

Acute Fornix Deep Brain Stimulation Improves Hippocampal Glucose Metabolism in Aged Mice

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Abstract

Background: A beneficial memory effect of acute fornix deep brain stimulation (DBS) has been reported in clinical studies. The aim of this study was to investigate the acute changes in glucose metabolism induced by fornix DBS.

Methods: First, the Morris water maze test and novel object recognition memory test were used to confirm declined memory in aged mice (C57BL/6, 20–22 months old). Then, four groups of mice were used as follows: aged mice with stimulation ($n = 12$), aged mice with sham-stimulation ($n = 8$), adult mice (3–4 months old) with stimulation ($n = 12$), and adult mice with sham-stimulation ($n = 8$). Ipsilateral hippocampal glucose metabolism and glutamate levels were measured *in vivo* by microdialysis before, during, and after fornix DBS treatment. Histological staining was used to verify the localization of electrodes and mice with inaccurate placement were excluded from subsequent analyses. The effects of fornix DBS on extracellular glucose, lactate, pyruvate, and glutamate levels over time were analyzed by repeated-measures analysis of variance followed by Fisher's least significant difference *post hoc* test.

Results: The aged mice had a higher basal lactate/pyruvate ratio (LPR) and lactate/glucose ratio (LGR) than the adult mice (LPR: 0.34 ± 0.04 vs. 0.13 ± 0.02 , $t = 4.626$, $P < 0.0001$; LGR: 6.06 ± 0.59 vs. 4.14 ± 0.36 , $t = 2.823$, $P < 0.01$). Fornix DBS decreased the ipsilateral hippocampal pyruvate and lactate levels ($P < 0.05$), but the glucose levels were not obviously changed in aged mice. Similarly, the LGR and LPR also decreased in aged mice after fornix DBS treatment ($P < 0.05$). Glucose metabolism in adult mice was not significantly influenced by fornix DBS. In addition, fornix DBS significantly decreased the ipsilateral hippocampal extracellular levels of glutamate in aged mice ($P < 0.05$), while significant alterations were not found in the adult mice.

Conclusions: The present study provides experimental evidence that fornix DBS could significantly improve hippocampal glucose metabolism in aged mice by promoting cellular aerobic respiration activity.

Key words: Deep Brain Stimulation; Fornix; Glucose Metabolism; Memory Decline

INTRODUCTION

Memory loss in older people is a widespread and serious problem that affects up to 50% of those over the age of 85 years. It is usually caused by Alzheimer's disease (AD) and other age-related dementia.^[1] Current pharmacological treatment does not cure or delay the progression of age-related memory impairment.^[2] Deep brain stimulation (DBS) has been used as a modulator for brain function and has a therapeutic effect on various movement disorders and psychiatric diseases. The fornix was chosen as a DBS target for dementia due to a serendipitous observation of memory improvement in patients receiving hypothalamic DBS for pathological

obesity.^[3] Clinical trials have confirmed the safety of fornix DBS and have shown therapeutic effects in patients older than 65 years.^[4–6] Chronic fornix DBS (12 months) has been reported to increase glucose metabolism and improve hippocampal atrophy in AD patients.^[5,7] Neurogenesis,

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Received: 01-11-2017 **Edited by:** Qiang Shi

How to cite this article: Wang X, Hu WH, Zhang K, Zhou JJ, Liu DF, Zhang MY, Zhang JG. Acute Fornix Deep Brain Stimulation Improves Hippocampal Glucose Metabolism in Aged Mice. Chin Med J 2018;131:594-9.

Access this article online

Quick Response Code:



Website:
www.cmj.org

DOI:
10.4103/0366-6999.226067

axonal remodeling, synaptogenesis, and increased vascularization may contribute to the chronic therapeutic effects of fornix DBS.^[8,9]

However, findings on chronic fornix DBS have not been able to explain the autobiographical recall triggered by intraoperative acute stimulation in awake patients.^[3,5] Source localization of the acute electroencephalographic (EEG) effects in AD patients,^[3,5] hippocampal c-fos and neurotrophic factor expression,^[10] and hippocampal acetylcholine release in animal models^[2] have been used as indicators in treatment mechanism research of acute fornix DBS. Until now, no studies have investigated the alteration *in vivo* of glucose metabolism induced by acute fornix DBS. In our study, we evaluated the effects of unilateral fornix DBS on glucose metabolism changes by intracerebral microdialysis, and we then tested the levels of extracellular glucose, lactate, and pyruvate in the hippocampus in both aged and adult mice. Our findings may provide additional insight into the possible mechanism of fornix DBS.

