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Original Article

The effect of spatial learning on the number of astrocytes in rat dentate gyrus

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ABSTRACT

In this study, we evaluated the effect of spatial learning on the number of astrocytes in the rat dentate gyrus with Morris water maze. Fifteen male albino Wistar rats were divided into three groups as control, reference memory and working memory groups. Each group was consisted of 5 rats. After spatial learning, the brains were histologically examined; the slides were stained with phosphotungstic acid hematoxylin (PTAH) staining to show the astrocytes.

We found significant difference in the number of astrocytes in dentate gyrus between control and reference memory groups, and between control and working memory groups as well. When compared two learning groups there was a significant difference in the number of astrocytes between them, being higher in the working memory group.

We concluded that the number of astrocytes increased due to spatial learning and this increase can be affected to the period of learning.

Our studies of spatial learning and effect of learning techniques (reference and working memory) showed that the technique that has longer period of learning has more effect on the number of astrocytes. ***Neuroanatomy; 2007; 6: 51–53.***

Key words [dentate gyrus] [astrocyte] [spatial learning] [PTAH staining]

Introduction

The dentate gyrus is part of the hippocampal formation. It contains granule cells, which project to the pyramidal cells and interneurons of the CA3 subfield of the hippocampus. The granule cells are the principal excitatory neurons of the dentate gyrus [1]. The hippocampal formation is a part of limbic system; it plays an important role in memory and learning. Learning needs some instrument for information storage and information maintenance mechanisms resemble to memory. On the other hand, the memory always accompany to learning [2].

The main cell type in the dentate gyrus is the granular cell. Apart from principal neurons, the dentate gyrus contains different types of glial cells especially the astrocytes [1]. Astrocytes, strategically positioned between the capillaries and neurons, are thought to play a role in neuronal energy metabolism [3,4]. Glycogen is localized in the brain almost exclusively in astrocytes [5,6]. Astrocytes and microglia play critical roles in central nervous system response to and recovery from injury [7-9]. Astrocytes have been shown to play important roles in nutrient supply, waste removal, and axonal guidance. More recent studies reveal 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 ensheatment (known as glia swelling) [9,10].

Astrocytes are the only cells in the brain that contain the energy storage molecule glycogen [11]. They also contain distinctive 9 nm intermediate filaments composed of a unique protein called glial fibrillary acidic protein (GFAP) [12].

Recently, the researches showed that the astrocytes, not only receive the information from environment, but also send signals to the neurons [13]. In this study, we aimed to evaluate the effect of spatial learning on the number of astrocytes in the rat dentate gyrus.

Materials and Methods

In our study, we used 15 male albino Wistar rats (200-250 g) obtained from Pasteur Institute of Iran. Rats were housed in large plastic cage where food and water were available. Animals were maintained under standard conditions and 12 hours of light/dark cycle with lights on at 07:00 a.m. After accommodated with environment, we divided rats to control, reference memory, and working memory groups. We used Morris water maze technique for spatial learning in reference memory, and working memory groups.

Reference memory testing in the water maze

On each trial, the rats were placed into the water at one of the four cardinal points of the compass (North, East, South, West), 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

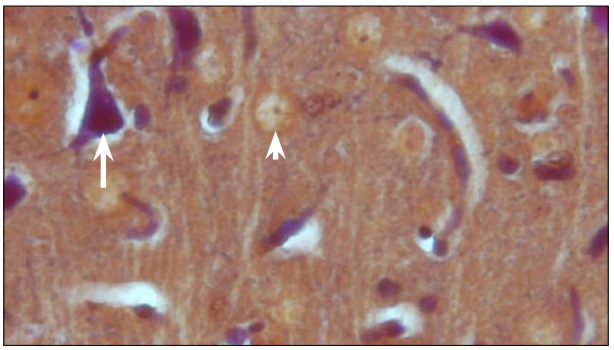

Figure 1. Astrocyte with PTAH staining (x100). Color version of figure is available online. ***(arrow:** astrocyte, **arrowhead:** neuron)*

rats were allowed to stay on the platform for 20 seconds. After the final trial, the rats were dried with towel and placed in a holding cage under a heating lamp before they were returned to the home cage. The route of rats was recorded by infrared digital camera and also route and time of each trial were recorded to computer.

Working memory testing in the water maze

Two day after the reference memory pre-training phase, training on the working memory version of the navigation task started. Only two trials per day were given until performance is stabilized. In the first trial (acquisition), the animal had to find the platform in a new position. The rats were allowed to stay on the platform for 20 seconds before they were returned to the home cage. In 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 [14,15].

