Short Term Effect of Mild Chronic Cerebral Hypoperfusion on Spatial Learning and Memory in Rats with/ without Electrical Stimulation

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Abstract : **The purpose of this study was to investigate the short-term effects of mild chronic cerebral hypoperfusion on spatial learning and memory in rats with or without electrical stimulation treatment. Male Sprague Dawley rats weighing 300 - 360 g were randomly assigned into seven groups (n = 8) of right common carotid artery occlusion (RCO), left common carotid artery occlusion (LCO), non-surgery (NS) and sham operated (sham) groups. Chronic cerebral hypoperfusion was induced by permanent ligation of either right or left common carotid artery. Electrical stimulation (stim) was delivered to the NS-stim, RCO-stim and LCO-stim groups for a period of 30 min, once a day from day 1 - 5 after arterial occlusion. Evaluations of spatial learning and memory were assessed in a Morris water maze at five days after arterial occlusion.**

Results revealed that five days of mild cerebral hypoperfusion induced either by permanent right or left common carotid occlusion had no effect on spatial learning and memory when compared with the sham group. But when we compared the RCO and the LCO rats, we found that the RCO rats performed significantly better than the LCO rats on the second day of learning; however, their performance tended to become persistently slower in the later phase than that of the LCO rats. Furthermore, the LCO rats tended to have better spatial memory than the RCO rats. Electrical stimulation therapy significantly enhanced spatial learning in NS and LCO rats but had no effect on the RCO rats.

These findings may suggest that unilateral carotid occlusion may induce only short- term mild deficit in spatial learning in rats, and electrical stimulation treatment may help in improving mild cognitive impairment caused by cerebral hypoperfusion.

Key words : Spatial learning and memory/Chronic cerebral hypoperfusion /Morris Water Maze/Electrical stimulation

เรื่องย่อ : การเรียนรู้และการจดจำตำแหน่งสถานที่ในหนูที่ได้รับเลือดมาเลี้ยงสมองลดลงเป็นเวลา 5 วัน และการรักษาด้วยการกระตุ้นไฟฟ้า

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โรคความจำเสื่อมที่มีสาเหตุจากโรคหลอดเลือดสมองมีจำนวนเพิ่มมากขึ้นทุกปี การรักษาในปัจจุบันยังไม่มีประสิทธิภาพเพียงพอ จึงเป็นปัญหาทางสาธารณสุขที่ต้องหาทางแก้ไขและป้องกัน การวิจัยเพื่อหาแนวทางป้องกันการเกิดโรคนี้จึงมีความสำคัญอย่างมาก

วัตถุประสงค์ : เพื่อศึกษาผลกระทบระยะสั้นที่เกิดขึ้นต่อการเรียนรู้และการจดจำตำแหน่งสถานที่ในหนู เมื่อสมองได้รับเลือดมาเลี้ยงลดลงเป็นเวลานาน 5 วัน และผลของการรักษา โดยวิธีการกระตุ้นไฟฟ้า

วัตถุและวิธีการ : ในการศึกษานี้ใช้หนูขาว เพศผู้ น้ำหนัก 300-360 กรัม แบ่งหนูออกเป็น 7 กลุ่ม กลุ่มละ 8 ตัว ได้แก่ กลุ่มที่ผูกหลอดเลือดคอมมอนคาโรติดข้างขวา (RCO) กลุ่มที่ผูกหลอดเลือดคอมมอนคาโรติดข้างซ้าย (LCO) กลุ่มที่ไม่ได้รับการผ่าตัด (NS) กลุ่มที่ได้รับการผ่าตัด แต่ไม่ได้ผูกหลอดเลือด (sham) และกลุ่มได้รับการกระตุ้นไฟฟ้าอีก 3 กลุ่ม คือ กลุ่ม NS-stim, RCO-stim และ LCO-stim การทำให้เลือดไปเลี้ยงสมองลดลง ทำโดยการผูกหลอดเลือดคอมมอนคาโรติดข้างซ้าย หรือ ข้างขวา เพียงข้างเดียว สำหรับการกระตุ้นไฟฟ้า ให้ครั้งละ 30 นาที / วัน ตั้งแต่วันที่ 1 ถึงวันที่ 5 หลังการผูกหลอดเลือด การประเมินความสามารถในการเรียนรู้และการจำตำแหน่ง ทดสอบด้วย Morris water maze โดยเริ่มทดสอบในวันที่ 6 หลังจากการผูกหลอดเลือด

