (Eurochrom 2000, Knauer, Berlin, Germany). The mobile phase consisted of 30/70 (v/v), methanol/water containing 0.6% glacial acetic acid and 0.008% triethylamine. The mobile phase was degassed through an in-line filter degasser supplied with 13 mm 0.45 M nylon 66 membrane filter (Phenomenex, Torrance, CA, USA) and operating by a model 0211 oil-less vacuum pump. The flow rate was 1 m1/min.

Statistical Analysis

All data are expressed as mean ± S.E.M. (*n*), where *n* refers to the number of animals. Statistical comparisons between the means of animals exposed to EMR and those of control animals were carried out by the independent t-test using SPSS (Statistical Package for Social Sciences, Inc., Chicago, IL, USA) version 14 for each of the adult and young rats in each time interval separately. Significance was determined at $p < 0.05$.

Results

Table I showed that, in the midbrain of adult rats, both glutamic and aspartic acid levels recorded an early significant increase after one hour of EMR exposure. Meanwhile, in case of young animals, a general decrease in both excitatory amino acid levels was observed being significant after one month of EMR exposure. As shown in Table II, GABA concentrations of adult rats recorded early and delayed significant increases after one hour and four months of EMR exposure, respectively. Taurine and glycine levels showed significant increases in adult animals after one hour and one month, respectively.

As shown in Table III, adult and young animals showed significant increases in cerebellar glutamate levels after one hour of EMR exposure. These significant increases were reversed into significant decreases after one month and four months in adult and young animals, respectively. As recorded in Table IV, EMR induced general significant increase in the adult inhibitory amino acids, GABA which was combined with significant increase in glycine level after one hour, 2 months and 4 months. Taurine levels in adult rats showed a significant decrease after one month that was reversed to significant increase after two months of EMR radiation. However, taurine levels in young rats showed significant increases after one hour and two months of exposure to EMR.

Glutamic and aspartic acid levels in the medulla of young rats (Table V) recorded significant decreases after one and two months of radiation. Meanwhile, as recorded in Table VI, the inhibitory amino acid, GABA showed general increase throughout the experimental periods in adult rats.

Stopping of EMR exposure revealed significant decreases in glutamine levels in the midbrain of both adult and young rats (Table I). In the cerebellum of young animals, a concomitant significant increase in aspartic acid (Table III) and GABA levels (Table IV) was observed after stopping exposure. In addition, cerebellar glutamine content showed significant decrease in adult rats (Table III). In the medulla of young animals, significant increases in glutamate (Table V) and GABA (Table VI) combined with significant decrease in glutamine level were observed after stopping exposure to EMR.

Discussion

The emission of low-level radiofrequency electromagnetic field leading to the absorption of radiation by the brain in users of handheld mobile phones has raised concerns regarding potential effects on health[24].

The present data showed a rapid significant increase in both excitatory and inhibitory amino acids in the cerebellum of adult and young rats after 1 hour of EMR exposure. This effect was also prominent in the midbrain of adult animals only.

It has been proven that both pulse-modulated and continuous RF-EMR including those of GSM have the potency to significantly increase the permeability of the blood brain barrier[13,14,25]. Therefore, the increase in the permeability of blood brain barrier due to exposure to EMR may increase the influx of glucose to the brain. It is well known that glucose represents the main source of glutamate, aspartate and glycine[26,27]. Accordingly, the significant increase in most of the amino acids in the midbrain and cerebellum recorded after 1 hour of EMR exposure, in the

