Electroacupuncture prevents cognitive deficits caused by topiramate (TPM) in rats

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Abstract: Objective Recent studies have demonstrated that acupuncture is feasible to treat cognitive impairments. The objective of this study was to present behavioral evidence that electro-acupuncture (EA) could improve the learning and memory of TPM administrated rats.

Methods The pattern of TPM-induced cognitive deficits in rats was made by administration of TPM intragastrically for 3 weeks. Of them the rats which showed damage in learning and memory (n = 45) were randomly allocated to 3 groups: impaired group (n = 15), EA group (n = 15) and placebo-EA group (n = 15). Moreover, normal group (n = 15) was set as control groups. EA stimulation was provided at acupoints located in either the midline of the back or of the head: Gv-20 (Baihui), Gv-14 (Dazhui). Morris water maze test was employed to assess spatial discriminative ability per group respectively and to analyze the curative effects of EA. Results Compared to the normal group, obvious cognitive deficits were found in the impaired and placebo-EA groups, and the statistic analysis showed that there were significant differences between normal and impaired groups in ANOVA. In the EA group, shortened mean escape latency was detected compared with the impaired effect on cognitive group during the same trial days; search strategy changed from random pattern adopted by impaired and placebo-EA rats to tendency or linear pattern popular in the normal group. Conclusion The present results suggested that EA exerted a protective impairment caused by TPM in rats, and EA has a specificity of cure. EA as a potential clinic method in treating TPM-induced cognitive impairment should be developed and investigated in the future.

Key words: electro-acupuncture; Topiramate; morris water maze; learning and memory

INTRODUCTION

A significant proportion of patients with epilepsy are at increased risk for cognitive impairment. A variety of factors contribute to their cognitive dysfunction[1]. It is known that antiepileptic drugs (AEDs) can have adverse effects on cognitive function [2,3]. AEDs have a dose-dependent effect on cognitive functioning, and AED polytherapy may result in even more striking adverse cognitive effects.

Topiramate (TPM) monotherapy has recently been found to be effective for seizure control in partial or generalized epilepsy [4,5]. However, the adverse effect of TPM on some components of cognition appears to be long-lasting, even at low dosages, and this could influence occupational functioning or academic achievement, and TPM has been associated with a high incidence of cognitive impairment, ranging from 11% to 20%, in those patients with refractory epilepsy under polytherapy[6,7]. This cognitive impairment is clinically relevant because it often requires drug discontinuation, even when the drug has a favorable effect on seizure frequency[6]. The major cognitive complaints are impaired attention/concentration, memory deficit, slow thinking, and word finding difficulties [6,7,8,9,10,11,12,13,14,15]. These effects are related to higher dosages, rapid titration, and polytherapy [2,3,6]. At present, there are fewer drugs that can prevent these side effects.

Acupuncture, a core component of Traditional Chinese Medicine (TCM), is becoming recognized as an effective way for treatment for many chronic diseases including depression, pain, addiction, with virtually no side effects in competent hands. Schwarz et al.[16] found that acupuncture could increase both cerebral oxygen saturation and cerebral blood flow velocity of patients with vascular dementia (VD). Recent study showed that EA could prevent atrophy of some limbic structures and improve cognitive deficits in pilocarpine-epileptic rats[17]. Wang et al. [18]reported that EA could modulate the production and clearance of free radicals, and improve the ability of learning and memory of the VD model rats made by 4-vessel occlusion method. It is reasonable to assume that EA can improve the cognitive deficits caused by TPM administration. Here we provided some evidence of neuroethology by investigating the effect of EA on cognitive performance of TPM-induced cognition impaired rats in Morris water maze test.

MATERIALS AND METHODS
1. Subjects

A total of 360 Wistar rats (340 ± 40 g) obtained from animal center of The Fourth Military Medical University were used in this experiment and randomly divided into 2 groups as follows: TPM administrated group \((n = 345)\), control group \((n = 15)\). After TPM administration and initial Morris water maze test, cognition impaired rats of the TPM administrated group was allocated to impaired group, EA group and placebo-EA group. All the rats were housed five to a cage with free access to food and water. The bedding and cages were changed twice a week. Animal room was maintained at 24±2°C.

2. Treatment

Animals in the two experimental groups were administrated topiramate intragastrically in doses of 80 mg/kg. These doses were chosen according to previous experiments with motor seizures induced by pentylenetetrazole[19]. TPM was freshly dissolved in saline in a concentration of 80 mg/ml. Control solvent groups were administrated saline intragastrically in a volume of 1ml/kg, corresponding to the volumes in the TPM administrated groups.

