The effect of spatial learning on the number of astrocytes in the CA3 subfield of the rat hippocampus

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ABSTRACT

Introduction: Astrocytes play an important role in the hippocampus, probably in memory and learning. The aim of this study was to evaluate the number of astrocytes in the CA3 subfield of the rat hippocampus after spatial learning using the Morris water maze with reference and working memory methods.

Methods: 45 male albino wistar rats were divided into three groups, with 15 rats in the control group and 15 rats in each of the other two groups. The two study groups of rats underwent spatial learning using the Morris water maze, with one group trained using the reference memory and the other, the working memory technique, respectively. After histological processing, the slides of the brains were stained with the phosphotanguestic acid haematoxylin staining method for detection of the astrocytes.

Results: There was a significant difference in the number of astrocytes in the CA3 area between the control and reference memory groups. The difference between control and working memory groups was significant as well. Additionally, when comparing the two learning groups, we also found significant differences between them.

Conclusion: The number of astrocytes increased due to spatial learning.

Keywords: astrocytes, CA3 subfield, hippocampus, Morris water maze technique, spatial learning

Singapore Med J 2008; 49(5): 388-391

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INTRODUCTION

The Morris water maze (MWM) is a test of spatial learning for rodents, which relies on distal cues to navigate from start locations around the perimeter of an open swimming arena to locate a submerged escape platform. Spatial learning is assessed across repeated trials, and reference memory is determined by preference for the platform area when the platform is absent.[1] The theory of neuron-astrocyte interaction may be useful in provoking a reformulation of concepts of some disorders affecting the nervous system. The presence of glial signaling in the brain and interactions between neurons and glia, were previously demonstrated.[2] Recent studies have shown that astrocytes are necessary for the formation, function, and stability of central nervous system (CNS) synapses *in vitro* and have provided increasingly provocative evidence that astrocytes also actively participate in synaptic plasticity within the developing brain.[3]

Astrocytes, strategically positioned between the capillaries and neurons, are thought to play a role in neuronal energy metabolism.[4,5] Glycogen is localised in the brain almost exclusively in astrocytes.[6,7] Astrocytes and microglia play critical roles in CNS response to, and recovery from, injury.[8-10] Astrocytes have been shown to play important roles in nutrient supply, waste removal, and axonal guidance. More recent work reveals that astrocytes play a more active role in neuronal activity, including regulating ion flux current, energy production, neurotransmitter release, and synaptogenesis. The latter includes the activity of glial cell apposition to synapses and the regulation of synapse elimination by ensheathment (known as glia swelling).[10,11]

The number of astrocytes in spatial learning has rarely been documented. The aim of this study was to evaluate the number of astrocytes in the CA3 subfield of the rat hippocampus after spatial learning by using the MWM with the reference and working memory learning methods.

METHODS

Between 2005 and 2006, 45 male albino Wistar rats (weighing 200–250 g) were obtained from Pasteur Institute of Iran, Tehran, Iran. Rats were housed in large plastic cages, and food and water were made available. Animals were maintained under standard conditions, with 12-hour light/dark cycles with lights switched on at 7.00 a.m. After adaptation to the environment, we divided the rats into control, reference and working memory groups. We used the MWM for spatial learning in the reference and working memory groups. For

MWM testing, the rats were placed in a circular plastic pool (diameter 120 cm) with white inside walls, located in a normally-equipped laboratory room, uniformly lighted by four neon lamps (40 W each) suspended from the ceiling (3 m). No modification was made to enhance or impoverish extra-maze cues, which were held in constant spatial relations throughout the experiments. The pool was filled with water (24°C), which was 50 cm deep and made opaque by the addition of 2 L of milk. A white steel escape platform (10 cm in diameter) was placed in the middle of one cardinal quadrant (NW, NE, SW, SE) 30 cm from the side walls; it was either submerged 2 cm below or elevated 2 cm above the water level. The animal was allowed to swim around to find the platform. Blocks of four trials were presented to each rat, two blocks of trials per day.[12]

In each trial of the reference memory test group, the rats were placed into the water at one of the four cardinal points of the compass (N, E, S, W), which varied from trial to trial in a quasi-random order. The rats had to swim until they climbed onto the escape platform. If they failed to locate the platform within 60 seconds, they were guided there. The rats were allowed to stay on the platform for 20 seconds. After the final trial, the rats were towel dried and placed in a holding cage under a heating lamp before they were returned to the home cage. The route of the rats was recorded by an infra-red digital camera, and the route and time of each trial were recorded with computer aid. In the working memory test group, training on the working memory version of the navigation task started two days after the reference memory pre-training phase. Only two trials per day were given until performance stabilised in the first trial (acquisition), where the animal had to find the platform in a new position. The rats were allowed to stay there for 20 seconds before they were returned to the home cage. On the second trial (retrieval), which was administrated 75 minutes later, the platform was in its previous position but the animals was started from a different place to the preceding trial.[13,14]

