Arch Biol Sci. 2016;68(3):567-573 DOI:10.2298/ABS150828047Z

PATCH-CLAMP RECORDINGS OF THERMAL EFFECTS OF MAGNETIC STIMULATION ON THE PHYSIOLOGICAL CHARACTERISTIC OF RAT HIPPOCAMPAL NEURONS

Yu Zheng[1,*], Lei Dong[1], Ying Kong[1], Hui Hong[1], Yang Gao[1], Zhe Zhao[1] and Hui-quan Wang[1]

[1] *School of Electronics and Information Engineering, Tianjin Polytechnic University*, Tianjin 300387, China

***Corresponding author:** zhengyu@tjpu.edu.cn

Received: August 28, 2015; **Revised**: October 14, 2015; **Accepted**: October 20, 2015; **Published online**: April 27, 2016

Abstract: Transcranial magnetic stimulation (TMS) has proven to be an invaluable tool both in clinical practice and basic brain research. However, many concomitant effects of TMS are still incompletely understood, including thermal effects induced by TMS. The present study investigated how thermal effects induced by magnetic stimulation influence the properties of the spontaneous excitatory postsynaptic current (sEPSC) of hippocampal CA1 pyramidal neurons. We have demonstrated that a 50-Hz low-frequency electromagnetic field with intensities of 7, 14, and 23 mT can induce thermal heating in artificial cerebrospinal fluid (aCSF) from 25 to 40°C over a period of 15 min. We also report that the thermal effects induced by TMS directly influence the properties of sEPSC in hippocampal CA1 pyramidal neurons. Double measures were taken to control the temperature across experiments in order to ensure the accuracy of the temperature measurement of the aCSF. These novel findings provide important insight into the thermal effects induced by TMS as well as their consequences.

Key words: thermal effects; TMS; spontaneous excitatory postsynaptic current (sEPSC); hippocampal CA1 pyramidal neurons; patch-clamp

INTRODUCTION

Since the advent of electromagnetic currents in neurophysiology in the early 20th century [1], techniques such as repetitive transcranial magnetic stimulation (rTMS) have been widely used as noninvasive neurostimulation tools in both clinical and research practices [2,3]. The TMS body of research has seen a rise in interest for its therapeutic potential and has since found applications in a range of neuropsychiatric disorders, including depression, Alzheimer's disease and Parkinson's disease [4-6]. In addition, many scientists have pioneered the use of TMS as a noninvasive means of modulating neuronal activity for neuroscientific investigations [7,8].

Previous TMS studies have demonstrated that hippocampal functioning is directly related to neuronal damage that is secondary to neurodegenerative diseases [9,10]. The hippocampus is known to play important roles in learning and memory [11-13]. Spontaneous excitatory postsynaptic current (sEPSC) is a physiological characteristic of the hippocampus, an important phenomenon believed to contribute to learning and memory processes within the hippocampus [26]. Following presynaptic stimulation, the physiological characteristics of the evoked sEPSC become transformed, which may suggest an influence of sEPSC on synaptic transmissions within the hippocampus. Both temperature and the introduction of magnetic fields have previously been found to impact the physiological characteristics of sEPSC [15-18].

Thermal effects caused by low-frequency electromagnetic stimulation are of great concern in clinical applications, as they may cause undesirable complex phenomena and side effects. Such biological thermal effects induced by electromagnetic stimulation are one of the main known causes of electromagnetic stimulation damage [14]. Furthermore, thermal effects may interfere with the analysis of electrophysiological signals during electromagnetic stimulation of the brain.

We demonstrate herein that TMS is capable of inducing thermal heating in aCSF and thermal effects influence the properties of the sEPSC in the hippocampal CA1 pyramidal neurons. Thermal heating was induced using 50 Hz rTMS for 15 min. The physiological properties of the sEPSC were measured across increasing temperatures. Evoked EPSCs in CA1 pyramidal cells have already been demonstrated for a temperature range of 26.5 to 33.5°C [19]. The present study additionally compared the difference in thermal effects by whole-cell patch clamp recording and analyzed the electrophysiological properties of the neurons in hippocampal CA1 slices.