ผลการศึกษา : พบว่า 1) ภาวะที่สมองได้รับเลือดมาเลี้ยงลดลงโดยการผูกหลอดเลือดคอมมอนคาโรติดข้างใดข้างหนึ่งเป็นเวลานาน 5 วัน ไม่เกิดผลกระทบต่อการเรียนรู้และการจำตำแหน่งเมื่อเปรียบเทียบกับหนูปกติ แต่เมื่อเปรียบเทียบระหว่างหนูกลุ่มผูกหลอดเลือดด้วยกัน พบว่าหนูกลุ่มที่ผูกหลอดเลือดข้างซ้ายมีประสิทธิภาพในการเรียนรู้ต่ำกว่าหนูกลุ่มที่ผูกหลอดเลือดข้างขวาอย่างมีนัยสำคัญในวันที่สองของการทดสอบ แต่มีแนวโน้มว่าหนูกลุ่มผูกซ้ายจะมีความสามารถในการเรียนรู้และความจำที่ดีกว่าหนูกลุ่มผูกขวาในวันท้ายๆ ของการทดสอบ 2) การรักษาด้วยการกระตุ้นไฟฟ้ามีผลทำให้การเรียนรู้ในหนูปกติและหนูที่ถูกผูก หลอดเลือดข้างซ้ายดีขึ้นอย่างมีนัยสำคัญในวันที่ 1-2 ของการทดสอบเมื่อเทียบกับกลุ่มที่ไม่ได้รับการกระตุ้น แต่การกระตุ้นไฟฟ้าไม่มีผลกับกลุ่มที่ผูกหลอดเลือดข้างขวา

สรุป : ภาวะที่สมองมีเลือดมาเลี้ยงลดลงโดยการผูกหลอดคอมมอนคาโรติดเพียงข้างเดียวในหนูขาวเป็นเวลานาน 5 วัน อาจไม่ทำให้เกิดความบกพร่องในการเรียนรู้ตำแหน่งสถานที่อย่างชัดเจน แต่อาการบกพร่องอาจเห็นได้ชัดขึ้นเมื่อเปรียบเทียบระหว่างหนูที่ผูกหลอดเลือดด้วยกัน และความบกพร่องนี้อาจแก้ไขได้ด้วยการกระตุ้นด้วยไฟฟ้า ซึ่งอาจมีผลไปเพิ่มเลือดมาเลี้ยงสมองโดยมีฤทธิ์ไปขยายหลอดเลือด

INTRODUCTION

Dementia, a common disabling disorder in the elderly, was viewed as an acquired chronic and irreversible deficit of intellectual ability such as impairment of memory. Its severity can interfere with daily functioning and quality of life. Hence, the economic burden of dementia is significant not only for patients, their families and friends, but also for society. Alzheimer's disease (AD) and vascular dementia (VaD) are the most prevalent dementia among the elderly. The etiology of dementia is still unclear. Recently, substantial growing evidence indicates that both VaD and nongenetic AD are initiated by a prolonged reduction in cerebral blood flow that precedes the neurodegenerative process.[1-3]

Vascular disease may produce a range of cognitive deficits from mild to severe, and early recognition of the deficits allows the clinician to intervene before the dementia occurs. Mild cognitive impairment (MCI) is an operational diagnostic term developed to describe the preclinical stage of AD. The rate at which MCI subjects convert to AD each year is ten times higher than the rate for normal subjects.[4,5]

At present, the available treatments after the onset of dementia are still ineffective and limited by the adverse side effects.[6-8] Hence, studying preclinical mild cognitive deficits and seeking any interventions that can be applied at the preclinal stage of MCI in order to prevent or delay dementia would be important for health care services, the potential sufferers and even for the health care providers themselves.

In order to address the question of whether mild

chronic cerebral hypoperfusion is a primary trigger of the mild cognitive impairment which may progress to dementia, animal models of the preclinical stage of mild cognitive impairment should be developed before the efficacy of interventions can be tested. Stroke is a major determinant of vascular dementia and persons with carotid artery disease, stenosis and occlusion are at possible risk.[3,9,10] However, in the case of asymptomatic persons, the question has been raised as to whether persons with asymptomatic carotid artery stenosis and occlusion truly are asymptomatic. The cognitive function has been addressed in a few studies examining atherosclerosis of common carotid and internal carotid arteries. Studies in middle-aged adults identified an association between the presence of atherosclerosis and cognitive decline. Breteler et al.[11] researched cardiovascular disease and the cognitive function in the elderly (55-94 years old), and concluded that atherosclerotic disease of the peripheral or carotid arteries accounts for considerable cognitive impairment in these individuals.

