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BEHAVIORAL AND NEUROBIOLOGICAL EFFECT OF WHOLE BODY EXPOSURE TO ELF ELECTROMAGNETIC FIELD IN THE RAT

SEYED MOHAMMAD MAHDAVI[1*]

[1]Department of Biosciences and Biotechnology, Malek Ashtar University of Technology, Tehran, Iran

***Address correspondence to: Seyed Mohammad Mahdavi; Seyed Mohammad Mahdavi; Tel: +98(21)22974606, Fax: +98(21)22974604, Malek Ashtar University of Technology, Tehran, Iran, E mail: Sm_mahdavi@mut.ac.ir**

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ABSTRACT

Nowadays, assessment of the non-ionizing wave's radiation effects emitted by machinery and equipment in the life and workplace on human cognitive and non-cognitive behaviors is one of the essential challenges. These waves can interfere with brain waves and causing changes in behavior and neural activity. In the present study, we investigated behavioral and neurobiological effect of whole body exposure to 12 Hz extremely low frequency electromagnetic field (ELF-EMF) in the rats. Body weight, rearing, Mc2R and glucose of irradiated rats not changed significantly, while their food intake, movement, adrenocorticotropic hormone (ACTH) and adrenaline significantly increased Sometimes. The animals showed significantly decreased numbers of sniffing in exposed rats at 1 and 7 days. Water intake of irradiated rats decreased in day 3 and then gradually increased during the period of study. In addition for more confirm of increasing plasma ACTH hormone, we evaluated ACTH gene expression in brain tissue of irradiated and control rats and significantly elevated gene expression observed in day one after irradiation in rats compared to controls. In conclusions, these data suggests that 12 Hz ELF-EMF may be induced stress-like behaviors in irradiated rats.

Keywords: Extremely low frequency electromagnetic field, adrenocorticotropic hormone, stress-like behaviors, brain

INTRODUCTION

In last decade, harmful and beneficial biological effects of electromagnetic field (EMF) were investigated by many studies in worldwide (Carpenter and Aĭrapetian 1994; Simkó and Mattsson 2004; Balmori 2005). Spectrum of electromagnetic fields with frequencies below 300 Hz is called Extremely Low Frequency Electro-Magnetic Fields (ELF-EMF), these frequencies are lower than intermediate frequencies (Tenforde 1992). People can be exposed to ELF fields from stationary sources that are operated in our environment, for example power lines (Portier and Wolfe 1998). At electric power industries workers can be exposed to high levels of ELF-EMFs on the job, or at home certain domestic appliances that contain motors, transformers and heaters can be emitted ELF electric and magnetic fields(Skotte and Hjøllund 1997; Kleinerman, Linet et al. 2005; Jovanović, Đinđić et al. 2010).

International Agency for Research on Cancer (IARC) reported ELF magnetic fields as "possibly carcinogenic to humans" (Group 2B) (Humans, Organization et al. 2002). This classification was due to statistical studies indicating children are more likely to develop leukaemia if their exposure to ELF-MFs, although experimental studies on animals did not support these findings(Dominici, Villarini et al. 2011; Schüz, Grell et al. 2012).Many epidemiological studies suggest that there is a possible link between ELF-MF exposures and clinically recognized medical disorders in people, such as leukemia, brain cancer, breast cancer, kidney cancer, and other kinds of cancers, as well as cardiovascular diseases (Smith 1987; Jelenković, Janać et al. 2006; Erdal, Gürgül et al. 2008). Despite the possible risks reported in the ELF-EMFs in different studies, ELF range of EMFs could have been beneficial medical applications in many cases such as stimulation of bone fracture healing process, wound healing, pain treatment or transcranial magnetic stimulation (Shupak, Prato et al. 2003; Markov 2007; Costin, A Birlea et al. 2012). One of the diagnostic applications of this range is detection of cancer by the bioimpedance measurement (Smith, Potter et al. 2000).

Studies suggest that ELF fields via interference to the cells membrane potential can induced biochemical changes, and consequently perturbed function of the trans-membrane ionic transfer with modified cell activities (Goodman and Shirley-Henderson 1991; Tenforde 1992; Jiang, Tsang et al.