METHODS

Ethical approval

The experiments were approved by the Ethics Committee of Beijing Neurosurgical Institute, Capital Medical University (No. 201502003), and conducted in accordance with the Guidance for Animal Experiment of the Capital Medical University and Beijing guidelines for the care and use of laboratory animals.

Animals

Female C57BL/6 mice (aged mice: 20–22 months, adult mice: 3–4 months; The Laboratory Animal Center, Academy of Military Medical Sciences, Beijing, China) were housed three per cage in an environmentally controlled room (20°C–23°C, 12-h light/12-h dark cycle, lights on at 7 a.m.). The mice were given free access to food and water. Twelve aged mice and 12 adult mice were tested using the Morris water maze (MWM) test and novel object recognition memory (NORM) test. Hippocampal glucose metabolism in mice was examined by microdialysis *in vivo* as follows: aged mice with fornix stimulation ($n = 12$), adult mice with fornix stimulation ($n = 12$), aged mice with fornix sham-stimulation ($n = 8$), and adult mice with fornix sham-stimulation ($n = 8$).

Learning and memory tests

Morris water maze test

The MWM was performed in a circular pool (diameter: 120 cm) filled with water and milk (200:1). One escape platform (diameter, 8 cm) was placed in the target quadrant. During the training period, the mice were allowed to swim for a maximum of 90 s with four sessions per day for 4 consecutive days. On the spatial probe test day, the mice were allowed to swim for 60 s with the platform removed. The latency to reach the platform, the total distance traveled, the distance traveled in the platform quadrant, and the frequency of platform crossing were recorded.

Novel object recognition memory test

After 2 days of adaptation (20 min/day) in a white square box (50 cm × 50 cm), the experiment composed of three sessions lasting 300 s each was started on the mice. During the training session, two identical objects were presented. Ninety minutes later, one of the familiar objects was replaced by a novel object (short-term memory test session). Then, 24 h later, the novel subject in the short-term memory test was replaced by a different novel subject (long-term memory test session). Exploration by the mice was defined as sniffing the objects at a distance of <2 cm. The exploration time spent with the familiar and novel objects during the test session was recorded.

Fornix deep brain stimulation and microdialysis

Mice were anesthetized with urethane (1 g/kg, i.p.) and mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, California, USA). A concentric bipolar stimulation electrode (outer diameter, 125 μm; inner diameter, 25 μm; CBASC30, FHC, USA) was implanted into the left fornix at a 45° angle off the sagittal plane according to coordinates in the Paxinos and Franklin mouse brain atlas (second edition, A: -0.1, L: -0.35, D: 3.75 mm). Then, a microdialysis probe (CMA7-1; active membrane length, 1.0 mm; diameter, 0.24 mm; CMA Microdialysis, Kista, Sweden) was implanted vertically in the ipsilateral hippocampus (A: -2.18, L: -2.35, D: -2.35 mm). The dialysis assembly consisted of Teflon tubing (CMA; Sweden) that connected the probe to a 2.5-ml gas-tight syringe (CMA; Sweden) mounted on a syringe pump (CMA; Sweden). The probe was then perfused with artificial cerebrospinal fluid at a flow rate of 1.5 μl/min for 0.5 h before dialysate collection. Dialysate samples were collected every 15 min from the anesthetic mice, and the first two samples were discarded. After stabilization, 10 samples were totally collected in each mice: the first two samples were collected to determine the basal value before stimulation (time points [T] 1 and 2), and the following six dialysates were collected during fornix stimulation (T3–T8) and the last two samples were collected after turning off the stimulation (T9–T10). The DBS stimulation time (130 Hz, 100 μA, and 90 μs; Master 8, AMPI, Israel) was 1.5 h. In sham-stimulation groups, electrodes were only implanted into the target without turning on the stimulation. Dialysates were automatically collected with a refrigerated autosampler and stored at -80°C.

Sample analysis

The concentrations of glucose, lactate, pyruvate, and glutamate in dialysate samples were analyzed in the ISCUS microdialysis analyzer (ISCUS^{flex}, Sweden) by batch analysis using standard ISCUS reagents. The lactate/pyruvate ratio (LPR) and lactate/glucose ratio (LGR) were also calculated in all samples.