In order to create an animal model similar to asympmatic carotid artery disease, an animal model of unilateral carotid artery occlusion was chosen for use to investigate cognitive ability. However, studies in both humans and animals revealed that mild reduction of cerebral blood flow (CBF) by unilateral carotid occlusion (UCO) alone typically does not induce ischemic damage; hence cognitive ability has not been tested. But several findings suggest that chronic reductions in the cerebral blood flow of a magnitude previously thought to be harmless to neurons (i.e., reduced by 25-50%) do alter neuronal function and affect total animal behavior. Such a scenario may be responsible for a symptomatology secondary to arteriovenous steal and severe carotid stenoses.[12]

Since suboptimal cerebral blood supply was a possible potential trigger of cognitive decline, various drugs, such as cholinergic compounds, hemorheologic agents and vascular smooth muscle relaxants, have already been tested in some instances for their efficacy in increasing brain perfusion. But uses of these drugs in the treatment of dementia are limited by their adverse side effects. Our unpublished results revealed that electrical stimulation of somatosensory afferent fibers at the cervical region was able to reduce hippocampal damage and improved fibrinolytic activity in the permanent right carotid artery occlusion rats.

Therefore, the present study aimed to investigate the effects of electrical stimulation on the spatial learning and memory in the animal model of MCI induced by permanent unilateral common carotid occlusion.

MATERIALS AND METHODS

Spraque Dawley rats, weighing 300-360 grams, were randomly divided into seven groups (n=8) of left common carotid artery occlusion (LCO), right common carotid artery occlusion (RCO), non surgery (NS), sham operated rats (sham), and three electrical stimulation groups of LCO-stim, RCO-stim and NS-stim. Chronic cerebral hypoperfusion was permanently induced by ligation of the right or left carotid artery. Electrical stimulation treatment was applied for a period of thirty minutes per day for five consecutive days, starting from the day of surgery. Behavioral tests began at the sixth day after surgery. Electrical stimuli were delivered by inserting two pairs of needle electrodes subcutaneously around the cervical area. Electrical stimuli of single pulses of frequency 2 Hz and intensity just above the threshold for muscle contraction were employed. Testing the ability of learning and memory was assessed by using the Morris water maze.

Morris water maze

A black circular pool (diameter 200 cm, height 50 cm.) was filled with water 30 cm in depth. The water was opaqued with kaolin (to stop the rat from seeing through the water) and the escape platform (20 × 21 cm) was submerged under 2 cm of water and 30 cm away from the pool side. The cues which were pictures of red circles, black triangles and blue rectangles, were placed 30 cm away from the pool side.

Determination of Spatial learning and Memory

On the sixth day after surgery, the ability in spa-

tial learning and memory was tested in the Morris water maze. The animals had a habituation trial with no platform for 1 minute before starting the first acquisition trial. Each rat performed 20 acquisition trials and 12 reversal trials over 8 days (4 trials a day; intertrial time interval (ITI) = 1 min) (124). During a particular trial, the rats were able to escape from the water only by climbing on an invisible platform which was submerged under water. In each acquisition trial, the location of the hidden platform was kept in the same quadrant. The platform in the acquisition trial was placed in the northwest quadrant (NW). The trial terminated as soon as the rat found the platform if the rats could not find the platform within 120 seconds, they were placed on the platform by hand. The latency to reach the platform called the escape latency was recorded. The animals were allowed to stay on the platform for 30 seconds in each trial; then they were dried with a bath towel and warmed with heating lamps. Two minutes after the last acquisition trial, the rats were subjected to a probe trial in which there was no platform for 60 seconds. The spatial memory was determined by the time spent in the quadrant of the platform position. All trials were recorded on videotape for analysis of the escape latency, the swimming distance of each trial and the time spent in the quadrant of the platform location in the probe trial.

Statistical analysis of the data

The Kolmogorov-Smirnov test was used to test whether the data obtained had normal distribution. All data were reported as mean ± standard error of mean (SEM). Testing of the difference between groups was performed by analysis of variance (ANOVA) with repeated measure and Tukey test for post - hoc testing. Statistical significance was set at $p < 0.05$.