2004). One of the bases of reactions between biomolecules in an EMF field is physical processes at the atomic level. Since EMF field can magnetically affect on chemical bonds that there are between adjacent atoms with consequent production of free radicals. We know that there are EMF fields in the internal of the bodies because of that the body formed by ionic and bipolar macromolecules, such as membrane lipids (Jelenković, Janać et al. 2006). The overall, associated with the use of EMF fields, an interaction between the external and internal field can induce changes in the biochemical signals transmitted across cell membranes, with unwanted effects at molecular and biochemical levels. Very small changes in the cell membrane potential induced by ELF-EMF can be of special interest in the brain signaling processes(Simkó 2004; Funk, Monsees et al. 2009). The aim of the present study was thus to test in vivo (in the rats), the effect of ELF-EMF exposure on some of the behavioral and neuronal functions such as body weight, water and food intake, locomotors activity, rearing and sniffing and hormonal analysis by Elisa and gene expression 1, 3, 7, 14 and 21 day during the period of study. Elisa technique was used for evaluation of Adrenocorticotropic hormone (ACTH), Adrenaline, melanocortin-2 receptor (MC2R) and D-Glucose. Then Real Time RT-PCR assay was used to evaluation of ACTH gene expression in rat's pre- and post-exposure with 12 Hz ELF-EMF.

MATERIALS AND METHODS

Animals and Experimental design

All experiments were performed on adult (12–13 weeks old) male wistar rats (Pasture Institute, Tehran, Iran) weighing 250±10 g. Animals were maintained under controlled conditions: temperature 23±2 ◦C, relative humidity 60–70%, 12-h dark/12-h light cycles, and with free access to food and water. All animals were initially untreated. One of two groups (n = 8 rats each) was left untreated (sham-exposed), and the other one was exposed to ELF-EMF with 12 Hz once a day, 75mW and 0.1 mT during 21 days. In each groups, on days 1, 3, 7, 14 and 21 the animals were sacrifice for biological assessments. Blood samples, brain, adrenal gland were collected for further analysis (Fig. 1). All procedures in this study are in accordance with the guide for the care and use of laboratory animals as adopted by the Ethics committee of Shahid Beheshti University of Medical Sciences (357: November 2000).

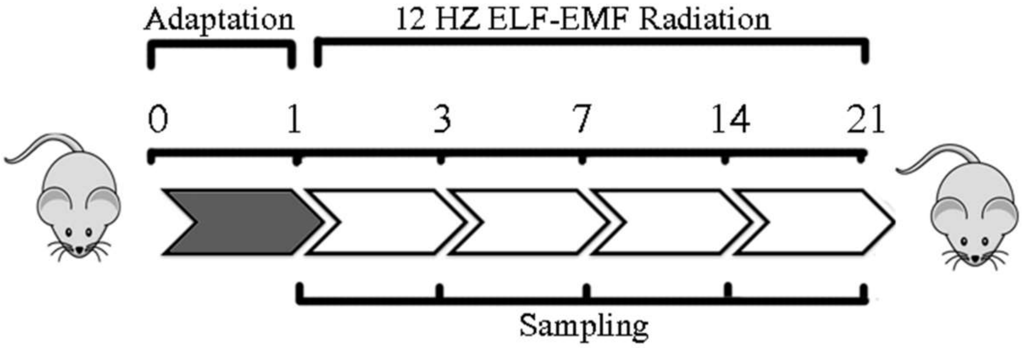

Fig 1.The design and time-line of the experiments as described in the text.

Device for electromagnetic field exposure and Shielded room

The radiation was carried out using the ELF electromagnetic field generator on six animals was placed in a cage made by Plexiglas (60×60×60 cm) once a day with 75mW and 0.1 mT. For possible intervention of external interference to testing wave a spatial room has been designed inasmuch as the whole room parts, including the ceiling and walls, windows, even the smallest openings fully covered by aluminum foil (0.4 mm diameter). For further conformation this step had been checked by a wave detector and lack of effective radiation in shielded room was confirmed. Then electromagnetic generating device antenna was fitted to the symmetrical (for waves uniformly irradiation) in the top boxes. All conditions have been done for control group except the irradiation.