MATERIALS AND METHODS

Animal subjects

Male Sprague-Dawley rats (14-18 days old at the time of surgery) from the Institute of Radiation Medicine Laboratory Animal Center of Tianjin, China were used. Animals were kept in a sterile room with a constant temperature (25±2°C) in individual cages with free access to standard food and water. All procedures were approved by the Institutional Animal Care and Use Committee protocol. We designed the experiments to minimize any animal suffering as well as the number of animals used in this study.

Solutions and drugs

Animals were deeply anesthetized with ether. During surgery, brains were rapidly removed into the ice-cold cutting solution containing, in mM: 90 sucrose, 87.2 NaCl, 2.5 KCl, 7 $MgCl_2$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$ and 16.7 glucose bubbled with 95% O_2 and 5% CO_2; 350-μm-thick horizontal slices were cut with a vibrating tissue slicer (Vibratome 3000, USA) in ice-cold cutting solution. Slices were incubated at 32-35°C in aCSF for at least 40 min before patch clamp recordings. Recording pipettes were filled with solution containing, in mM: 134 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 EGTA, and 2 Na_2ATP (pH 7.2-7.25).

Magnetic stimulator

A carbon dioxide incubator was modified to allow for magnetic stimulation [20] and further modified to monitor the real-time temperature of chambers. The solution (aCSF) was placed in the magnetic stimulator with 95% O_2 and 5% CO_2 (Fig. 1). We recorded the temperature 10 times every three minutes. The experimental results showed that 50-Hz low-frequency electromagnetic irradiations with magnetic field intensities of 7, 14, and 23 mT could induce thermal heating in aCSF from 25 to 40°C across 15 min (Fig. 2). We also demonstrated that the stronger the magnetic field, the higher the increase in temperature. According to these results and in keeping with current literature, 25°C (25±1°C), 30°C (30±1°C), 35°C (35±1°C) and

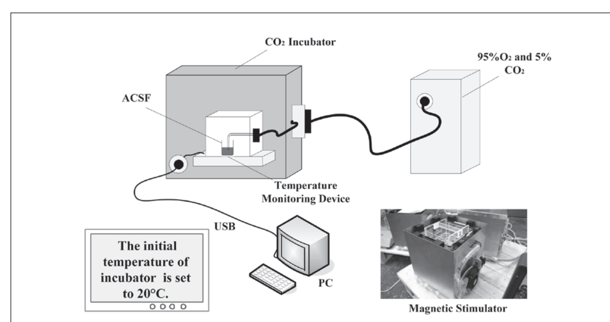

Fig. 1. An incubator modified to allow real-time monitoring of the temperature of aCSF displayed on the computer. The first temperature of the incubator was set to 20°C. The solution (aCSF) was placed in the magnetic stimulator bubbled with 95% O_2 and 5% CO_2. The temperature was recorded every three minutes and displayed digitally.

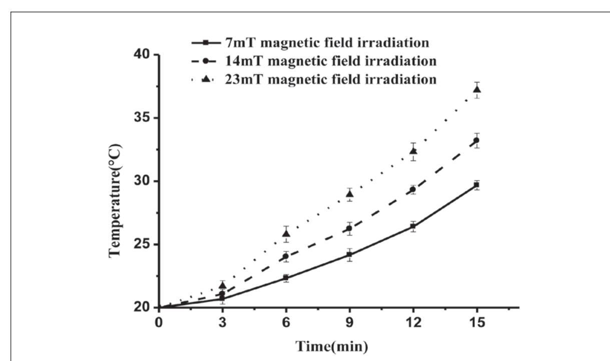

Fig. 2. The temperature distribution of aCSF. The temperature distribution was measured after exposure to a 50-Hz magnetic field and 7, 14, 23 mT across 15 min. The temperature at the start time (0 min) was set to 20°C. Each magnetic field was recorded 10 times.