RESULTS

There was no motor performance deficits in any of the groups studied. All rats were able to swim for a period of one minute without any difficulty.

Spatial learning and memory in rats with chronic cerebral hypoperfusion

Testing ability of spatial learning was determined by the time spent (escape latency) and swimming distance used to find the hidden platform. It was found that the mean escape latency and swimming distance of the RCO and LCO groups were not significantly different from those of the sham groups

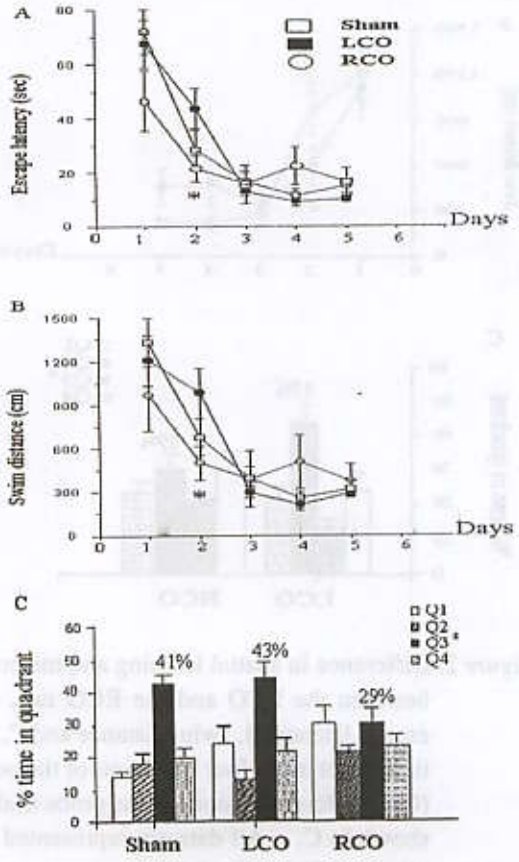

Figure 1. Effects of unilateral carotid occlusion (CO) for five days on the spatial learning and memory of rats (RCO, LCO and sham groups). A: escape latency and B: swim distance to find the hidden platform during acquisition. In addition, the % time spent in the four quadrants of the pool (Q3, platform position) in the probe trial is shown in C. All data are presented as mean + SEM. *P<0.05

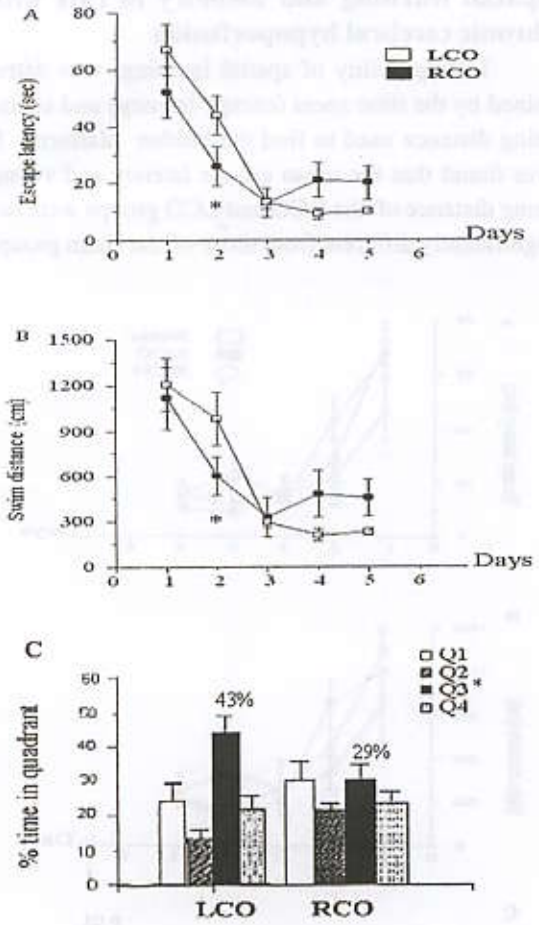

Figure 2. Difference in spatial learning and memory between the LCO and the RCO rats. A, escape latency, B, swim distance and C, % time spent in the four quadrants of the pool (Q3, platform position) in the probe trial is shown in C. All data are represented as mean + SEM. * P < 0.05