Histological verification

After completion of the experiment, all mice were transcardially perfused while deeply anesthetized with

0.1 mol/L and 37°C phosphate-buffered saline and 4% paraformaldehyde (4°C). Hematoxylin and eosin staining was performed for histological verification of the localization of electrodes on coronal sections (20- μ m thick).

Statistical analysis

All data are expressed as the mean \pm standard error (SE). Behavioral data between the adult and aged group in the MWM probe test and NORM test were analyzed using the Student's *t*-test. Microdialysis data were presented as the folds of the mean of two baseline samples prior to fornix DBS. One-way analysis of variance (ANOVA) followed by Fisher's least significant difference *post hoc* test was used to analyze changes in hippocampal glucose, lactate, pyruvate, and glutamate levels. Obvious changes were considered if significant differences were found when comparing the value with both baseline T1 and T2. All analyses were performed using SPSS 24.0 (IBM Corp., Armonk, NY, USA), and $P < 0.05$ was considered statistically significant.

RESULTS

Behavioral data

During the spatial probe test of MWM, the adult mice retained the reference memory of the platform location more effectively than the aged group with respect to the percentage of the path length in the platform quadrant ($36.00\% \pm 4.32\%$ vs. $22.42\% \pm 2.73\%$, $t = 2.707$, $P < 0.05$) and the frequency of platform crossings (2.5 ± 0.3 vs. 1.5 ± 0.3 , $t = 2.253$, $P < 0.05$). During the test phase of the NORM, adult mice spent more time exploring the novel object than the aged mice in the short-term (percentage of exploration time spent with novel objects: $60.64\% \pm 2.58\%$ vs. $46.23\% \pm 3.92\%$, $t = 3.067$, $P < 0.01$) and long-term ($63.39\% \pm 2.58\%$ vs. $49.32\% \pm 4.24\%$, $t = 2.834$, $P < 0.01$) memory tests.

Histological results

After histological verification, data of mice with inaccurate electrode placement were discarded. Nine, six, eight, and six mice were finally included in the statistical analysis in groups of aged mice with fornix stimulation, aged mice with fornix sham-stimulation, adult mice with fornix stimulation, and adult mice with fornix sham-stimulation, respectively.

Baseline level of glucose metabolism

Due to the lack of knowledge regarding the recovery rate of the microdialysis probe in the brain, it is inappropriate to compare the extracellular concentration difference by directly examining the microdialysis samples. We only compared the differences in the baseline LGR and LPR. Taking both baseline T1 and T2 together (including stimulation and sham-stimulation groups), aged mice had a significantly higher LPR than adult mice (0.34 ± 0.04 vs. 0.13 ± 0.02 , $t = 4.626$, $P < 0.0001$). A similar finding was observed in the LGR between

the two groups (6.06 ± 0.59 vs. 4.14 ± 0.36 , $t = 2.823$, $P < 0.01$).

Effect of unilateral fornix DBS on glucose metabolism

The glucose levels [Figure 1a] were not changed significantly during and after fornix DBS in adult ($P = 0.590$) and aged mice ($P = 0.927$). In addition, neither pyruvate ($P = 0.775$) nor lactate ($P = 0.734$) were significantly altered during and after fornix DBS in adult mice. However, hippocampal pyruvate in aged mice [Figure 1b] began to decrease in the fourth 15-min period after fornix DBS and lasted until 15 min after DBS was turned off (T6: 0.49 ± 0.10 , T9: 0.47 ± 0.10 , $F = 2.259$, $P < 0.05$). Furthermore, lactate levels in aged mice [Figure 1c] were reduced earlier in the third 15-min period after DBS was turned on and lasted until DBS was turned off (T5: 0.37 ± 0.08 , T8: 0.51 ± 0.12 , $F = 2.010$, $P < 0.05$). No significant alterations of glucose metabolism were presented during microdialysis in both adult and aged sham-stimulation groups.

The LGR was not significantly changed by DBS in the adult group ($P = 0.972$). However, DBS significantly decreased the LPR in the adult group when compared with that at baseline T1, but no significant difference was found when compared with that at baseline T2. As a result, we did not consider this change to be significant. In the aged mice, fornix DBS decreased the LGR [Figure 1d] in the third 15-min period, which lasted until the second 15-min period (T5: 2.12 ± 0.54 , T10: 2.40 ± 0.62 , $F = 2.062$, $P < 0.05$) after DBS was turned off (T9 was not significant). Fornix DBS also decreased the LPR [Figure 1e] of aged mice at T3 (0.12 ± 0.03), T4 (0.11 ± 0.03), T5 (0.15 ± 0.05), and T7 (0.08 ± 0.03 ; $F = 2.112$, $P < 0.05$).