Three weeks after TPM administration, all rats were given the screening trial twice daily during consecutive 5 days. The trail was a part of Morris water maze test (hidden platform) and detected how much time the rats needed to find the submerged platform. The mean of the escape latency values of each TPM administrated rat \((value 1)\) and the total mean escape latency for the normal rats \((value 2)\) on the fifth day were calculated, respectively. Screening ratio (SR) was selected as index for evaluating the cognitive deficit for each TPM insult rat, SR = \((value 1 − value 2) / value 2 \times 100\%\). The rat was considered as cognitive deficit if its SR was greater than 0.2[20]. Those TPM administrated rats with a ratio larger than 0.2 \((n = 43)\) were randomly allocated to impaired group \((n = 15)\), acupuncture group \((n = 15)\) and placebo-acupuncture group \((n = 15)\), together with the normal group \((n = 15)\) mentioned above, there were 4 groups used for next research. The EA group was given EA at points Gv-20 (Baihui), Gv-14 (Dazhui), Gv-2 (Yaoshu) and M-HN-3 (Yin Tang), respectively. For the placebo-EA group, stimulus spots (non-acupuncture points) sited at 4 non-acupoints located in close vicinity to the acupoints. The EA and placebo-EA were given once daily and each point was needled for 30 minutes. The rats in the other groups were grasped in the same amount of time and with the same extent of strength as that in the EA group. The process had been continued for 21 days with a rest every 7 day.

3. Morris water maze

Cognitive function was tested by the water maze[22,23,24], which was a circular tank with a diameter of 120 cm and a height of 50 cm. It was positioned in the middle of a well-lit testing room enriched with distal visual stimuli. The bottom of the maze was raised 0.35 m above the room floor. At the beginning of each day, the tank was filled with a mixture of cold and hot tap water to a depth of 30 cm, and the water at 22±1°C was made opaque by adding 2 kg of milk in order to prevent the animals from seeing the submerged platform. A circular escape platform, measuring 9.5 cm in diameter and 28 cm in height, was submerged 2 cm below the surface of the water hidden from the rat's view. Four points, equally spaced along the circumference of the pool, were arbitrarily assigned as: N, E, S and W, on this basis, the pool area was divided into 4 quadrants (NE, SE, SW and NW). These points served as the starting positions at which the rat was lowered gently into the water, with its head facing the wall of the water maze. A video camera, connected to an image analysis system (provided by Chinese Academy of Medical Sciences) which in turn was connected to a microcomputer running the maze software, was mounted above the center of the water maze. The swim path of the animal was tracked, digitized and stored for subsequent behavioral analysis using the same software. Each trial was started and ended manually by the experimenter, who operated a remote switch connected to the microcomputer.

3.1. Measuring procedure

The water maze test consisted of 5 stages organized as follows: 5 (before EA) and 2 (after EA) days of hidden platform trial, 1 day of probe trial, 3 days of reversal trial, and finally 1 day of visible platform trial.

3.2. Hidden platform trial

Rats were given two trials per day with a submerged platform that the rats could climb onto, to escape from the water. The location of the escape platform was fixed (in the middle of quadrant NE, 30 cm from the wall) throughout training. Two starting points, equidistant from the platform location were used. The starting points were different for consecutive trials, but were counterbalanced to prevent order effects. At the beginning of each trial, the rat was gently placed into the water at the start location, always facing the edge of the tank. A trial ended when the rat escaped onto the platform and the escape latency for each trial was recorded. If a rat failed to escape within 90 s, it was either guided to the platform, or placed onto it, by the experimenter. On such trials, an escape latency of 90 s was recorded. The rat was allowed to spend 10 s on
the escape platform and then placed for 20 s in a holding cage before the next trial began, resulting in an inter-trial interval of 35 s. At the end of the session, the rat was dried with a towel before being returned to its home cage. On each trial, the time needed to reach the platform was measured. The mean of the escape latencies of every daily trials was elaborated statistically.

3.3. Probe trial

In this 1-day test, each rat was subjected to a probe trial (60 s) in which there was no platform present. One of two starting positions in the hidden platform trial was used; this position was consistent for all rats. For the probe trial, two measurements were made: (1) the time spent in the quadrant of the former platform position; (2) the number of crossings of the exact place where the platform had been located.

3.4. Reversal trial

Rats were given two trials per day similar to those described for the hidden platform trial, but the escape platform was located in a novel position, opposite to the location used for the hidden platform trial.

3.5. Visible platform trial

To exclude the effect of motivational or sensorimotor factors in rats on learning performance, all experimental animals performed the visible trial on finally 1 day. Rats were given two trials per day similar to those described above for the hidden platform trial, but the escape platform was elevated above water surface 2 cm.