After the learning examinations, animals were decapitated after ether anaesthesia and the brains were removed for histological assessment. At first, the brains were fixed in 10% formaldehyde and two weeks later, they were impregnated with paraffin wax. After histological processing, 7-µm thick coronal slices (serial section of anterior to posterior hippocampus) were produced with a Leitz rotary microtome (HM 325, Microm International GmbH, Walldorf, Germany). One of ten sections were selected for staining; therefore, we had about 40 slides for morphometeric measurement). For astrocyte staining, we used phosphotanguestic acid haematoxylin (PTAH) staining[15] because it is the special staining method for astrocyte cells and their processes. In this method the astrocytes appeared blue and the neurons appeared pink (Fig. 1). Morphometric measurement were carried out using on Olympus DP 12 digital camera and BX 51 microscope (Olympus Optical, Tokyo, Japan). We selected a field (75,000 µm²) within the pyramidal layer of the hippocampus subfield CA3.

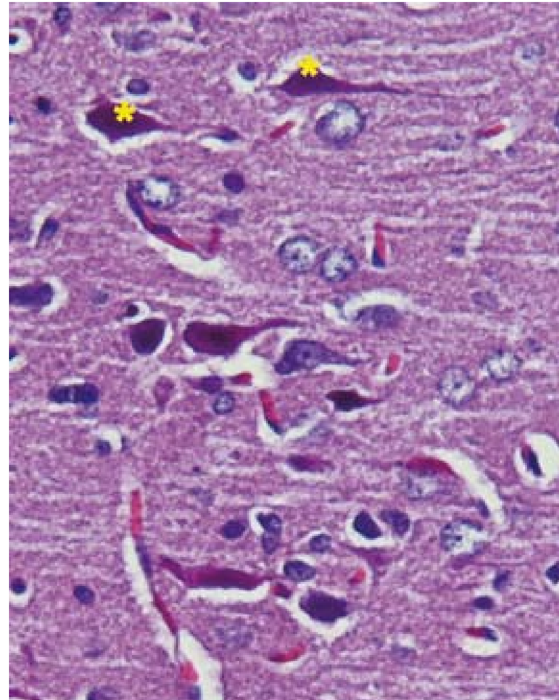

Fig. 1 Photomicrograph shows the astrocytes (PTAH stain, ×100).

Randomly-selected, non-overlapping photographs using a 40× objective lens were taken from the designated areas. Images were saved by the Bioreporter programme and further processed using the Adobe Photoshop 6.0 programme (Adobe System Inc, San Jose, CA, USA). For cell counts, photographs at a magnification of × 40 (objective lens) were taken throughout the longitudinal axis of the hippocampus CA3 subfield and further processed as described above. All of the astrocytes shown on this field were counted, and then the mean and standard deviation (SD) of astrocytes number were measured. Data was expressed as mean ± SD. Differences between areas were statistically evaluated using the one-way analysis of variance (ANOVA). p-value < 0.05 was considered significant.

RESULTS

The mean, SD and standard error of the mean (SEM) of the number of astrocytes in the CA3 area of the hippocampus (per 75,000 µm²) in the control, reference and working memory study groups are depicted in Table I. There were significant differences in the number of astrocytes between

Table I. The mean, SD and SEM of the number of astrocytes in the CA3 area of the hippocampus in the three groups.

Group	Mean astrocyte no.	Area (μm²)	SD	SEM
Control	41.95	75,000	11.22	0.846
Reference memory	116.6	75,000	31.14	2.34
Working memory	164.3	75,000	30.51	2.3