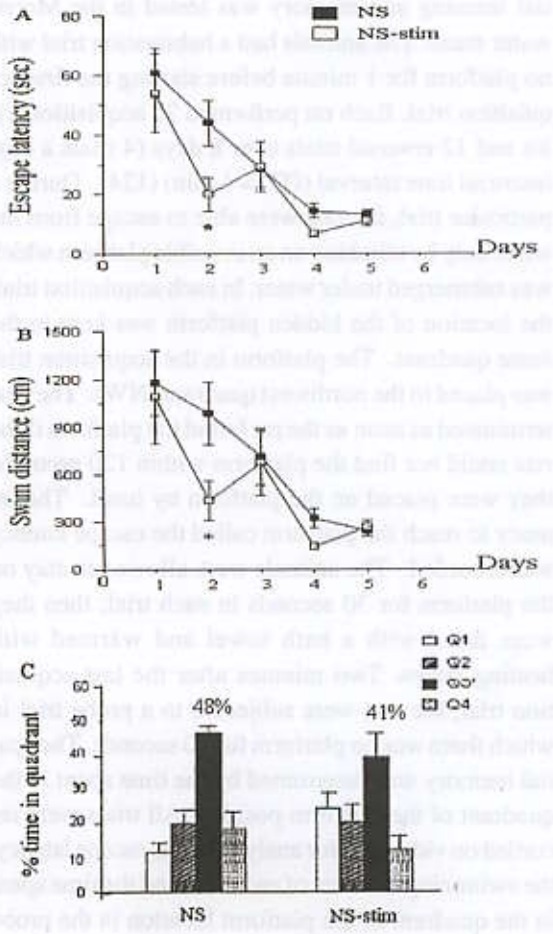

Figure 3. Effect of electrical stimulation on spatial learning and memory of NS and NS-stim rats. A, escape latency, B, swim distance to find the hidden platform , during acquisition. C, % time spent in the four quadrants of the pool (Q3, platform position) in the probe trial is shown in C. All data are presented as mean + SEM. *P < 0.05

(Figure 1A, B). However, the spatial memory was indicated by the time spent in the quadrant which contained the platform. It was found that the percentage of time spent by the sham rats (41%) and the LCO rats (43%) was longer than that of the RCO rats (29%) but not significantly different (Figure 1C). These finding suggested that one-week reduction of cerebral blood flow by unilateral carotid occlusion produced no effect on spatial learning but may have had a mild effect on the spatial memory of the RCO rats.

Asymmetric effect on the spatial learning and memory after right / left carotid occlusion

In comparing the spatial learning ability between the RCO rats and the LCO rats, we found

that the LCO rats performed significantly more slowly in finding the hidden platform than the RCO rats on the second day of the learning session, but the LCO rats tended to perform faster in the later phase during acquisition as shown in Figure 2A, B. In addition, the LCO rats tended to have better memory than the RCO rats, as indicated by the percentage of time spent in the platform quadrant, LCO (43%), RCO (29%) (Figure 2C)

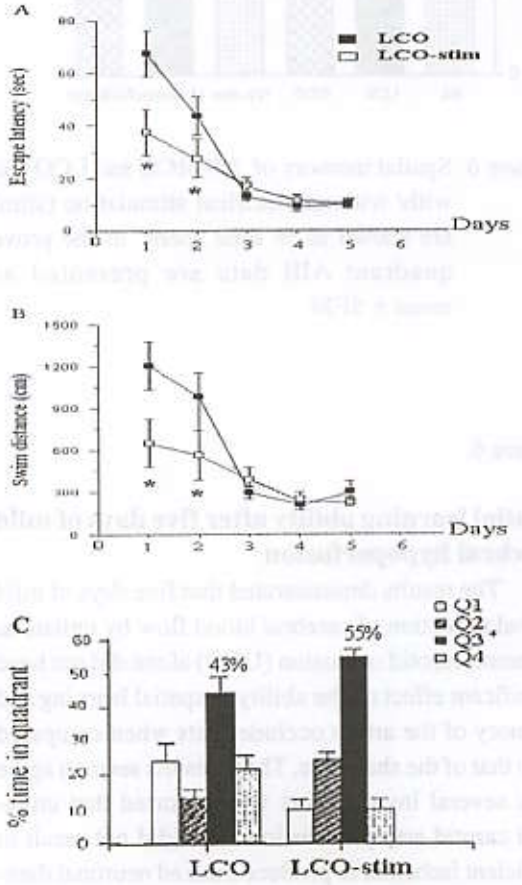

Figure 4. Effect of electrical stimulation on spatial learning and memory of LCO and LCO-stim rats. A, escape latency, B, swim distance to find the hidden platform during acquisition. C, % time spent in the four quadrants of the pool (Q3, platform position) in the probe trial is shown in C. All data are presented as mean + SEM. *P < 0.05