Table I. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on excitatory amino acid neurotransmitter concentrations in the midbrain of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
Glutamic acid	1 hour	7.76 ± 0.11(7)	8.23 ± 0.14(6)*	+6.06	6.65 ± 0.12(6)	6.18 ± 0.21(6)	-7.07
	1 month	5.30 ± 0.44(7)	4.92 ± 0.18(7)	-7.17	7.91 ± 0.36(6)	6.33 ± 0.37(7)*	-19.97
	2 months	6.56 ± 0.33(6)	6.45 ± 0.29(7)	-1.68	4.55 ± 0.27(6)	4.28 ± 0.19(7)	-5.93
	4 months	7.68 ± 0.63(6)	7.08 ± 0.20(7)	-7.81	6.61 ± 0.27(6)	6.15 ± 0.21(6)	-6.96
	Stopping for 1month	5.58 ± 0.18(5)	5.18 ± 0.17(7)	-7.17	5.23 ± 0.09(7)	5.14 ± 0.13(7)	-1.72
Aspartic acid	1 hour	4.82 ± 0.19(7)	5.43 ± 0.18(7)*	+12.66	3.09 ± 0.11(6)	2.69 ± 0.13(6)*	-12.94
	1 month	2.21 ± 0.26(7)	1.99 ± 0.14(7)	-9.95	3.99 ± 0.29(7)	3.08 ± 0.27(6)*	-22.81
	2 months	3.46 ± 0.39(6)	3.31 ± 0.20(7)	-4.34	2.06 ± 0.19(5)	1.53 ± 0.11(6)*	-25.73
	4 months	3.45 ± 0.41(5)	2.98 ± 0.11(6)	-13.62	2.96 ± 0.22(5)	2.44 ± 0.11(6)	-17.57
	Stopping for 1 month	2.09 ± 0.09(5)	1.96 ± 0.08(8)	-6.22	2.23 ± 0.06(7)	2.18 ± 0.10(7)	-2.24
Glutamine	1 hour	2.92± 0.10(8)	2.81 ± 0.10(7)	-3.77	3.66 ± 0.12(7)	3.56 ± 0.15(7)	-2.73
	1 month	4.17 ± 0.34(7)	3.77 ± 0.06(7)	-9.59	4.76 ± 0.18(6)	3.91 ± 0.30(6)*	-17.86
	2 months	4.30 ± 0.24(6)	3.59 ± 0.15(5)*	-16.51	3.06 ± 0.17(7)	3.15 ± 0.08(7)	+2.94
	4 months	3.42 ± 0.18(6)	2.99 ± 0.05(7)*	-12.57	4.15 ± 0.11(7)	3.55 ± 0.09(6)*	-14.46
	Stopping for 1 month	3.82 ± 0.15(5)	3.28 ± 0.16(7)*	-14.14	3.05 ± 0.07(7)	2.74 ± 0.03(7)*	-10.16

Values represent mean ± SEM with the number of animals between parentheses. *Significant at *P*-value <0.05; %D represents comparison with reference to control level.

Table II. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on inhibitory amino acid neurotransmitter concentrations in the midbrain of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
GABA	1 hour	2.91 ± 0.14(7)	3.48 ± 0.14(7)*	+19.59	3.54 ± 0.15(6)	3.73 ± 0.15(7)	+5.37
	1 month	3.72 ± 0.30(7)	3.65 ± 0.10(7)	-1.88	3.30 ± 0.12(7)	3.30 ± 0.14(7)	0.00
	2 months	4.34 ± 0.13(6)	4.14 ± 0.27(7)	-4.61	3.29 ± 0.15(7)	3.29 ± 0.09(6)	0.00
	4 months	3.35 ± 0.12(5)	3.87 ± 0.09(6)*	+15.52	4.26 ± 0.22(6)	4.29 ± 0.09(6)	+0.70
	Stopping for 1 month	3.68 ± 0.43(5)	4.15 ± 0.06(7)	+12.77	3.40 ± 0.07(7)	3.54 ± 0.08(7)	+4.12
Glycine	1 hour	2.03 ± 0.06(7)	2.25 ± 0.09(7)	+10.84	2.08 ± 0.07(7)	2.15 ± 0.07(7)	+3.37
	1 month	2.03 ± 0.06(6)	2.29 ± 0.08(7)*	+12.81	2.00 ± 0.12(7)	2.06 ± 0.03(7)	+3.00
	2 months	2.33 ± 0.11(6)	2.39 ± 0.13(7)	+2.58	2.39 ± 0.11(6)	2.31 ± 0.06(7)	-3.35
	4 months	1.88 ± 0.16(6)	2.13 ± 0.07(6)	+13.30	2.30 ± 0.12(7)	2.16 ± 0.08(7)	-6.09
	Stopping for 1 month	2.32 ± 0.16(5)	2.13 ± 0.08(8)	-8.19	1.79 ± 0.03(7)	1.90 ± 0.05(7)	+6.15
Taurine	1 hour	4.31 ± 0.10(7)	4.61 ± 0.08(7)*	+6.96	3.93 ± 0.14(7)	3.78 ± 0.15(7)	-3.82
	1 month	3.02 ± 0.23(7)	3.09 ± 0.09(7)	+2.32	3.93 ± 0.33(7)	3.76 ± 0.30(7)	-4.33
	2 months	4.71 ± 0.28(6)	4.54 ± 0.19(7)	-3.61	3.06 ± 0.08(7)	3.15 ± 0.08(7)	+2.94
	4 months	3.48 ± 0.23(6)	3.33 ± 0.10(7)	-4.31	3.87 ± 0.11(6)	3.90 ± 0.17(7)	+0.78
	Stopping for 1 month	3.78 ± 0.24(6)	3.99 ± 0.20(8)	+5.56	2.58 ± 0.06(7)	2.51 ± 0.05(7)	-2.71

