Effect of Electroacupuncture on Synaptic Transmission in the Dentate Gyrus of the Hippocampus After Cerebral Ischemic Injury in rats

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Abstract **Objective:** To observe the Effect of Electroacupuncture on Synaptic Transmission in the Dentate Gyrus in rats. **Methods**: Using electrophysiological technique to record the long-term potentiation (LTP) in both basic synaptic transmission and 200Hz high-frequency stimulation (HFS) induced LTP of the dentate gyrus in rat Cerebral Ischemic Injury. **Results:** There were not significant changes of magnitude of population spikes (PS) in 120 min in sham surgical group, but magnitude of PS in the Cerebral Ischemic Injury group begun to descend was 60%±7% at 10^{th} min, which were significant difference (P<0. 01) compared with sham operation group, then magnitude of PS step down, magnitude of PS in electroacupuncture group begun to descend obviously was 93%±5% at 10 min and 168%±25% at 120 min. which were significant difference (P<0. 01) compared with Cerebral Ischemic Injury group, 30 min after LTP induced by HFS, the magnitude of PS obviously increased and continued for about 180 min in sham operation group , 40 min after treatment with Electroacupuncture (was 70 min after HFS), magnitude of PS begun to increase and was 171%±22% at 90 min (was 120 min after HFS) in Electroacupuncture group, and there was no significant difference compared with sham operation group (178%±24%, P>0. 05).
Conclusion: Electroacupuncture can enhance the basic synaptic transmission and the magnitude of LTP induced by HFS of the dentate gyrus in rats.
Key words: Electroacupuncture; long-term potentiation; Dentate Gyrus

Long-term potentiation synaptic transmission is an important form of plasticity for the central nervous system (CNS). It is considered to be the cellular basis for learning and recollection. LTP formation can be created by multiple externally induced factors, especially through the action of electrical stimulation (1). In order to investigate the external factors that influence brain plasticity, we used an electrophysiological technique to observe the effect of electroacupuncture treatment on basic synaptic transmissions and the LTP induced by high frequency stimulation (HFS) within the dentate gyrus [of the hippocampus] in rats, in order to further investigate the effect of electroacupuncture on cerebral plasticity under cerebral ischemic [attack] conditions.

1. Material and methods

1.1 Test animals and groups

We chose to use 60 healthy male Wistar rats weighing between 150 - 250g, furnished by the Guangzhou TCM University Center for test animals. The 60 animals were randomly divided into 2 groups, a basic (transmission) and a High Frequency Stimulation (HFS) group which were further subdivided into a sham-surgical group, an [cerebral] ischemia group and an electroacupuncture group (each group therefore containing 10 animals): (1) the sham surgical group only had surgery to expose the cerebrum common carotid artery bilaterally without blocking the circulatory system; (2) the ischemia group had surgery to expose the cerebrum common carotid artery bilaterally

and also had it obstructed; (3) the ischemia and electroacupuncture group had surgery to interrupt the cerebrum common carotid artery's flow.

1.2 Testing apparatus

The RM-86 polygraph recording machine (Japan's Guangdian Coorporation), RB-5 biophysical amplifier (Japan's Guangdian Corporation) and Shanghai Medical University Average software of SMUP, the SS-202J stimulation isolator (Japan's Konden [Corporation]), SEN 7130 constant source stimulator and SR-6N 3-D imaging system (Japan's Narishige [Corporation]).

1.3 Electrode implantation and the formation of cerebral ischemia

A 10% chloral dehydrate solution, at 400 mg/kg dose, was administer to anesthetized animals; using fixed 3-D imaging equipment, electrodes were placed according to the Pelligrino rat brain diagrams, bilateral stimulating electrode implantation within the entorrhinnal perforate pathway (PP), the fixed location coordinates being: AP 7.5mm, L4 2mm and H 3.0mm and the recording electrode coordinates being AP 3.8mm, L 2.5mm and H 3.5mm unilaterally in the granular cell layer of the dentate gyrus. At the above coordinates a 1.5mm diameter hole was drilled for implanting the recording and stimulating electrodes which were adjusted appropriately until the maximum population spike (PS) was found for both the recording and stimulating electrodes, then the electrodes were fixed.