40°C (40±1°C) were selected as temperature recording points in the following experiment.

Temperature control design

Given that temperature may fluctuate over a period of time, double measures were taken to control the temperature throughout the experiments in order to ensure temperature measurements are accurate. Four kinds of aCSF with temperatures of 25°C (25±0.3°C), 30°C (30±0.3°C), 35°C (35±0.3°C) and 40°C (40±0.3°C) were prepared. The aCSF was inserted into an incubator (BPN-80CH, Blue pard, ShangHai, China) at a temperature of 25°C. After 30 min, the temperature of the solution was measured using a thermometer. Subsequent temperature measurements were taken in the same manner. Slices were then transferred to the recording chamber and superfused with aCSF at the rate of 2 mL/min. Next, the recording chamber was controlled to maintain a constant temperature point using a temperature control instrument (TC-324B, Warner, USA). Each temperature point was recorded at 15 min intervals. The temperature was then raised from 25°C to 40°C. Data was collected with Path-Master software (HEKA, German). With each increase of temperature point, the aCSF of the corresponding temperature in the incubator was controlled for perfusion and temperature.

Electrophysiological recordings

Whole-cell patch clamp recordings were amplified using an EPC10 amplifier (HEKA, Germany) and performed in hippocampal CA1 pyramidal neurons. Cells were viewed with an upright microscope (BX51-WI, Olympus, Japan) equipped with a long-range water immersion objective (40×) and an infrared video camera (710 M, DVC, USA). The pyramidal cells situated in the pyramidal stratum were identified morphologically for recordings. After stable recording was carried out, sEPSCs were recorded for 15 min while the membrane potential was held constant at 70mV throughout [21].

Statistical analysis

Data were transformed and the different electrophysiological properties sorted by the ABF utility of Minianalysis (Synaptosoft, USA). The data were statistically analyzed using Origin8.0 and are expressed as means±SEM. One-way ANOVA was performed with temperature as the dependent variable. Conditional on a significant F value, post-hoc Scheffé tests were used to further analyze the direction of effects and significance among groups. The significance level (α) was set to 0.05, and the following supplementary identifiers were used throughout: *p<0.05, **p<0.01 and ***p<0.001.

RESULTS

Thermal effects on sEPSCs

The representative traces of the sEPSC of hippocampal CA1 pyramidal neurons at 25°C, 30°C, 35°C and 40°C were investigated (Fig. 3A-D). This was followed by recording the average amplitude of sEPSCs, which were (660.91±9.62) pA, (247.64±3.88) pA, (34.03±0.17) pA and (12.20±6.13%) pA (n=10) at 25°C, 30°C, 35°C and 40°C, respectively (Fig. 4A). One-way ANOVA revealed significant inhibition in the average amplitude of sEPSCs at 25 to 40°C ($F_{(3,6621)}$=32.98, P<0.001). A subsequent Scheffé test showed that the extent of inhibition was more pronounced at temperatures above 30°C (Table 1).

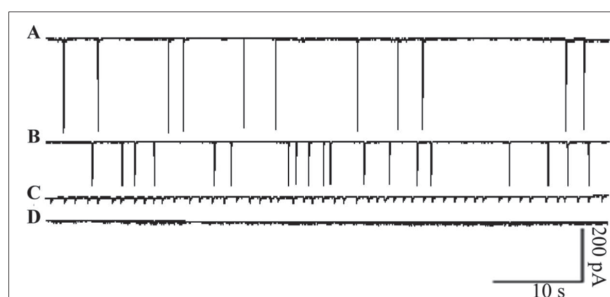

Fig. 3. Representative sEPSC traces recorded at different temperatures. A, B, C, D, sEPSC properties at 25°C, 30°C, 35°C, and 40°C, respectively. The unit of time is 10s and the unit of amplitude is 200 pA.