Effect of Electrical stimulation (ES) on Spatial learning and Memory

The results demonstrated that ES treatment helped in facilitating the spatial learning ability in the normal healthy rats (NS-stim rats). The average time spent as well as on the swimming distance to find the hidden platform of the NS-stim rats was significantly shorter on the second day of the acquisition (Figure 3A,B). However, the ES treatment had

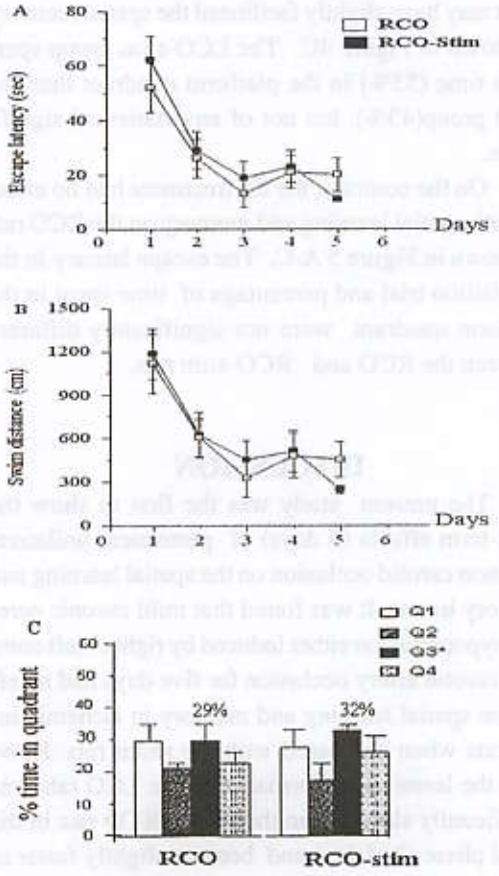

Figure 5. Effect of electrical stimulation on spatial learning and memory of RCO and RCO-stim rats. A, escape latency, B, swim distance to find the hidden platform during acquisition. C, % time spent in the four quadrants of the pool (Q3, platform position) in the probe trial is shown in C. All data are presented as mean + SEM. *P < 0.05

no effect on the spatial memory as shown in Figure 3C. These finding suggested that ES treatment helped in facilitating the spatial learning ability in normal healthy rats.

Interestingly, the ES treatment after occlusion in the LCO rats resulted in significantly improved spatial learning ability. As shown in Figure 4A, B, the LCO-stim rats spent significantly shorter time and lesser distance than the LCO rats did on the 1st and 2nd day of the acquisition. Furthermore, the ES treatment may have slightly facilitated the spatial memory, as shown in Figure 4C. The LCO-stim group spent more time (55%) in the platform quadrant than the LCO group(43%), but not of any statistical significance.

On the contrary, the ES treatment had no effect on both spatial learning and memory on the RCO rats as shown in Figure 5 A-C. The escape latency in the acquisition trial and percentage of time spent in the platform quadrant were not significantly different between the RCO and RCO-stim rats.

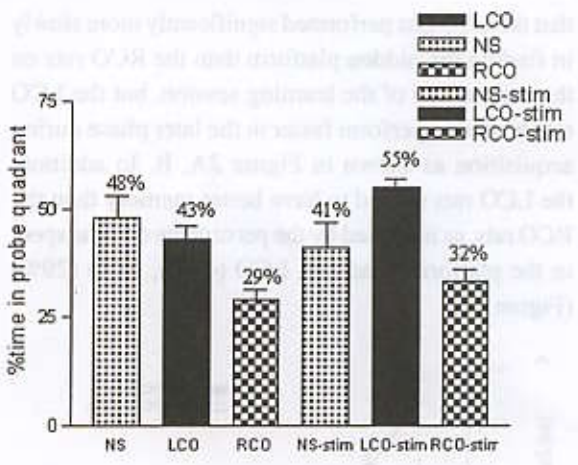

Figure 6. Spatial memory of NS, RCO and LCO rats with/ without electrical stimulation (stim) are shown as % time spent in the prove quadrant Alll data are presented as mean ± SEM.