The bilateral common carotid arteries were separated out and 2 strands of #0 silk thread were passed down through them. After injecting 0.25 mg / 100g of body weight of sodium nitroprusside via the abdominal cavity, the thin silk was pulled slightly to cut off the blood circulation of the common carotid arteries; after 10 minutes the silk thread was untied and circulation in the arteries was continued; after another 10 minutes the thread was pulled through a second time to obstruct the arteries and then unclamped after the next 10 minutes. The silk thread was removed, the cut open areas sutured and the ambient temperature was maintained at 25 C. For the sham surgical group, the common carotid arteries were separated out, then after two strands of #0 silk thread were passed through the common carotid arteries and then after 30 minutes the open areas were sutured closed.

1.4 Needling technique

Base group: Electroacupuncture observation group animals had electroacupuncture done for 10 min prior to the blockage of the common carotid artery and for 60 minutes after its blockage; the high frequency group animals were given the high frequency [for 30 minutes] and electroacupuncture for 60 minutes. The point locations were GV-20 (*bai hui*) and GV-14 (*da zhui*) of the Governing vessel (du mai) and CV-24 (cheng jiang) and CV-4 (*guan yuan*) of the ren mai. The locations of the points followed those of *Acupuncture Testing* (People's Health Press, 1997 1st edition) as related to the point location of a rat. We employed the PCEA electroacupuncture machine from the Anhui TCM Hospital Jingluo Research Institute using a 2-5 Hz, dense wave formation, with enough stimulation to make the musculature vibrate slightly, generally between 3-5v sustained for 60 minutes, and took measurements of PS once every 5 minutes.

1.5 Recording and observing the induced electrical potential.

We used an extracellular recording method. After generating a test stimulus (a square wave form with a frequency of 1/30 Hz and a wave length 0.1ms) via an electronic

stimulator, it was conveyed via the perforant pathway (PP) through the stimulus isolator and stimulus electrode. The induced electrical activity was displayed through the bioamplifier onto the physiological recorder, and simultaneously fed into a computer that continuously analyzed and recorded the magnitude of the Population Spikes (PS). This served as the dentate gyrus (DG) granular cellular excitation index. We adjusted the stimulus strength, using 1/2 of the required stimulus strength for maximum PS as the test stimulus strength. Throughout the entire experimental process the test stimulus parameter remained constant.

1.6 High Frequency Stimulus (HFS)

The long-term potentiation (LTP) of the dentate gyrus granular cellular layer was induced by a series of 10 200 Hz High Frequency Stimuli (HFS), a series of stimuli consists of square waveform stimulus of 5 waves 0.1ms in width, with an interval of 200ms between each series, the stimulus strength and test stimulus were identical.

1.7 Testing Procedure

Both the sham surgical group and pre-ischemia groups had a normal PS recording, which served as the control group's normative comparative value. After blocking the common carotid artery, the PS recording was continued for 1 hour, with the acupuncture group receiving electric acupuncture for 10 minutes prior to the blockage of the artery and for 60 minutes afterwards. The PS changes for both common carotid artery blockage groups are compared. The high frequency group, after 30 minutes of receiving HFS, received electric acupuncture, continuously for 1 hr. Each test stimulus produced a PS; the mean value of 10 PS groups became the PS value for one time interval, each time interval's synaptic transmission level was compared to the base level giving a relative percentage indicator. The group's comparison was carried out by the t test statistical process (we used the SMUP Stat software of the Shanghai TCM University).