Values represent mean ± SEM with the number of animals between parentheses.*Significant at *P*-value <0.05; %D represents comparison with reference to control level.

Table III. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm2) on excitatory amino acid neurotransmitter concentrations in the cerebellum of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
Glutamic acid	1 hour	7.16 ± 0.15(7)	7.74 ± 0.15(7)*	+8.10	7.03 ± 0.12(6)	8.01 ± 0.36(6)*	+13.94
	1 month	6.29 ± 0.29(6)	5.34 ± 0.16(6)*	-15.10	7.40 ± 0.28(7)	7.78 ± 0.45(7)	+5.14
	2 months	7.33 ± 0.43(7)	7.74 ± 0.34(6)	+5.59	6.09 ± 0.25(7)	6.31 ± 0.20(7)	+3.61
	4 months	8.29 ± 0.15(5)	8.52 ± 0.29(6)	+2.77	8.03 ± 0.15(6)	7.39 ± 0.16(5)*	-7.97
	Stopping for 1month	7.81 ± 0.58(6)	6.89 ± 0.36(8)	-11.78	5.77 ± 0.14(6)	6.02 ± 0.14(7)	+4.33
Aspartic acid	1 hour	3.68 ± 0.22(7)	3.76 ± 0.18(8)	+2.17	1.77 ± 0.08(7)	1.86 ± 0.12(7)	+5.08
	1 month	1.44 ± 0.16(7)	1.13 ± 0.07(7)	-21.53	1.86 ± 0.11(7)	1.88 ± 0.12(7)	+1.08
	2 months	2.39 ± 0.20(6)	2.85 ± 0.22(6)	+19.25	1.39 ± 0.10(7)	1.38 ± 0.09(7)	-0.72
	4 months	1.86 ± 0.14(6)	1.87 ± 0.09(6)	+0.54	1.53 ± 0.07(6)	1.62 ± 0.05(5)	+5.88
	Stopping for 1 month	1.71 ± 0.13(6)	1.44 ± 0.11(8)	-15.79	1.24 ± 0.05(6)	1.40 ± 0.05(6)*	+12.90
Glutamine	1 hour	2.72 ± 0.14(8)	2.19 ± 0.08(7)*	-19.49	5.25 ± 0.12(6)	4.76 ± 0.11(6)*	-9.33
	1 month	5.07 ± 0.20(7)	4.34 ± 0.23(6)*	-14.40	4.02 ± 0.09(7)	4.20 ± 0.14(7)	+4.48
	2 months	4.55 ± 0.12(5)	3.98 ± 0.09(6)*	-12.53	3.84 ± 0.10(6)	4.22 ± 0.11(6)*	+9.90
	4 months	4.24 ± 0.12(6)	3.53 ± 0.07(6)*	-16.75	5.24 ± 0.07(6)	4.66 ± 0.10(6)*	-11.07
	Stopping for 1 month	4.80 ± 0.42(6)	3.77 ± 0.15(7)*	-21.46	3.54 ± 0.09(7)	3.28 ± 0.11(7)	-7.34

Values represent mean ± SEM with the number of animals between parentheses.*Significant at *P*-value <0.05; %D represents comparison with reference to control level.