Measurement of body weight and water and food intake

Before beginning of the study in each day, rats were weighed by the scales with ±0.1 g accuracy and then turned to their home cages. During the period of study 400 g rat chew and 150 mL tap water were placed in each cage. Twenty four hours before and after the irradiation the reminder food and water were calculated as water and food intake indexes at 3, 7, 14 and 21 days.

Measurement of locomotors activity, rearing and sniffing

Behavioral tests were performed in an open field container (30 × 30 × 40 cm high), which its floor was divided to 16 equal squares by thin black lines. A video camera was placed on the top of the apparatus at 120 cm heights for video typing. Each animal was placed in the apparatus and after 5 min for habituation; its activity was recorded for 10 min. The types then were analyzed for locomotion (number of line crossing) sniffing and rearing off line.

Biochemistry analysis

D-Glucose, Adrenaline, MC2R and ACTH as major stress hormones were determined by an ELISA method. Blood samples were collected in the Ependorf tubes with 5% EDTA and were centrifuged in 4 °C for 5 min in 3000 RPM. The supernatant was collected for ELISA assay using the appropriate kites (all from CUSABIO CO., Japan).

Real-Time RT-PCR

Relative Quantification of ACTH genes expression were carried out on irradiated rats compare to controls at 1, 3, 7, 14 and 21 days. Total RNA was extracted from rat's brain tissue using Qiazol reagent (Qiagen) according to manufacturer's protocol. The first-strand cDNA was synthesized using random hexamers (Vivantis, USA) in a total reaction volume of 25 µl using M-MLV Reverse Transcriptase (Vivantis Cat No: RTPL12). The PCR parameters included denaturation at 95°C for 3 min, then 40 cycles at 95°C for 20 s, annealing at 60°C for 30 s, and elongation at 72°C for 30 s. For PCR amplification and real- time RT-PCR, primer sequences are illustrated in Table 1. Real-time RT-PCR was performed using Takara SYBR Premix Ex Taq Master. The relative quantification model was applied to calculate the expression of the target gene in comparison to TBPused as an endogenous control. Gene expression levels were quantified by Rotor Gene 6000 (Corbett, Concorde, NSW, and Australia).

Table 1: Primers used in real-time RT-PCR

Name	Host	Sequence 5′ ⟶ 3′	products' Length
ACTH	Rat	GAGGTTAAGGAGCAGTGACTAAG CGTCTATGGAGGTCTGAAGCA	125
TBP (internal control)	Rat	CCTTGTGTTGACCCACCAGCAGTTCAGTAG ACAGGAGTCCATGGCAGACAACTATGTGG	224

Statistical analysis

The Real-time RT-PCR data were analyzed by REST 2009 software. Each experiment was repeated independently at least three times invitro. MSCT and H&E data were reported as mean ± standard deviation (SD). The statistical significance was determined by a Mann Whitney U test as a non-parametric equivalent of an independent sample for Student's t test. Simple one-way analysis of variance and its non-parametric equivalent (Kruskal Wallis test) was used to compare the results among rats. All analyses were performed using SPSS 17.0 software.

P-values less than 0.05 were considered as statistically significant.

RESULTS

Body weight and Food and water intake

Exposure to ELF-MF changed body weight of irradiated rats during the period of study but these changes not significant compared to control (Fig. 2A).As showed in fig.2B, food intake of irradiated rats in days 3 and 7 significantly increased compared to control group and highest food intake observed in day 7. However, no significant changes detected in food intake of irradiated rats at 14 and 21 days.No uniform trends observed in water intake of irradiated rats, as significantly decrease detected at day 3, while significantly increased observed at day 21 compared with controls (Fig. 2C). Also, no changes were observed on days 7 and 14 compared to control group.

Exploratory-like behavior

For exploratory-like behavior evaluation movements, rearing and sniffing of animals investigated during the period of study and results demonstrated significantly increase in movement of irradiated rats at day 14 compared to control groups (Fig 3A). Sniffing results demonstrate a significantly decrease in days 1 and 7 in rats were exposure with 12 Hz ELF-EMF compared to controls (Fig 3B). No significant change observed in rearing of irradiated rats compare with controls during the period of study (Fig 3C).