2. Results

2.1 The control value for the normal rats

The sham surgical and the pre-ischemia group animals used the mean PS value created by the 1/30 Hz test control stimulus at 6 time intervals (at 5, 10, 15, 20, 25 and 30 min) that served as the normative value for the synaptic transmission levels. The commonly seen waveforms were from positive to negative to positive; due to relatively large variations in the amplitude among the different rats, the normative value did not calculate. For this reason we calculated the test rats PS comparative value; this was done by comparing synaptic transmission level for each animal group's time interval to the normative synaptic transmission level [thus] giving the comparative value (%); that is the PS segment for each time period equaled the test value divided by the base value.

2.2 Changes in the PS post cerebral ischemia

After the cerebral artery ischemic event, besides a significant decrease in the brains' PS magnitude, there occurred definite changes within each PS wave, of which the early stage ischemic amplitude change was the most significant. After the cerebral ischemia, all subsequent amplitude gradually decreased following the reperfusion intervals extended. The PS amplitude fell from the pre-ischemic event norms by 10-40% in the rats within 1 hr of the ischemia event; while in a few rats, the PS amplitude gradually fell by 50% within 10 minutes after the ischemic event ($P<0.01$). Following the ischemia, the

reperfusion interval was prolonged, each PS wave's decline was significantly reduced approaching the pre-ischemic event level, and when compared to the control group's showed no significant difference ($P>0.05$).

2.3 The effect on the basic synaptic movement of the dentate gyrus

We observed a different series of effects on the base PS magnitudes in all the groups of animals using the 1/30Hz HFS stimulation. For instance, referring to table 1A, for the sham surgical group, there were no significant changes within 120 minutes and the PS values at the 10, 30, 60, 90, and 120 minute marks were 100%±5%, 102%±6%, 105%±7%, 105%±9%, and 108%±11% respectively ($P>0.05$). Referring to Table 1B, in the ischemia animals, the PS magnitude began to drop 60%±7% within 10 minutes, which, when compared to the control group, was a significant difference ($P<0.01$). Subsequently, at the 30, 60, 90, 120 minute marks the [rate of] decline in the PS magnitude gradually decreased to 90%±3% ($P<0.05$), 93%±4%, 95%±3%, 96%±7%, 98%±8% and 101%±10% ($P>0.05$). Referring to table 1C, with the electric acupuncture group, after using electric acupuncture prior to the ischemia, and 10 minutes after the ischemic event, the PS value did not significantly drop, 93%±5%, a significant difference compared to the ischemia group ($P<0.05$). The subsequent values gradually increased at the 30, 60, 90, and 120 minutes, marks were 106%±10%, 123%±16%, 145%±20%, and 168%±25%, which is a significant difference compared to the ischemia group ($P<0.01$). These results demonstrate that electric acupuncture can increase the effect of the synaptic transmissions within the dentate gyrus.

2.4 The effect of an induced HFS on the LTP of the dentate gyrus.

The form of HFS used in this test is very close to the theta brainwaves of hippocampus which animals spontaneously generate naturally and can effectively induced LTP in the dentate gyrus. Referring to table 2A, after having induced the HFS in the sham surgical group, the PS magnitude significantly increased without any significant diminution over a 3-hour period. At $t = 0$ minutes, the PS magnitude was 100%±6%; after the use of HFS, the PS magnitudes for the 30, 60, 90, and 120 minute marks were 182%±21%, 179%±18%, 177%±18%, 175%±23% and 178%±24%. Referring to table 2B for ischemia group at $t = 0$ minutes, the PS magnitude was 60%±7%, 30 minutes after the induction by the HFS, LTP can appear with the PS magnitude being 138%±11%, and thereafter at the 60, 90, 120, and 180 minute marks the magnitudes were 141%±13%, 139%±14%, 140%±13%, and 138%±15% ($P<0.01$).