Table IV. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on inhibitory amino acid neurotransmitter concentrations in the cerebellum of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
GABA	1 hour	1.79 ± 0.06(7)	2.17 ± 0.04(7)*	+21.23	1.62 ± 0.05(6)	1.83 ± 0.04(6)*	+12.96
	1 month	2.13 ± 0.12(6)	1.72 ± 0.09(6)*	-19.25	1.70 ± 0.02(6)	1.80 ± 0.06(7)	+5.88
	2 months	1.77 ± 0.10(6)	2.09 ± 0.03(6)*	+18.08	1.86 ± 0.06(7)	1.81 ± 0.05(7)	-2.69
	4 months	1.75 ± 0.04(6)	1.99 ± 0.04(7)*	+13.71	2.03 ± 0.03(7)	2.03 ± 0.07(7)	0.00
	Stopping for 1 month	2.70 ± 0.27(6)	2.32 ± 0.06(7)	-14.07	1.77 ± 0.02(6)	1.91 ± 0.02(6)*	+7.91
Glycine	1 hour	0.98 ± 0.02(7)	1.14 ± 0.04(7)*	+16.33	1.12 ± 0.06(7)	1.17 ± 0.03(7)	+4.46
	1 month	1.17 ± 0.03(6)	1.10 ± 0.08(7)	-5.98	1.06 ± 0.03(7)	1.11 ± 0.04(7)	+4.72
	2 months	0.97 ± 0.04(6)	1.21 ± 0.02(6)*	+24.74	1.05 ± 0.14(7)	1.15 ± 0.03(7)	+9.52
	4 months	1.04 ± 0.03(5)	1.22 ± 0.05(6)*	+17.31	1.09 ± 0.03(6)	1.31 ± 0.04(6)*	+20.18
	Stopping for 1 month	1.46 ± 0.16(6)	1.27 ± 0.05(7)	-13.01	0.97 ± 0.04(7)	1.09 ± 0.06(7)	+12.37
Taurine	1 hour	5.82 ± 0.26(8)	5.71 ± 0.25(8)	-1.89	5.99 ± 0.20(6)	6.66 ± 0.15(6)*	+11.19
	1 month	4.48 ± 0.25(6)	3.80 ± 0.16(6)*	-15.18	4.63 ± 0.11(7)	4.76 ± 0.17(7)	+2.81
	2 months	4.65 ± 0.15(6)	5.18 ± 0.09(6)*	+11.40	4.50 ± 0.15(6)	5.12 ± 0.13(5)*	+13.78
	4 months	4.78 ± 0.10(6)	4.68 ± 0.08(7)	-2.09	5.81 ± 0.18(7)	5.75 ± 0.20(6)	-1.03
	Stopping for 1 month	6.82 ± 0.59(6)	6.39 ± 0.19(7)	-6.30	3.87 ± 0.16(7)	3.72±0.11(7)	-3.88

Values represent mean ± SEM with the number of animals between parentheses.*Significant at *P*-value <0.05; %D represents comparison with reference to control level.