Effect of unilateral fornix deep brain stimulation on hippocampal glutamate levels

Fornix DBS had no influence on extracellular glutamate levels in the ipsilateral hippocampus of the adult mice. However, there was a significant decrease in the 3rd (T5: 0.46 ± 0.07), 4th (T6: 0.39 ± 0.12), 5th (T7: 0.48 ± 0.16), and 6th (T8: 0.44 ± 0.10) 15-min period after DBS was turned on in the aged mice ($F = 2.561$, $P < 0.05$). The glutamate levels returned to normal after DBS was turned off [Figure 1f].

DISCUSSION

The present findings confirm the results of previous studies showing progressive age-related memory impairment in aged mice.^[1,11] We also attempted to explain the instant memory improvement associated with intraoperative acute stimulation^[3,5] by using microdialysis *in vivo*. We found that fornix DBS could significantly improve hippocampal glucose metabolism in aged mice by promoting cellular aerobic respiration activity.

In our study, we used the aged mouse model to investigate the regulation mechanism of fornix DBS. Aging is

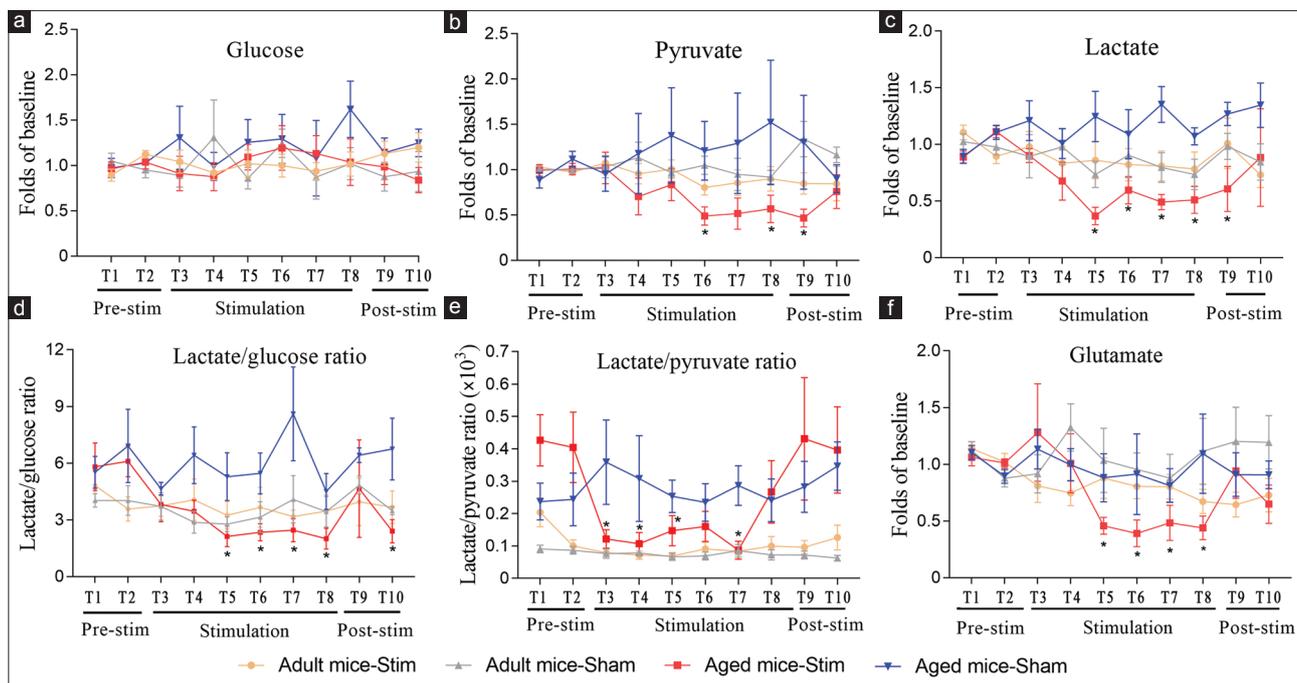


Figure 1: Alterations of glucose metabolism and glutamate release in ipsilateral hippocampus before, during, and after fornix DBS. Glucose levels did not change in adult and aged mice after fornix stimulation (a). Hippocampal levels of pyruvate (b), lactate (c), LGR (d), and LPR (e) were decreased after fornix DBS in aged mice. Glutamate levels were significantly decreased in aged mice 45 min after fornix stimulation, whereas did not change in adult mice after fornix stimulation (f). Data points of glucose, pyruvate, lactate, and glutamate are mean \pm standard error expressed as folds of baseline (Adult mice-Stim, $n = 8$; Adult mice-Sham, $n = 6$; Aged mice-Stim, $n = 9$; Aged mice-Sham, $n = 6$). * $P < 0.05$, compared with both baseline T1 and T2. Pre-stim: Samples collected before DBS ON, Stimulation: Samples collected during DBS ON, Post-stim: samples collected after DBS OFF. X-axis refers to time points (T) of sample collection and every time point means a period of time lasting 15 min. Stim: Stimulation; Sham: Sham-stimulation; LPR: Lactate/pyruvate ratio; LGR: Lactate/glucose ratio.

associated with cognitive impairments and increased risks of neurodegenerative disorders, and aged mice could present, to some extent, the neuropathological changes associated with memory decline. A previous study showed that mitochondrial dysfunction in aging leads to a metabolic shift from aerobic respiration to glycolytic metabolism in the brain, and increased lactate levels were detected in the hippocampus of aging mice.^[12] Lactate level is a marker for anaerobic metabolism. In our study, we found a significantly higher LPR and LGR in aged mice than in adult mice, also suggesting that hippocampal aerobic respiration is inhibited. Neuronal glucose/energy metabolism is of central significance to normal cellular and molecular reactions. Human studies have shown a decline in the regional cerebral metabolic rate of glucose in the temporal lobe among elderly women with subjective memory impairment^[13] and in patients who received a diagnosis of AD.^[5] Dysfunction of neuronal glucose energy metabolism in aging or AD may result from the inhibition of insulin signal transduction.