Table 1. Results of the Scheffé test.

	Amplitude			Frequency		
	F Value	Prob	Sig	F Value	Prob	Sig
30°C *vs* 25°C	14.43	P<0.001	1	0.59	0.57	0
35°C *vs* 25°C	24.77	P<0.001	1	2.86	0.02<0.05	1
35°C *vs* 30°C	1.96	0.16	0	2.27	0.053	0
40°C *vs* 25°C	23.36	P<0.001	1	2.96	0.02<0.05	1
40°C *vs* 30°C	2.13	0.14	0	2.38	0.04<0.05	1
40°C *vs* 35°C	0.02	0.99	0	0.11	0.92	0

Sig=1 – significant difference; Sig=0 – No significant difference

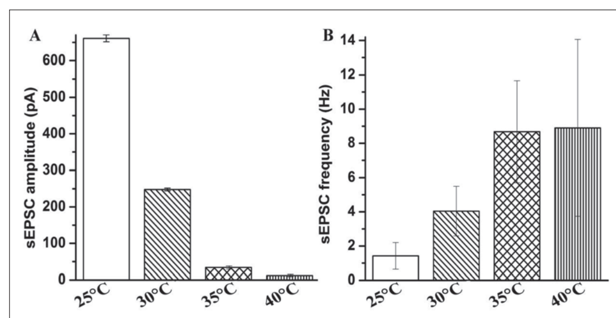

Fig. 4. Comparisons of average amplitude and frequency of sEPSC across the different temperatures. **A** – comparisons of average amplitudes of sEPSC at 25°C, 30°C, 35°C and 40°C. **B** – comparisons of average frequencies of sEPSC at 25°C, 30°C, 35°C and 40°C.

The average frequency of sEPSCs in the hippocampal CA1 slices at 25°C, 30°C, 35°C and 40°C was 1.42±0.78 Hz, 4.04±1.45 Hz, 8.68±2.98 Hz and 8.90±5.16 Hz (n=10), respectively (Fig. 4B). One-way ANOVA revealed significant increases in the average frequency of sEPSCs when the temperature was increased from 25°C through to 40°C ($F_{(3,8391)}$=4.68, P<0.05). A Scheffé test showed that these were circumscribed to temperatures of 30°C or lower (Table 1).

Thermal effects on single sEPSCs

We further studied the electrophysiological properties of single sEPSCs, which were compared and recorded at different temperatures (Fig. 5A). The rise time of sEPSCs at 25°C, 30°C, 35°C and 40°C were 1.91±3.74% ms, 2.28±2.21% ms, 2.20±1.32% ms and 1.84±2.27% ms (n=10), respectively (Fig. 5B). Results from the one-way ANOVA showed that there were significant differences in the rise time of sEPSCs with respect to the different temperatures ($F_{(3,4486)}$=40.14, P<0.001). Subsequent comparisons with the Scheffé test revealed that there were significant increases in the rise time of sEPSCs at temperatures 25-30°C, and significant inhibition at temperatures above 30°C (Table 2).

The decay times of sEPSCs were 1.65±2.54% ms, 2.48±2.79% ms, 4.19±2.28% ms and 2.40±4.05% ms (n=10) at 25°C, 30°C, 35°C and 40°C, respectively (Fig. 5C). Results from the one-way ANOVA showed that these differences were significant ($F_{(3,4486)}$=40.14, P<0.001). There were significant increases in decay times of sEPSC at temperatures 25-35°C, and significant inhibition at temperatures above 35°C (Table 2).