Table V. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on excitatory amino acid neurotransmitter concentrations in the medulla of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
Glutamic acid	1 hour	5.04 ± 0.16(8)	5.32 ± 0.24(7)	+5.65	5.59 ± 0.12(7)	5.51 ± 0.15(7)	-1.43
	1 month	3.73 ± 0.09(7)	3.63 ± 0.07(7)	-2.68	4.28 ± 0.14(5)	3.72 ± 0.11(6)*	-13.08
	2 months	4.62 ± 0.16(7)	4.80 ± 0.10(6)	+3.90	4.29 ± 0.07(6)	3.98 ± 0.11(6)*	-7.23
	4 months	5.82 ± 0.13(6)	5.67 ± 0.11(6)	-2.58	4.74 ± 0.15(6)	5.27 ± 0.14(6)*	+11.18
	Stopping for 1month	4.34 ± 0.16(6)	4.34 ± 0.18(8)	0.00	3.85 ± 0.06(6)	4.18 ± 0.13(6)*	+8.57
Aspartic acid	1 hour	2.86 ± 0.11(7)	3.11 ± 0.15(7)	+8.74	3.00 ± 0.15(7)	2.75 ± 0.09(6)	-8.33
	1 month	1.65 ± 0.07(7)	1.49 ± 0.06(7)	-9.70	2.47 ± 0.11(5)	1.92 ± 0.10(6)*	-22.27
	2 months	2.58 ± 0.12(7)	2.81 ± 0.11(6)	+8.91	2.17 ± 0.10(6)	1.83 ± 0.07(5)*	-15.67
	4 months	2.69 ± 0.13(6)	2.63 ± 0.06(7)	-2.23	2.07 ± 0.10(6)	2.26 ± 0.09(7)	+9.18
	Stopping for 1 month	1.76 ± 0.11(6)	1.67 ± 0.10(7)	-5.11	1.76 ± 0.08(7)	1.88 ± 0.06(6)	+6.82
Glutamine	1 hour	2.08 ± 0.08(7)	1.77 ± 0.05(7)*	-14.90	3.51 ± 0.07(7)	3.40 ± 0.11(7)	-3.13
	1 month	3.06 ± 0.09(6)	2.84 ± 0.01(6)*	-7.19	2.25 ± 0.10(7)	2.30 ± 0.09(7)	+2.22
	2 months	3.19 ± 0.07(7)	3.17 ± 0.06(6)	-0.63	2.81 ± 0.04(6)	2.85 ± 0.08(7)	+1.42
	4 months	2.89 ± 0.08(6)	2.66 ± 0.08(6)	-7.96	3.35 ± 0.11(7)	3.28 ± 0.15(7)	-2.09
	Stopping for 1 month	2.78 ± 0.06(6)	2.70 ± 0.10(8)	-2.88	2.68 ± 0.06(6)	2.41 ± 0.04(6)*	-10.07

Values represent mean ± SEM with the number of animals between parentheses.*Significant at *P*-value <0.05; %D represents comparison with reference to control level.

Table VI. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on inhibitory amino acid neurotransmitter concentrations in the medulla of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
GABA	1 hour	1.31 ± 0.03(7)	1.54 ± 0.09(7)*	+17.56	1.72 ± 0.05(7)	1.67 ± 0.05(7)	-2.91
	1 month	1.47 ± 0.04(7)	1.45 ± 0.03(7)	-1.36	1.20 ± 0.06(7)	1.15 ± 0.04(7)	-4.17
	2 months	1.56 ± 0.05(5)	1.76 ± 0.04(6)*	+12.82	1.66 ± 0.07(6)	1.51 ± 0.07(7)	-9.04
	4 months	1.43 ± 0.04(6)	1.63 ± 0.06(6)*	+13.99	1.98 ± 0.05(7)	1.98 ± 0.08(7)	0.00
	Stopping for 1 month	1.69 ± 0.05(6)	1.78 ± 0.06(7)	+5.33	1.51 ± 0.03(6)	1.67 ± 0.04(6)*	+10.60
Glycine	1 hour	3.29 ± 0.07(8)	3.40 ± 0.09(8)	+3.34	4.35 ± 0.09(7)	4.50 ± 0.09(7)	+3.45
	1 month	3.92 ± 0.08(7)	3.74 ± 0.09(7)	-4.59	3.02 ± 0.12(7)	3.01 ± 0.07(7)	-0.33
	2 months	3.66 ± 0.08(7)	3.86 ± 0.09(7)	+5.46	4.13 ± 0.12(6)	3.88 ± 0.05(6)	-6.05
	4 months	3.82 ± 0.08(5)	4.20 ± 0.13(6)*	+9.95	4.16 ± 0.09(6)	4.08 ± 0.06(7)	-1.92
	Stopping for 1 month	3.91 ± 0.08(6)	3.92 ± 0.04(8)	+0.26	3.36 ± 0.12(7)	3.51 ± 0.07(7)	+4.46
Taurine	1 hour	2.62 ± 0.12(8)	2.64 ± 0.19(7)	+0.76	3.46 ± 0.07(7)	3.46 ± 0.10(7)	0.00
	1 month	2.22 ± 0.04(7)	2.25 ± 0.04(7)	+1.35	1.92 ± 0.10(7)	1.85 ± 0.07(7)	-3.65
	2 months	2.35 ± 0.11(7)	2.48 ± 0.08(7)	+5.53	2.40 ± 0.11(6)	2.46 ± 0.09(7)	+2.50
	4 months	2.47 ± 0.09(6)	2.58 ± 0.10(7)	+4.45	2.75 ± 0.08(7)	2.70 ± 0.05(7)	-1.82
	Stopping for 1 month	2.31 ± 0.12(6)	2.24 ± 0.08(8)	-3.03	1.96 ± 0.07(7)	1.95 ± 0.03(7)	-0.51