In the electric acupuncture group, we began giving electric acupuncture 30 minutes after giving the HFS. Referring to table 2C, 40 minutes after the electric acupuncture stimulation (70 minutes after the HFS), the PS magnitude began to increase reaching 154%±16%, which when compared to the sham surgical group (172%±21%) there were not significant differences ($P>0.05$). 90 minutes after the electric acupuncture (120 minutes after the HFS), the PS magnitude was 171%±22%, which when compared to the sham surgical group (175%±23%) there were not significant differences ($P>0.05$). At 150 minutes (180 minutes after the HFS), the PS magnitude was 181%±25%, which when compared to the sham surgical group (178%±24%) there were not significant differences ($P>0.05$). The results show that electric acupuncture enhanced the function of the already created LTP induced by HFS.

3. Discussion

Recent research reveals [3] that plasticity is an important characteristic of the human nervous system, [that] it is the ability to adapt that occurs within the neurons and nerve networks. The plasticity of the nervous system is the physiological foundation for behavioral adaptability; at the microscopic level there are changes within the fine structure and function of the neuron synapses and nerve networks, within their electrophysical activity as well as in their submicroscopic structures. At the higher ganglionic levels of the brain, each neuron already possesses the potential ability to continue forming synaptic connections; this ability is the foundation for the nerve networks plasticity. The emphasis of this research is that we observed the pattern of the recovery process during an ischemic attack, the synaptic transmission within dentate gyrus and the activity of acupuncture. Also, the hippocampus is the most susceptible region of the brain to ischemia. Especially important to note is Bliss, et al [4] in 1993, who first observed the onset of LTP phenomena in both anesthetized and conscious rabbit hippocampus structures after using short series (10 sec) of high frequency (15 Hz) electrical stimulation through the PP; and further [5] data demonstrates that the LTP appearance within the hippocampus is an important kind of synaptic plasticity change. Thompson et al [6] discovered that while giving nictitating membrane (winking) conditioned reflex training to rabbits, a similar kind of LTP phenomena arises within the hippocampus; and Berger et al [7] reported earlier induced LTP in rabbit dentate gyrus. From these can be seen that LTP may be the basis for the plasticity found within the hippocampus nerve synapses.

That the controlling (*ren mai)* and governing vessels (*du mai*) effectively treat cerebral blood vessel ischemic diseases is very well confirmed[8]. They are not only responsible for the embryonic gestation but are also closely related to physiological diseases of the brain, thus we conjectured that needling the controlling vessel must produce some growth factors related to development, and that synaptic plasticity must be related to them. Therefore, we chose commonly used points on the controlling vessels used to treat wind stroke (*zhong feng*) to carry out the experiment, namely CV-4 (*guan yuan*) and *shen que* (CV-8) and *cheng jiang* (CV-24); and because the controlling and governing vessels are closely related, we chose GV-20 (*bai hui*) and GV-14 (*da zhui*). Referring to related theories of Chinese medicine, we tentatively put forth that the primary function of the controlling vessel is for pregnancy and recuperation, while those of the governing vessel initiate warming and unblocking. All of these are important for accelerating the development of cerebral plasticity.

The electrical activity recorded using electrophysiological methods for this paper was field Excited Post Synaptic Potential (fESPS) in the dentate gyrus conic cells of the hippocampus. By means of electric acupuncture applied to different groups of rats, we observed the effect of electroacupuncture on LTP induced by HFS and dentate gyrus synaptic transmissions as well as the relationship between electric acupuncture action mechanism and LTP in the hippocampus. Whether induced by HFS or by electroacupuncture, if the magnitude of the PS compared to that produced spontaneously by the control group was more than 30% over at least a 30 minute time period, then it demonstrated that the LTP phenomenon has already been created. In the testing we