Serum biochemistry evaluation

Investigation of 2 hormones, one hormone receptors and glucose concentration in rats showed in fig 4 (A-D). The significantly highest concentration of ACTH hormone detected in day 1 after irradiation, but in the other time ACTH concentration was uniform (Fig. 4A). As showed in Fig 4B, adrenaline concentration was constant one day after irradiation, but in day 3 significant increased hormone concentration observed in irradiated rats compare with controls. After that adrenaline concentration gradually declined during the period of study.MC2R (Fig. 4C) and Glucose (Fig. 4D) concentrations were increased after irradiation but changes not significant in compared to control groups.

Gene Expression

Evaluation of ACTH gene expression in irradiated rats with 12 Hz ELF-EMF showed in fig. 5. The expression of ACTH gene one day after irradiation significantly increased in rats compared to control groups. Trends of gene expression in others days was not uniform and also no significantly changed observed.

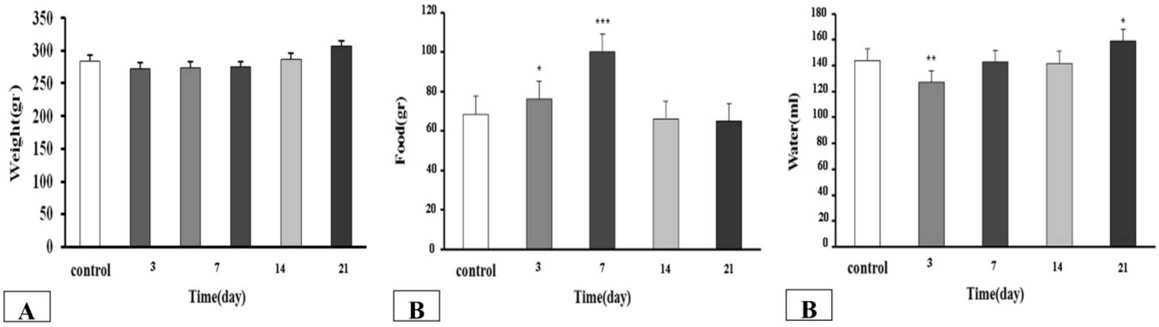

Fig. 2: Change in rat's body weight (A), Food intake (B) and water intake (C) during 21 days exposure to 12 Hz electromagnetic field. Asterisks show significant difference between groups on each day (* = p < 0.05, ** = p < 0.01 and *** = p < 0.001).

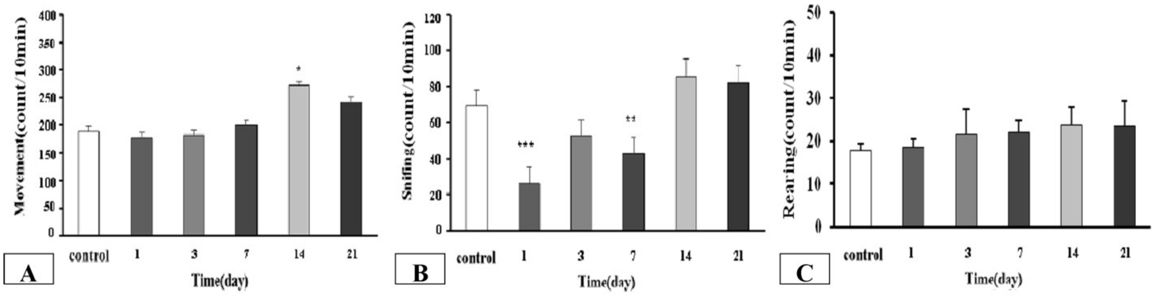

Fig. 3: Change in rat's dopamine-related behaviors (locomotors activity, A), (sniffing, B) and (numbers of rearing, C) during 21 days exposure to 12 Hz electromagnetic field. Asterisks show significant difference between groups on each day (* = p < 0.05, ** = p < 0.01 and *** = p < 0.001)