Values represent mean ± SEM with the number of animals between parentheses.*Significant at *P*-value <0.05; %D represents comparison with reference to control level.

present study, may probably result from the increase in cerebral glucose content.

The present investigation showed that cerebellar glutamine content in both adult and young animals was significantly decreased after 1 hour of EMR exposure. It has been known that the amide, glutamine is stored in glial cells, then taken up by neurons and converted into glutamate and ammonia via the phosphate-dependent glutaminase (PDG) pathway[28-30]. In addition, it has been suggested that 50% of the endogenous GABA released is derived from glutamine[31]. Thus, the accompanied significant increases in cerebellar glutamate and GABA in the same time segment in both adult and young rats may be due to an enhancement of the glutamine-to-GABA and -glutamate conversion pathway.

It has been found that excessive glutamate release from presynaptic neurons or reduced clearance of glutamate from the synaptic cleft may result in chronic depolarization of the postsynaptic neurons[32,33] which lead to a state of hyperexcitability. Taurine has been suggested to act as a neuroprotector against the excitotoxicity induced by excitatory amino acids[34,35].

Therefore, it could be suggested that the increase in taurine level after 1 hour of EMR exposure in the midbrain of adult animals and cerebellum of young animals may represent a feedback mechanism to protect against the concomitant increase in glutamate level.

In the light of the present data of midbrain in young animals, it could be suggested that the exposure to EMR may lead to a state of neurochemical inhibition. This suggestion is supported by the calculation of ER% between the inhibitory and excitatory amino acids (Table VII) which indicates a state of neurochemical inhibition[22,36,37].

The reticular formation is a complex of network of nuclei and nerve fibers within the medulla, pons, midbrain, thalamus and hypothalamus that functions as the reticular activating system (RAS). The RAS, through its nonspecific arousal of the cortex, helps to maintain a state of alert consciousness[38]. It has been concluded by Augner et al.[39] that short-term exposure to GSM base station signals may have an impact on well being by reducing psychological arousal. Therefore, as shown from ER% calculations (Table VII) which showed a state of inhibition in the midbrain of young rats, it could be suggested that the young users of mobile phones may be under the risk of impaired alertness.

Regarding the present data of midbrain of young animals, it can be noted that after 4 months, as the young rats reached adulthood, glutamate and aspartate recorded nonsignificant changes and returned to control-like values after stopping EMR exposure. The accompanied decrease in glutamine may help in restoring the levels of glutamate and aspartate after 4 months and normalizing them after stopping radiation.

Amino acid concentrations in the cerebellum of adult rats showed fluctuating changes throughout the experimental period. The significant decrease recorded in glutamate level after 1 month was normalized to nearly a control-like value after 2 and 4 months of exposure. Thus, it could be suggested that the parallel significant decrease in glutamine level may play an important role in restoring the normal level of glutamate. On the other hand, the significant decrease in GABA concentration observed after 1 month was reversed to a significant increase after 2 months which was accompanied by the same pattern of change in case of taurine.

Kamisaki et al.[40] provided evidence that taurine plays an important role in neuromodulation with which it regulates the depolarization-evoked release of GABA through the activation of presynaptic GABA-B receptor. This could explain the present changes in both GABA and taurine after 1 and 2 months of exposure. After 4 months, the persisted significant increase in GABA could be mainly at the expense of glutamine.