4. Electro-acupuncture stimulation

Electrical stimulation was delivered with an EA stimulator (Model G6805-02, Smeif, Shanghai, China) at voltage 0.7 V, frequency 2 Hz, duration 0.5 ms, to the groups receiving it at Gv-20 (Baihui), Gv-14 (Dazhui). The needle used for stimulation was 0.25mm in diameter and 2 cm long, made of stainless steel stem and copper handle. For bilateral points, the positive output lead from the EA apparatus was connected to the left acupoint and the negative terminal was connected to the right acupoint and the polarity between these points was continuously alternated. For midline points the pairs were constituted between adjacent points.

5. Data analysis

Data analyses were performed with SPSS10.0. Data obtained over training days from hidden platform trial and reversal trial was analyzed by two-way analysis of variance (ANOVA). Mean escape latency was the dependent variable, day was the within-subjects variable, and 4 groups were the between-subject variables. When appropriate, post hoc comparisons were assessed using the LSD test (equal variances assumed) or Dunnett's T3 test (equal variances not assumed). The other data were analyzed by one-way ANOVA. All results were shown as means ± S.E.M. In all statistical comparison, \( P < 0.05 \) was used as the criterion for statistical significance.

**RESULTS**

1. Hidden platform trial

In the hidden platform trial before EA, the time to find the platform (escape latency) for normal group decreased in a day-dependent manner. Although TPM administrated group followed this pattern (Fig.1), group comparisons revealed that TPM administrated group presented a longer latency to find the platform than normal group \( n=15, \ P < 0.01 \). After treatment, EA group showed significant decrease in escape latency compared to impaired rats and placebo-EA rats \( (P < 0.01) \) (Fig.2, on Days 1–2).
Fig. 1. Comparison of average escape latency time for each group before acupuncture.

Fig. 2. Performance in Morris water maze test for each group after acupuncture.

Note: Points represent average latency to find the platform. Days 1–2: hidden platform trial, showed a significant difference between normal, EA group and impaired group, placebo-acupuncture group ($P < 0.01$); Days 4–6: reversal trial, showed a significant difference among groups ($P < 0.01$); Day 7: visible platform trial, showed no significant difference among the groups ($P > 0.05$).

2. Probe trial

The data obtained from two measures of probe trial performance was shown in Fig.3A and B. From Fig.3A, we can see that normal, EA groups spent significantly more time ($P < 0.05$) in the training quadrant where the platform was previously located than impaired and placebo-EA groups, which visited all quadrants equally during 60 s of free swimming. Similar results were obtained from the former platform crossings (Fig.3B). EA group crossed over the platform location more frequently than impaired and placebo-EA groups ($P < 0.05$). Gallagher et al. [25] defined spatial learning in the water maze by applying a combination of probe trial criteria. A rat was considered to have learned the
location of the platform if, during a probe trial, it spent one third of its time in the goal quadrant and crossed over the platform location at least twice. Using this criteria, both normal (23.25 s, 2.95) and EA (20.55 s, 2.65) groups had learned the location of the platform, and were better at solving the task than the impaired (16.35.21 s, 1.50) and placebo-EA (16.72 s, 1.45) groups.

![Fig. 3. Probe trial performance for each group after EA.](image)

Note: (A) The time spent in the quadrant of the former platform position. (B) Crosses over the former platform location. *P < 0.05, **P < 0.01, significantly different from normal group; #P < 0.05, ##P < 0.01, significantly different from impaired group.

3. Reversal trial

Fig.2 (on Days 4–6) showed the average latency to find the platform, which was located in a different quadrant (SW) from the one used in hidden platform (NE). In the successive 3 training days, there was a significant difference among groups ($P < 0.01$). It revealed that normal and EA groups spent markedly less time ($P < 0.05$) to find the platform than impaired and placebo-EA groups.

4. Visible platform trial

Training in the visible platform trial took place after spatial training was finished. Fig.2 (on Day 7) showed that there was no significant difference of performance ($P > 0.05$) among the groups in visible platform trial for escape latency, indicating that motivation and motor skills were essentially intact.

5. Spatial strategy

The swimming patterns were used as an indication of the search strategy employed by the rats in Morris water maze task. Fig.4 showed typical swim patterns in hidden platform trial. The patterns were chosen according to the criterion that escape duration of a given rat should be as close as possible to the mean group value on that day. On Day 1, all groups happened to reach the platform in marginal and random patterns and the swimming track was either large loop along the pool border or random across each quadrant, exhibiting a fairly high thigmotaxis. On Days 3 and 5, normal group found the platform mainly by tendency and linear patterns, almost straight toward the platform, whereas, animals in impaired group frequently searched the platform using marginal and random patterns. After treatment, rats exposed to EA improved very rapidly, and hunted for platform mainly by tendency and linear patterns, but not as accurate as the normal animals.
Fig. 4. Typical swimming patterns found in hidden platform trial.