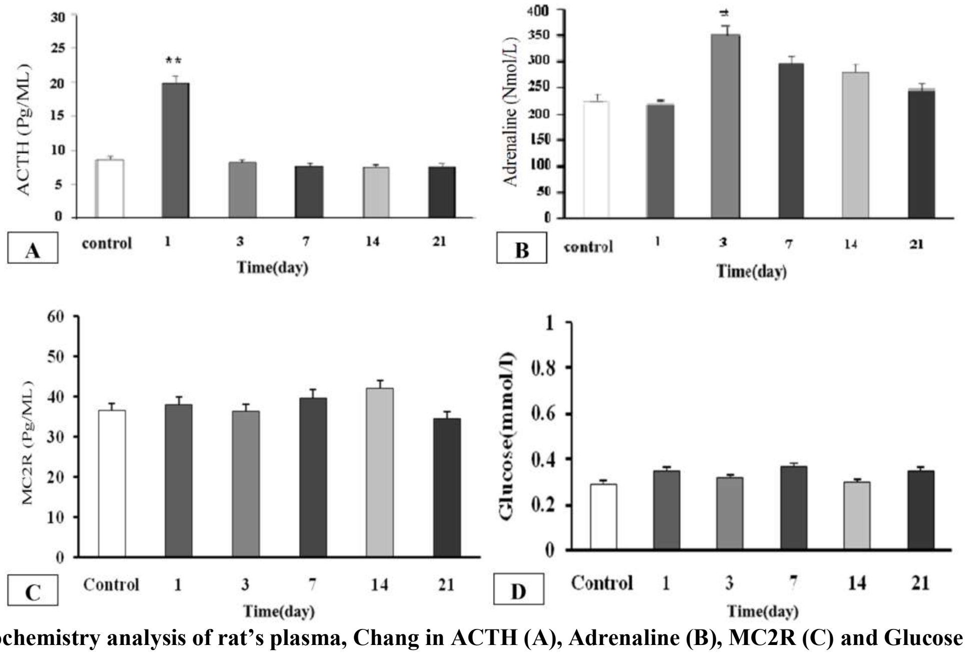

Fig. 4: Biochemistry analysis of rat's plasma, Chang in ACTH (A), Adrenaline (B), MC2R (C) and Glucose (D) in rats during 21 days exposure to 12 Hz electromagnetic field. Asterisks show significant difference between groups on each day (* = p < 0.05, ** = p < 0.01 and *** = p < 0.001).

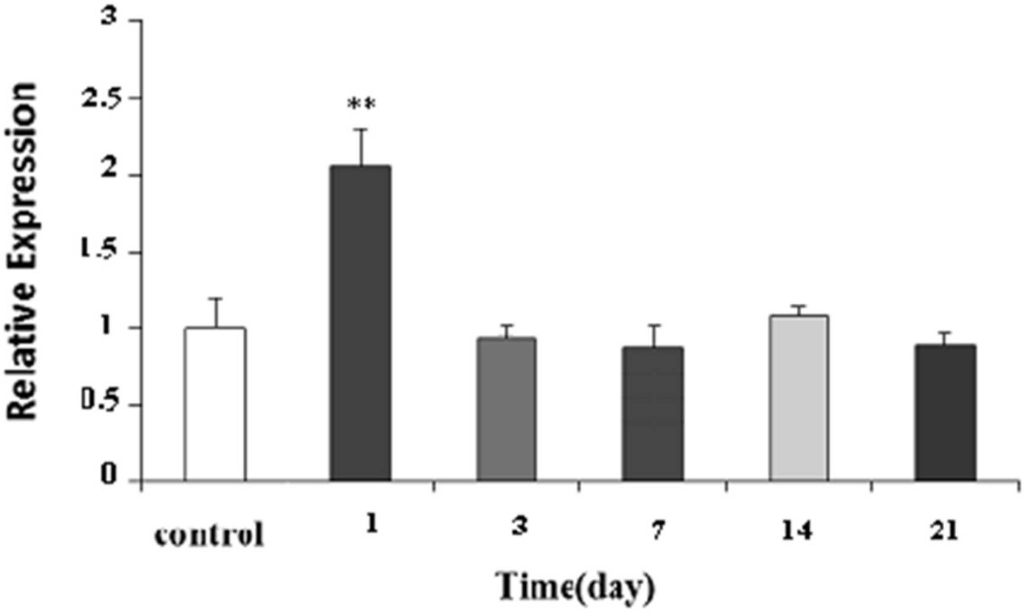

Fig. 5: Relative expression of ACTH gene in irradiated rat's brain during 21 days exposure to 12 Hz electromagnetic field. Asterisks show significant difference between groups on each day (* = p < 0.05, ** = p < 0.01 and *** = p < 0.001).