observed that dentate gyrus synaptic transmission was increased after 10 minutes of electroacupuncture, and that within 120 minutes the PS had continued to increase. This demonstrated that electroacupuncture can increase the functionality of synaptic transmission within the Central Nervous System (CNA), increase basic activity of synaptic transmissions. During the testing we also observed that LTP was induced through HFS after 30 minutes, when electroacupuncture was added, that it could continue to cause in increase in the LTP even after it had already been created by the HFS and that the PS magnitudes increased. This research results indicate that even though the LTP induced by HFS was made larger, that electroacupuncture, given 30 minutes after the induction of LTP by HFS, still could effectively increase the scope of synaptic transmissions within the CNS. The research indicates that electroacupuncture is closely related to the function of cerebral plasticity and to the enhancement of the CNS synaptic transmissions and this could very well be one of the mechanisms in the CNS. The creation and continuation of LTP involve pre- and post-synaptic mechanisms, post-synaptic mechanism is the main one and among the important factors for inducing LTP are intracellular calcium, calcium-binding protein, NMDA (N-methyl-D-aspartate) receptors and retro messengers (NO and CO). In addition, maintenance of LTP still involves synthesis of protein, protein phosphoric acid transformation and the changes of synaptic structure, for this reason ongoing investigation into the exact mechanism for LTP still needs to be more deeply researched.

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table 1 A

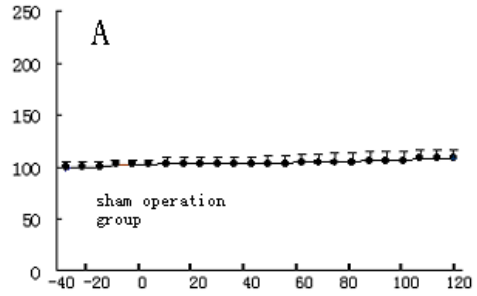
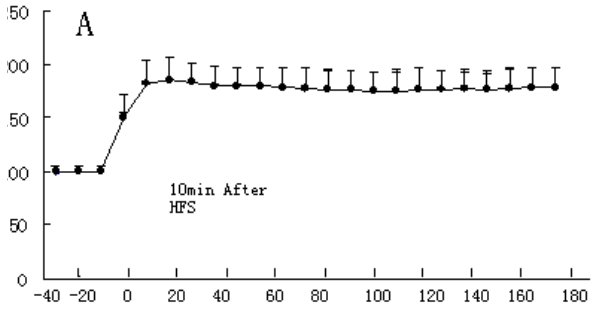


table 2 A



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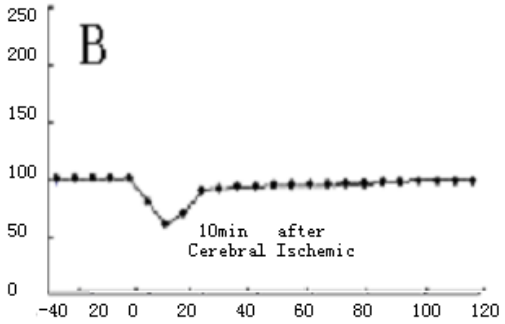

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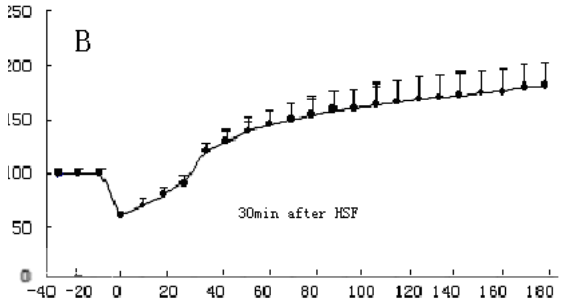

table 1 C

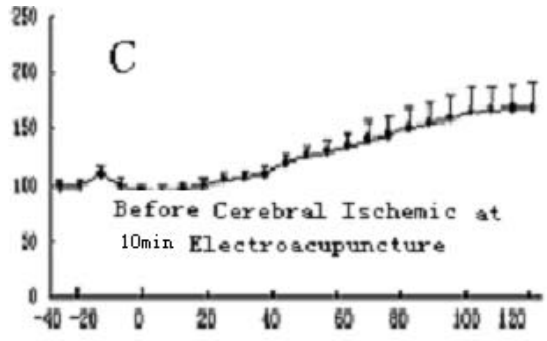

table 2 C