The present data concerning the amino acid levels in the cerebellum of young rats showed that the significant increase in glutamate and GABA recorded after 1 hour returned to control levels after 1 and 2 months of radiation.

It has been reported that glutamate in astrocytes is predominantly converted to glutamine through an ATP-requiring reaction catalyzed by the astrocyte-specific enzyme, glutamine synthase[41]. Therefore, the significant increase observed in cerebellar glutamine content of young rats after 2 months may help in keeping the normal levels of glutamate and GABA as the excess of their concentrations may be converted to glutamine. However, after 4 months of exposure, young animals showed a state of inhibition as a result of the significant decrease in glutamate and the significant increase in glycine levels. This state of inhibition was returned to normal-like ratio percent after stopping EMR as the increase in aspartate was compensated by the increase in GABA level.

Table VII. Effect of EMR (frequency 900 Mz modulated at 217 Hz, SAR 1.165 W/kg, power density 0.02 mW/cm^2) on the calculated ER% between inhibitory & excitatory amino acid concentrations in the brain areas of albino rats.

Amino acid	Time before decapitation	Adult			Young		
		Control	Treated	%D	Control	Treated	%D
Mid-Brain	1 hour	39.27	41.95	+6.82	57.70	66.29	+14.89
	1 month	76.56	85.96	+12.28	44.54	56.96	+27.89
	2 months	66.57	66.91	+0.51	85.93	96.39	+12.17
	4 months	46.99	59.64	+26.92	68.55	75.09	+9.54
	Stopping for 1 month	78.23	87.96	+12.44	69.57	74.32	+6.83
Cerebellum	1 hour	25.55	28.78	+12.64	31.14	30.40	-2.38
	1 month	42.69	43.59	+2.11	29.81	30.12	+1.04
	2 months	28.19	31.16	+10.54	38.90	38.49	-1.05
	4 months	27.49	30.90	+12.40	32.64	37.07	+13.57
	Stopping for 1 month	43.70	43.10	-1.37	39.09	40.43	+3.43
Medulla	1 hour	58.23	58.60	+0.64	70.66	74.70	+5.72
	1 month	100.19	101.37	+1.18	62.52	73.76	+17.98
	2 months	72.50	73.85	+1.86	89.63	92.77	+3.50
	4 months	61.69	70.24	+13.86	90.16	80.48	-10.74
	Stopping for 1 month	91.80	94.84	+3.31	86.81	85.48	-1.53

%D represents a % difference between treated and control animals.

Amino acid concentrations in the medulla of both adult and young animals revealed slight changes after EMR exposure. According to ER% calculations, these changes induced a state of inhibition after 4 months in case of adult animals due to the significant increase in GABA and glycine levels. However, in young animals, the inhibition state was observed earlier after 1 month of exposure due to the significant decrease in glutamate and aspartate levels. This was reversed to a state of excitation after 4 months due to the increase in glutamate level. After stopping EMR exposure for 1 month, GABA was significantly increased at glutamine expense to overcome the increase in glutamate level which returned the ER% to a control-like value (Table VII).

It has been stated that the medulla contains groupings of neurons required for the regulation of breathing and of cardiovascular responses[38]. It was shown that occupational exposure to EMF can cause fluctuations in heart rate and heart rate variability[42-44]. In addition, the findings of Braune et al.[19] did not support the assumption of a non-thermal influence of EMFs emitted by mobile phones on the cardiovascular autonomic nervous system in healthy humans. However, results of Andrzejak et al.[45] demonstrated that the call with a mobile phone may influence heart rate variability and change the autonomic balance. They also concluded that the influence of speaking cannot be excluded. Moreover, results of Tahvanainen et al.[18] indicated that exposure to a cellular phone (35 min.), using 900 MHz or 1800 MHz with maximal allowed antenna powers, does acutely change arterial blood pressure and heart rate.

Therefore, it could be suggested that the present changes in amino acid concentrations in the medulla might play a role in the reported cardiovascular effects of EMR exposure. The young users may be more influenced than adults.

In conclusion, although the present data indicate some changes in amino acid neurotransmitter concentrations due to EMR exposure, further studies are recommended to investigate whether or not these changes are implicated in any neural disorder.

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