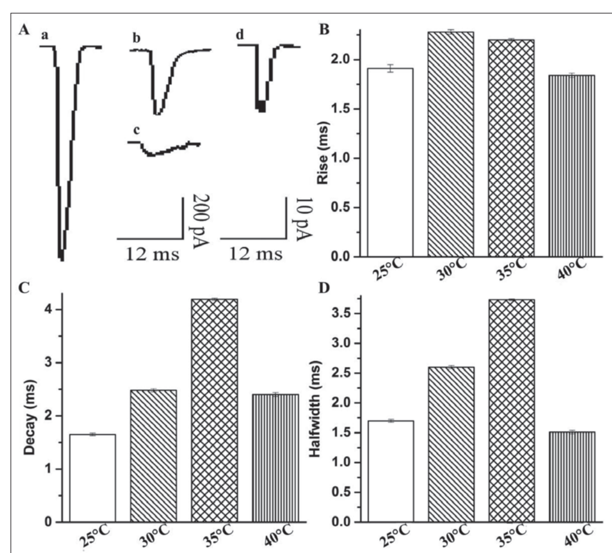

Fig. 5. Comparison of properties of single sEPSC at 25°C, 30°C, 35°C and 40°C. **A** – representative single sEPSC trace recorded at 25°C (a), 30°C (b), 35°C (c), and 40°C (d). **B**, **C**, and **D** – comparisons of the rise time, decay time and half-width at 25°C, 30°C, 35°C and 40°C.

Table 2. Results of the Scheffé test.

	Rise			Decay			Half-width		
	F Value	Prob	Sig	F Value	Prob	Sig	F Value	Prob	Sig
30°C *vs* 25°C	20.23	P<0.001	1	15.25	P<0.001	1	22.26	P<0.001	1
35°C *vs* 25°C	14.08	P<0.001	1	49.57	P<0.001	1	33.77	P<0.001	1
35°C *vs* 30°C	2.49	0.06	0	43.42	P<0.001	1	25.67	P<0.001	1
40°C *vs* 25°C	0.96	0.41	0	14.31	P<0.001	1	4.96	P<0.001	1
40°C *vs* 30°C	37.72	P<0.001	1	2.38	0.33	0	13.43	P<0.001	1
40°C *vs* 35°C	32.22	P<0.001	1	42.69	P<0.001	1	36.58	P<0.001	1

Sig=1 – significant difference; Sig=0 – no significant difference

The half-widths of sEPSCs in the hippocampal CA1 slices were 1.70±2.55% ms, 2.60±2.83% ms, 3.73±1.43% ms and 1.51±2.96% ms (n=10) at 25°C, 30°C, 35°C and 40°C, respectively (Fig. 5.D). One-way ANOVA showed that these differences were significant ($F_{(3,4486)}=40.14$, $P<0.001$). There were significant increases in the half-width of sEPSCs at temperatures ranging from 25-35°C, and significant inhibition at temperatures above 35°C (Table 2).

DISCUSSION

To our knowledge, this is the first study to demonstrate that a 50-Hz low-frequency electromagnetic stimulation with magnetic field intensities of 7, 14, and 23 mT can induce thermal heating in artificial cerebrospinal fluid (aCSF) from 25 to 40°C during 15 min. Moreover, the resulting thermal effect appears to change the properties of sEPSC in the hippocampal CA1 pyramidal neuron. Double measures were taken to control the temperature throughout the experiments to ensure the accuracy of temperature measurements. The present results suggest that the function of hippocampal CA1 pyramidal neurons is influenced by the thermal effect induced by magnetic stimulation. The effect this may have on the neural excitability and synaptic plasticity of hippocampal CA1 pyramidal neurons is discussed below.