Several studies have focused on the acute effect of fornix stimulation in the past. EEG analysis in AD patients has shown that acute fornix DBS electrodes produce short latency-specific and localized changes in the activity of ipsilateral mesial temporal lobe structures.^[5] Animal

studies have shown that acute fornix DBS increases the expression of *c-fos* as early as 1.0–2.5 h after stimulation^[2,10] and rapidly modulates the expression of neurotrophic factors.^[10] Moreover, Heschem *et al.* found in their *in vivo* microdialysis study that fornix DBS caused a significant increase in hippocampal acetylcholine levels within 20 min.^[2] In our study, lactate levels and the LPR, markers of anaerobic metabolism, significantly decreased in the early stage of stimulation period. These findings suggest that unilateral fornix DBS could inhibit the high level of glycolysis in aged mice. Lactate dehydrogenase can metabolize lactate back to pyruvate, which can enter the mitochondrial tricarboxylic acid cycle.^[14] Despite the inhibition of anaerobic metabolism, the level of pyruvate decreased significantly at the fourth 15 min of DBS, and the levels of extracellular glucose remained stable during and after fornix DBS. This phenomenon indirectly suggests that fornix stimulation might increase lactate utilization, promote lactate metabolism back to pyruvate, and enhance aerobic metabolism at the mitochondrial level. As mitochondrial oxidative phosphorylation provides the major source of adenosine triphosphate (ATP) in neurons,^[15,16] increased glucose aerobic metabolism in the hippocampus of aged mice contributes to enhanced level of ATP production and may result in restoration

of normal function in neurons with energy deficiency. In our previous microdialysis study using anterior nucleus of the thalamus (ANT) stimulation in a model of temporal lobe epilepsy, we also found inhibition of hippocampal anaerobic metabolism during ANT DBS.^[14] These results may provide evidence for the use of ANT DBS for memory improvement in AD patients. In the study of fornix stimulation in AD patients, volume changes of fornix and mammillary bodies were highly correlated with hippocampal volume change after fornix stimulation,^[7] which strongly suggests the critical role of Papez circuit in memory function regulation. Thus, both ANT-DBS and fornix-DBS could improve hippocampal activity through Papez circuit. Unexpectedly, in the present study, we did not find any significant changes in glucose metabolism induced by stimulation in the adult group. These results could potentially be explained by the “ceiling effect,” in which adult mice already have an appropriate glucose metabolic level and enzyme activity, while the respiratory chain enzymes in the aged hippocampus have declined.^[12] Combined with previous studies, we postulate that fornix DBS may be protective for neurons in the aged hippocampus.