Figure 6.

DISCUSSION

The present study was the first to show the short-term effects (5 days) of permanent unilateral common carotid occlusion on the spatial learning and memory in rats. It was found that mild chronic cerebral hypoperfusion either induced by right or left common carotid artery occlusion for five days had no effect on spatial learning and memory in ischemic insult rats when compared with the sham rats. However, the learning performance of the LCO rats was significantly slower than that of the RCO rats in the initial phase (2nd day) and became slightly faster in the later phase. In addition, the LCO rats tended to have better spatial memory than the RCO. Furthermore, we also demonstrated that electrical stimulation treatment applied after ischemic insult was able to facilitate spatial learning significantly in normal healthy and left common carotid occlusion rats, but no effect on right common carotid occlusion rats. Furthermore, electrical stimulation may help in facilitating spatial memory of the LCO-stim (55%) when compared that of the LCO (43%) as shown in

Spatial learning ability after five days of mild cerebral hypoperfusion

The results demonstrated that five days of mild global reduction of cerebral blood flow by unilateral common carotid occlusion (UCO) alone did not have significant effect on the ability in spatial learning and memory of the artery occluded rats when compared with that of the sham rats. These results seem to agree with several investigators who reported that unilateral carotid artery occlusion alone did not result in sufficient ischemia to produce marked neuronal damages in rats.[13-16] Although acute UCO only slightly reduced the hemispheric blood flow on the side of the occlusion (20-30%), the circulatory reserve was also depressed as indicated by inadequate adaptation of CBF to hypoxia, hypercapnia and hypotension. The resting hemispheric blood flow was progressively restored rather rapidly (bilateral normalization on the fifth day) whereas restoration of the cerebrovascular reserve proceeded more slowly and a nearly normal hypercapnic response was reached

on day thirty. This short-term reduction in hemispheric blood flow combined with the reduction in cerebrovascular reserve may have disturbed the functioning of neurons that were very sensitive to ischemia and this could explain our finding of mild deficit in spatial learning on the first and second day of learning in the LCO rats, this mild deficit can be restored by electrical stimulation treatment.[13,17]

Therefore, mild chronic hypoperfusion induced by unilateral carotid occlusion combined with these adverse consequential factors may result in cognitive impairment in the long term. Hence, in our study we have assessed the cognitive deficit only at five days after hypoperfusion, more long-term investigation should be done in the future to prove this suggestion.

Effect of Electrical stimulation on Spatial learning and Memory

This study found that the electrical stimulation (ES) that was modified from spinal cord stimulation (SCS) and acupuncture facilitated spatial learning in normal healthy rats. This may imply that ES may increase cerebral perfusion, resulting in accelerated learning capability. It has been shown that somatosensory fiber stimulation via SCS can enhance cerebral blood flow and the effect of SCS in inducing central and peripheral vasodilation was mediated by the release of nitric oxide.[18-20] Nitric oxide plays an important role in the basal cerebral blood flow regulation by modulating the haemostatic system. Because the rats in the electrical stimulation groups (NS-stim and LCO-stm) received more cerebral blood flow than did the NS group, their spatial learning improved. The effect of ES in enhancing the cognitive function may act similarly to regular exercise that improves CBF and the cognitive function in normal subjects and in rats.[21] It has been suggested that exercise maintains cerebrovascular integrity[22], increases capillary growth, increases dendritic connections and enhances the efficiency of the processing functions of the central nervous system.[23] This suggestion needs further proof in future studies.

Differences in spatial ability between the LCO and RCO rats

This study found difference in spatial ability between the LCO and RCO rats. We found that the LCO rats may learn significantly more slowly than the RCO rats in the initial phase of learning, but they seem to perform better in the later phase. The possible explanations for this difference could be either that left and right carotid occlusion produced different degree of damage in the brain, or rat's brain has lateralization in spatial ability. The right brain is dominant in cognitive function therefore the right brain damage will result in greater deficit than the left brain damage. This suggestion may agree with those reported by Arteni et al.[24] Their studies demonstrated that adult rats with neonatal right cerebral hypoxia-ischemia resulted in significantly greater deficit in the inhibitory avoidance task and the working memory than those of the left brain damage group, suggesting lateralization in cognitive function of the rodent brain.

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