The presentesd results point to a remarkable turning-point of temperature affecting the electrophysiological properties of hippocampal CA1 neurons. The changes in temperature could increase and decrease the excitability of synapses [22,23], as well as convert silent synapses into active ones [24]. sEPSC consists of two components, AMPA (α-amnio-3-hydorxy-5-methyl-4-isoxazoleporpionate) and NMDA (N-methyl-d-aspartate) [25]. With these findings, we have demonstrated that the maximum amplitude and the minimum frequency are observed at 25°C with two separate outcomes. On the one hand, the number of postsynaptic receptor channels induced by excitatory synapses is increased, while the frequency of opening channels is decreased. On the other hand, the released quantity of presynaptic neurotransmitter is increased, but the frequency is decreased. Because of the mild temperatures, the properties of sEPSCs are able to transform silent synapses into active ones. By recording the release of sEPSCs at different temperatures, we demonstrated that the amplitude and frequency of sEPSCs present opposite tendencies with rising temperature (amplitude decreases and frequency increases).

The present study further showed that temperature changes have a significant impact on the properties of sEPSCs. The possible mechanism of spontaneous synaptic activity is dependent on the excitability of cells induced by temperature changes. At the same time, the number of postsynaptic receptors is decreased, while the open frequency is increased. Analysis of the characteristics of single sEPSCs showed that rise time, decay time and half-width of sEPSC all exhibited a similar pattern. The sensitivity of postsynaptic receptors markedly changed as temperatures increased. All appeared to steadily increase at temperatures below 30°C, culminating near the 30°C point. Taken together, this suggests that thermal effects play an important role in the function of postsynaptic receptors.

In conclusion, this study demonstrates that there are a number of significant changes in the electrophysiological properties of sEPSCs or single sEPSCs of hippocampal CA1 neurons associated with the thermal effects induced by magnetic stimulation. These findings also demonstrate that the excitation of synaptic activity is significantly increased at temperatures between 25 and 30°C. There are undoubtedly a number of secondary effects incompletely understood concerning the application of TMS. For example, magnetic stimulation may affect a more distributed neuronal system than may be initially intended. Nonetheless, the present study offers a deeper understanding of some the consequences of TMS application in clinical and research practices.

Acknowledgments: This research was supported by the Research Program of Application Foundation and Advanced Technology of Tianjin (No. 14JCYBJC16300), the National Natural Science Foundation of China (No. 41206031).

Authors' contributions: All authors contributed equally. Experimental design and analyses were done by Yu Zheng and Lei Dong, while temperature control and patch-clamp experiments were done by Ying Kong, Hui Hong, Yang Gao, Zhe Zhao, Hui-quan Wang.

Conflict of interest disclosure: We declare no conflict of interests.