Note: On Day 1, all groups happened to reach the platform in marginal and random patterns, exhibiting a fairly high thigmotaxis. On Days 3 and 5, normal group found the platform mainly by tendency and linear patterns; impaired and placebo-EA groups' swimming patterns always remain marginal and random patterns. After EA, EA group employed tendency and linear patterns more often than impaired and placebo-acupuncture groups.

During the probe trial, the EA rats shuttled back and forth in the middle of each quadrant to look for the platform. Contrarily, impaired and placebo-EA rats swam some large loops along the pool border to hunt for the target using marginal pattern (Fig.5).

Fig. 5. Typical swimming patterns in probe trial for each group.

Note: The rats in normal, and EA groups looked for the platform by means of shuttling back and forth. In contrast to the above rats, impaired and placebo-acupuncture rats swam some large loops along the pool border, and looked for the platform using marginal pattern.

DISCUSSION

The harmful cognitive effects of AEDs are especially important to those who require maximal cognitive efficiency for their job, school, and daily activities. Decreased cognition was recognized as being one of the most severe side effects related to TPM administration. In this study, impaired rats showed obvious cognitive impairment in Morris water maze, i.e., they reached the platform with longer latency, spent less time in the quadrant where the platform had been located, and adopted a spatial strategy different from that of normal group, indicating that the impairment in learning and memory was produced by administrating TPM intragastrically. These observations were particularly important in view of the significant cognitive impairment characteristic of TPM administrated epileptic patients.

Generally, learning and memory at least included three primal processes: acquisition, consolidation and retention. In this study, the hidden platform trial was mainly designed to measure acquisition of spatial memory, reversal trial was to reflect relearning ability and probe trial was to evaluate retention. After treatment, EA rats presented an improvement in the acquisition in hidden platform trial and reversal trial; they reached the platform with lower latency than impaired and placebo-EA groups. Simultaneously, acupuncture rats also showed melioration in retention of
memory, they spent more time to swim in former quadrant and across over the former platform more frequently. On the contrary, impaired and placebo-EA groups did not display quadrant bias for visiting any one of the four quadrants of the pool, confirming that they had no better knowledge of the platform's precise location, which suggested acupuncture could improve the learning and memory ability in impaired rats.

Searching strategy is used as a measure to reflect the difference in thinking, analysis and judgment. EA group displayed less thigmotaxis in the Morris water maze than impaired and placebo-EA groups. In probe trial, an obvious difference was detected in searching strategies among the groups (Fig.5). The normal, and EA groups rats went or retraced immediately to the target quadrant of the swimming pool to look for the platform, exhibiting a kind of typical “shuttling type” search pattern. In contrast, impaired and placebo-EA rats required more time to choose the target quadrant and continuously persevered on their task, and swim some large loops along the pool border, i.e., using marginal pattern. These results provided evidence that TPM administration impaired cognition of rats and EA partly improved analyzing, judging, and straining ability of the rats.

As can be seen in Fig.4, the search strategies for normal animals exhibited “marginal pattern→random pattern→tendency pattern→linear pattern” along training days elapsed. But for impaired rats, their search strategies had no significant changes: frequency of occurrence of tendency and linear patterns was much less than that for normal rats. EA treatment could improve this abnormality and the search strategy adopted by EA group changed from marginal pattern to tendency pattern, indicating that EA could raise analysis, judgment, and ability of solving problem of impaired rats so they could find the submerged platform with the best strategies in the shortest time. Placebo-EA had little or no effect on any of the neurobehavioral parameters analyzed, indicating that the therapeutic properties of acupoints show “relative specificity”.

In summary, the present results suggested that the EA stimulation at acupoints Gv-20 (Baihui), Gv-14 (Dazhui) exerted a protective effect on cognitive impairment caused by TPM in rats, and EA has a specificity of cure. Our previous work found that EA stimulation at the same acupoints decreased the frequency of spontaneous recurrent seizures in Li-pilocarpine epileptic model of rats. Therefore, for TPM administrated epileptic patients, EA stimulation at acupoints Gv-20 (Baihui) and Gv-14 (Dazhui) could not only improve cognitive deficits, but also prevent seizures. Further studies are needed to clarify the mechanism of EA stimulation for maximizing treatment effectiveness and improving quality of life.

REFERENCES