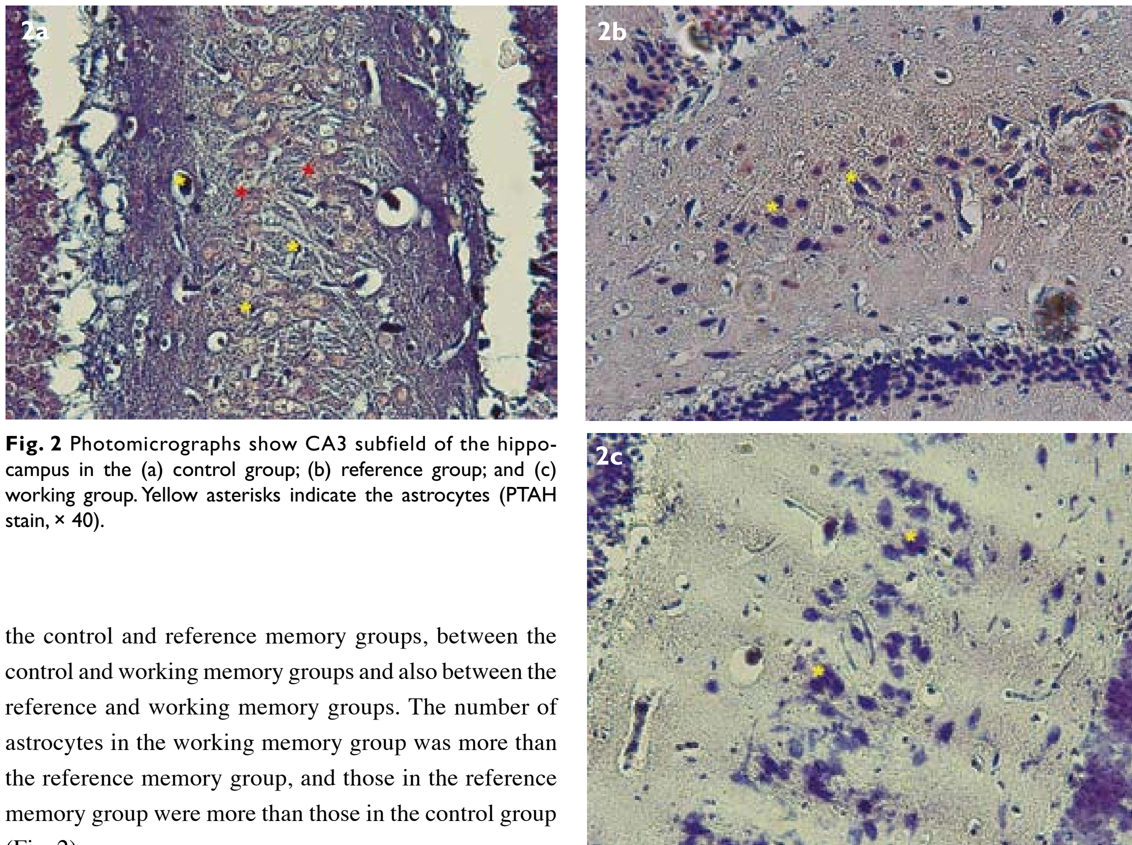

Fig. 2 Photomicrographs show CA3 subfield of the hippocampus in the (a) control group; (b) reference group; and (c) working group. Yellow asterisks indicate the astrocytes (PTAH stain, × 40).

the control and reference memory groups, between the control and working memory groups and also between the reference and working memory groups. The number of astrocytes in the working memory group was more than the reference memory group, and those in the reference memory group were more than those in the control group (Fig. 2).

DISCUSSION

The differences in the astrocyte numbers among all groups were significant. These results indicated that the reference and working memory methods of spatial learning can cause an increase of astrocyte number in the CA3 subfield of the hippocampus. Physiologically, our results are similar to and resemble those of many researchers who have worked on spatial learning.(13-18) Many studies have provided the relationship between exercise and neurogenesis in the hippocampus, and especially in the dentate gyrus.(19) Physical exercise increases the neurogenesis in the hippocampus, apart from genetic factors.(19,20) One of the exercise and learning methods, which can increase neurogenesis in dentate gyrus is the MWM.(21) Keuker et al used the MWM technique and the reference and working methods (similar to our research), and reported that the working memory in aged animals differed significantly from that in young animals, while the reference memory did not change with age.(22)

Rusakov et al found that memory formation is believed to alter neural circuitry at the synaptic level. Although the hippocampus is known to play an important role in spatial learning, no experimental data exist on the synaptic correlations of this process at the ultrastructural level. Analysis of synaptic spatial distribution showed a training-associated increase in the frequency of shorter distances (i.e. clustering) between synaptic active zones in the CA1, but not the dentate, thus indicating alterations in local neural circuitry. This finding indicates subtle changes in the synaptic organisation in area CA1 of the hippocampus following a learning experience, suggesting that spatial memory formation in mammalian hippocampus may involve topographical changes in local circuitry without synapse formation *de novo*.(23)

In conclusion, according to our hypothesis, the number of astrocytes is increased in the CA3 subfield after learning, because they are the third structure of synapses.(24) The knowledge of changes in astrocyte

numbers can help us study the extent of how these cells are involved in memory. Our study corroborated with previous research, which showed that spatial learning can increase the synaptic location and indirectly showed that the increase of synaptic numbers can also increase the number of astrocytes.

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