REFERENCES

1. Thompson SP. A physiological effect of an alternating magnetic field. Proc R Soc Lond B Biol Sci. 1910;82(557):396-8.
2. Wassermann EM, Zimmermann T. Transcranial magnetic brain stimulation: therapeutic promises and scientific gaps. Pharmacol Ther. 2012;133(1):98-107.
3. Mottaghy FM, Keller CE, Gangitano M, Ly J, Thall M, Parker JA, Pascual-Leone A. Correlation of cerebral blood flow and treatment effects of repetitive transcranial magnetic stimulation in depressed patients. Psychiatry Res. 2002;115(1-2):1-14.
4. Gross M, Nakamura L, Pascual-Leone A, Frengi F. Has repetitive transcranial magnetic stimulation (rTMS) treatment for depression improved? A systematic review and meta-analysis comparing the recent vs. the earlier rTMS studies. Acta Psychiatr Scand. 2007;116(3):165-73.
5. Nardone R, Tezzon F, Höeller Y, Golaszewski S, Trinka E, Brigo F. Transcranial magnetic stimulation (TMS)/ repetitive TMS in mild cognitive impairment and Alzheimer's disease. Acta Neurol Scand. 2014;129(6):351-66.
6. Wu AD, Fregni F, Simon DK, Deblieck C, Pascual-Leone A. Noninvasive brain stimulation for Parkinson's disease and dystonia. Neurotherapeutics. 2008;5(2):345-61.
7. Fregni F, Pascual-Leone A. Technology insight: noninvasive brain stimulation in neurology-perspectives on the therapeutic potential of rTMS and tDCS. Nat Clin Pract Neurol. 2007;3(7):383-93.
8. Sandrini M, Umiltà C, Rusconi E. The use of transcranial magnetic stimulation in cognitive neuroscience: a new synthesis of methodological issues. Neurosci Biobehav Rev. 2011;35(3):516-36.
9. Cotelli M, Manenti R, Cappa SF, Zanetti O, Miniussi C. Transcranial magnetic stimulation improves naming in Alzheimer disease patients at different stages of cognitive decline. Eur J Neurol. 2008;15(12):1286-92.
10. Julkunen P, Jauhiainen AM, Westeren-Punnonen S, Pirinen E, Soininen H, Könönen M, Pääkkönen A, Määttä S, Karhu J. Navigated TMS combined with EEG in mild cognitive impairment and Alzheimer's disease. J Neurosci Methods. 2008;172(2):270-6.
11. Banta Lavenex P, Lavenex P. Spatial memory and the monkey hippocampus: not all space is created equal. Hippocampus. 2009;19(1):8-19.
12. Kumaran D. Short-term memory and the human hippocampus. J Neurosci. 2008;28(15):3837-838.
13. Schulz K, Korz V. Emotional and cognitive information processing: relations to behavioral performance and hippocampal long-term potentiation in vivo during a spatial water maze training in rats. Learn Mem. 2010;17(11):552-60.
14. Katsuki S, Mitsutake K, Yano M, Akiyama H, Kai H, Shuto T. Non-thermal and transient thermal effects of burst 100 MHz sinusoidal electric fields on apoptotic activity in HeLa cells. IEEE Trans Dielectr Electr Insul. 2010;17(3):678-84.
15. Paul A, Narasimhan A, Kahlen FJ, Das SK. Temperature evolution in tissues embedded with large blood vessels during photo-thermal heating. J Therm Biol. 2014;41:77-87.
16. Hyun NG, Hyun KH, Hyun KB, Han JH, Lee K, Kaang BK. A computational model of the temperature-dependent changes in firing patterns in Aplysia neurons. Korean J Physiol Pharmacol. 2011;15(6):371-82.
17. Pappas TC, Motamedi M, Christensen BN. Unique temperature-activated neurons from pit viper thermosensors. Am J Physiol Cell Physiol. 2004;287(5):C1219-C1228.
18. Duan B, Xu TL. Transient receptor potential channels and signal transduction. Acta Biophysica Sinica. 2005; 21(4):245-60.
19. Hestrin S, Sah P, Nicoll RA. Mechanisms generating the time course of dual component excitatory synaptic current recorded in hippocampal slices. Neuron. 1990;5(3):247-53.
20. Schuderer J, Oesch W, Felber N, Spät D, Kuster N. *In vitro* exposure apparatus for ELF magnetic fields. Bioelectromagnetics. 2004;25(8):582-91.
21. Hou JF, Yu LC. Blockade effects of BIBN4096BS on CGRP-induced inhibition on whole-cell K^+currents in spinal dorsal horn neuron of rats. Neurosci Lett. 2010;469(1):15-8.

22. Makkar SR, Zhang SQ, Cranney J. Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology. 2010;35(8):1625-52.
23. Griffin JD. Central thermosensitivity and the integrative response of hypothalamic neurons. J Therm Biol. 2004;29(7):327-31.
24. Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993;361(6407):31-9.
25. Zhang L, Warren RA. Muscarinic and nicotinic presynaptic modulation of EPSCs in the nucleus accumbens during postnatal development. J Neurophysiol. 2002;88(6):3315-30.
26. Luo QT, Fujita T, Jiang CY, Kumamoto E. Carvacrol presynaptically enhances spontaneous excitatory transmission and produces outward current in adult rat spinal substantia gelatinosa neurons. Brain Research. 2014;1592:44-54