Our study did not find a significant influence of fornix DBS on the release of glutamate in adult mice, which is in accordance with results seen in a study on adult rats.^[2] Interestingly, we found that fornix DBS could significantly reduce the extracellular release of glutamate in aged mice. The basal levels of extracellular glutamate in aged rodents have been found to be 94% greater^[17] or 60% lower^[18] than those in adult rodents according to *in vitro* studies. An *in vivo* study reported an increase in the basal glutamate level in aged rats compared with adults.^[19] It is impossible to conclude whether fornix DBS reduced the neurotoxicity of enhanced glutamatergic signals or disrupted a memory-related mechanism by glutamate and ionotropic glutamate receptors (e.g., N-methyl-D-aspartate). Therefore, studying changes in the glutamatergic system after fornix DBS is of great importance.

The main limitations of this study pertain to whether glucose metabolism measured by microdialysis under anesthesia reflects the metabolic state in awake animals. We could not conclude that improvement in the glucose metabolic levels in aged mice contributes to the neuromodulatory mechanism of fornix DBS for memory enhancement because we did not include any memory tests after acute DBS in the present study.

In conclusion, the present study provides experimental evidence that fornix DBS could significantly improve hippocampal glucose metabolism in aged mice by promoting cellular aerobic respiration activity, while no significant alterations were observed in adult mice. Our results suggest that acute fornix stimulation may induce favorable modulations of the neural activity in hippocampus of aged mice, which might be an

important mechanism underlying the effects of memory improvement induced by acute fornix stimulation.

Financial support and sponsorship

This work was supported by grants from the Scientific Research Common Program of Beijing Municipal Commission of Education (No. KZ201510025029), the National Natural Science Foundation of China (No. 81301183), and the Beijing Municipal Administration of Hospitals' Ascent Plan (No. DFL20150503).

Conflicts of interest

There are no conflicts of interest.

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穹窿急性电刺激对老龄小鼠海马葡萄糖代谢的影响研究

摘要

背景: 既往临床研究发现穹窿脑深部电刺激 (deep brain stimulation, DBS) 可以改善阿尔茨海默病患者记忆功能。本研究拟探讨急性穹窿刺激对海马葡萄糖代谢的影响, 为术中急性穹窿刺激改善记忆功能现象提供理论基础。

方法: 研究应用老龄小鼠 (C57BL/6, 20-22月龄) 作为记忆下降动物模型, 首先应用Morris水迷宫实验和新物体识别实验验证老龄小鼠与成年小鼠 (C57BL/6, 3-4月龄) 相比存在记忆功能下降; 随后实验小鼠分为4组: 老龄小鼠刺激组 (n=12), 老龄小鼠假刺激组 (n=8), 成年小鼠刺激组 (n=12) 和成年小鼠假刺激组 (n=8), 于左侧穹窿靶点植入DBS电极, 并在刺激前、刺激中和刺激后应用在体微透析方式采集小鼠同侧海马透析液, 检测透析液中葡萄糖及其代谢产物、谷氨酸的含量, 并应用重复测量的方差分析进行统计比较, 组织学检查发现电极刺激部位不准确的小鼠对应数据不纳入统计学分析。

结果: 老龄小鼠海马乳酸/丙酮酸比值 (LPR) 和乳酸/葡萄糖比值 (LGR) 明显高于成年小鼠 (LPR: 0.34 ± 0.04 vs. 0.13 ± 0.02 , $t = 4.626$, $P < 0.0001$; LGR: 6.06 ± 0.59 vs. 4.14 ± 0.36 , $t = 2.823$, $P < 0.01$)。穹窿电刺激可以显著降低老龄小鼠海马内丙酮酸和乳酸水平 ($P < 0.05$), 而对葡萄糖含量无显著影响, 此外, 老龄小鼠海马LGR和LPR因穹窿刺激而出现明显下降 ($P < 0.05$), 而穹窿电刺激对小鼠海马的葡萄糖代谢水平无显著影响。此外, 穹窿电刺激同样可以抑制老龄小鼠海马谷氨酸的释放 ($P < 0.05$), 而对成年小鼠无明显影响。

结论: 穹窿脑深部电刺激通过改善有氧呼吸活动提高了老龄小鼠海马的葡萄糖代谢水平, 可能是术中急性刺激改善记忆功能的机制之一。