DISCUSSION

All measurements of this study were carried out in rats irradiated for 21 days, the field strength levels used for this study was 12 Hz, 75mW and 0.1 mT. Body weight, water and food intake, locomotors activity, rearing and sniffing and serum hormonal analysis (corticosterone, glucose, adrenaline, α1-Receptor, MC2R and ACTH) and ACTH gene expression determination were carried out at day 1, 3, 7, 14 and 21 during the period of study. Blood, brain, adrenal gland samples were collected for further analysis. Our results indicate that despite food and water intake on some days has shown significant changes, but average body weight of irradiated rats in comparison to control was not significantly changed. Reported results of body weight in other studies is contradictory, for example in agreement our results Zecca et al. reported that mean body weight of rats irradiated with 1 kV/m - 5 mT and 5 kV/m - 100 mT were not statistically changed in compared to sham exposed rats at any time throughout the experimental periods. Margonato et al. also reported that both electric and magnetic fields were not significantly effect on irradiated rat body weight(Margonato, Veicsteinas et al. 1993). In another study Grisset demonstrated monkeys exposed to 76 Hz ELF-MF with 0.2 mT and 20 kV/m field strength showed an enhanced growth rate (Grissett 1979). Marino also reported alterations in growth rate were observed in mice exposed to 60 Hz with 0.5–100 kV/m field strength (Marino

1990). In the following, movement, rearing and sniffing of animals evaluated as locomotors activity. Results demonstrated that two weeks after radiation with 12 Hz ELF-EMF movements of rats increased significantly, rearing of irradiated rat not changed significantly, but sniffing of irradiated rats in days 1 and 7 significantly increased.

Many several studies have been investigated the effects of electric and magnetic fields on the metabolism of cell (Fiorani, Cantoni et al. 1992; Reiter and Richardson 1992; Berg 1993; Volkow, Tomasi et al. 2011). Their results demonstrated disturbances in the metabolism of protein, carbohydrate and lipid reflected by altered blood glucose levels and by accelerated glycolysis and glycogenolysis with a metabolic block of conversion of pyruvic acid to acetyl coenzyme A. In this study glucose concentration levels increased during two weeks after irradiation, but these changes not significant. This change could have resulted from food intake or catabolic action that is an important factor in the development of muscle atrophy. According to the results, it's due to food intake because of irradiated rats showed increased significantly food intake in days 1 and 3, but also body weight not changed after irradiation.

Plasma ACTH concentration significantly increased one day after irradiation, this increased secretion of ACTH hormone from anterior pituitary gland causes to increase of adrenaline, MC2R concentration respectively. However irradiated rats adapted during the period of study. Increased plasma ACTH is response to stress in irradiated rats and this increase could be causes to increase in adrenaline hormone and glucose concentration in plasma. Increasing the feed rate also explains the increase in ACTH hormone levels as known stressor index.

In agreement with our results, Arnetz & Berg in a preliminary study showed that ACTH levels elevated in workers (sex not specified) exposed to ELF electric and magnetic fields (exposure not given), authors note that EMF might act as a stressor(Arnetz and Berg 1996). Several studies has been investigated possible effects of EMF exposure on the release of hormones involved in stress responses, particularly ACTH, cortisol, corticosterone and adrenaline released from the adrenal cortex. Hackman & Graves reported that a transient increase in serum corticosterone levels observed in young rats immediately following the onset of exposure to ELF electric fields (Hackman and Graves 1981). Their results also showed that the exposure for longer durations had no effect on serum corticosterone concentration. Although, Portet & Cabanes demonstrated prolonged exposure to ELF fields has no effects on ACTH levels (Portet and Cabanes 1988)and also several other studies reported that ELF fields has no effect on cortisol or levels (Quinlan, Petrondas et al. 1985; Portet and Cabanes 1988; Thompson and Jeggo 1995; Burchard, Nguyen et al. 1996). We also checked ACTH in the levels of gene, for this reasons we used Real Time RT-PCR for evaluation of ACTH gene expression and results showed that one day after irradiation expression levels of ACTH increased in rats compared to controls.

In conclusion, present study results and literature review allow us to make a suggestion that ELF EMF induces enhanced reversible and transient social recognition performance in rats. We also suggested that the primary effects of ELF EMF were on the neurobiological substratum of the short-term social memory in rats.

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