After learning examinations, animals were decapitated with ether anesthesia, and the brains were removed for histological examination. At first, we fixed the brains in 10% formalin and two week later, processed them for embedding with paraffin. After embedding, we took serial sections in 7 μm of thickness.

For staining of astrocytes, we used phosphotungstic acid hematoxylin (PTAH) [16]. We preferred PTAH because it is a special staining for astrocyte cell bodies and processes. In this staining, the astrocytes had blue and the neurons had pink dye (Figure 1).

Morphometric measurement was carried out with an Olympus DP 12 digital camera and BX 51 microscope. We selected a field within the specified cell layer and counted all the astrocytes that were seen on the monitor.

Statistical analysis

Data expressed as mean ± SD differences among the areas were statistically evaluated using the one-way analysis of variance (ANOVA). Probabilities of <5% were considered as significant ($p<0.05$).

Results

The average number of astrocytes in control group's dentate gyrus (per 36000 μm2) was 73.73 ± 22.61. In reference memory group, it was 300.57 ± 5.98 and in working memory group it was 375.77 ± 4.11 (Table 1).

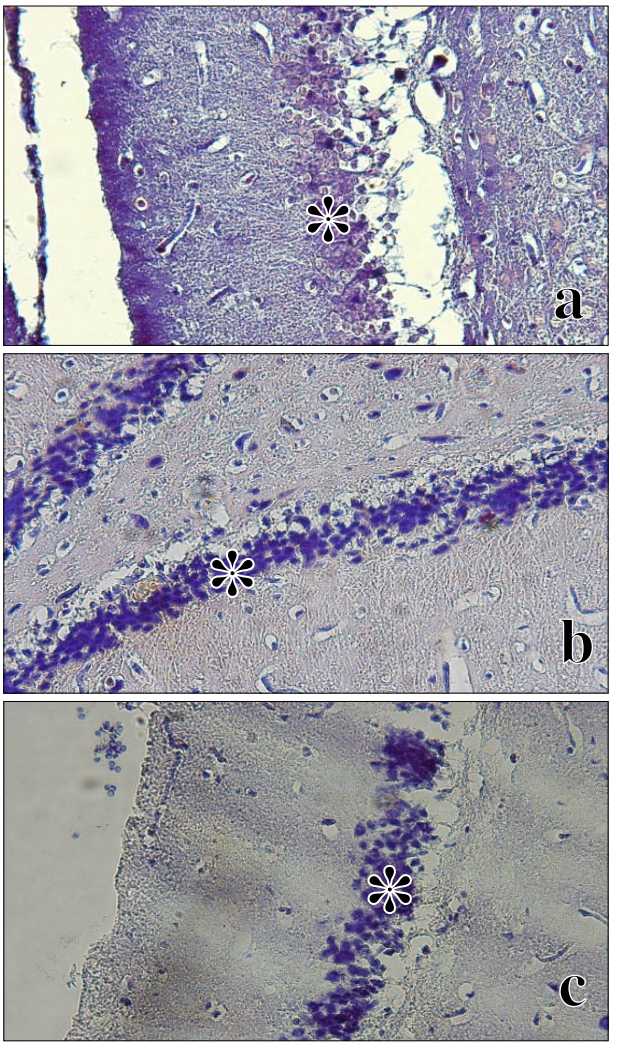

Figure 2. Dentate gyrus in all groups (PTAH staining x40). Color version of figure is available online. ***(a:** control group; **b:** reference memory group; **c:** working memory group; **asterisks** show astrocytes)*

The difference between control group and learning group was significant; the difference between two learning groups was also significant. The microscopic features for each group are depicted on Figure 2.

We divided the dentate gyrus into anterior one-third, middle one-third, and posterior one-third due to the different function between anterior and posterior hippocampal formation [17,18]. Then we analyzed differences in the number astrocytes between these parts. The average numbers of astrocytes in different parts (anterior one-third, middle one-third, and posterior one-third) of dentate gyrus in all groups are depicted on Table 2.

In all groups, significant differences were obtained between anterior, middle and posterior parts of dentate gyri.

Discussion

In the dentate gyri of control group, the highest number of astrocytes was in the anterior one-third and the lowest number was in the middle one-third. The differences between the corresponding parts (anterior, middle and

posterior one-thirds) of the reference memory and working memory groups were significant. Also in reference memory group the highest number of astrocytes was in the anterior one-third, but in working memory group it was in the posterior one-third. The number of astrocytes in working memory group was more than reference memory group, and the number of astrocytes in reference memory group was more than control group.

This data indicate that spatial learning such as Morris water maze technique increases the number of astrocytes in the dentate gyrus and this increasing is related to the length of the learning period.

Physiologically, our results were similar to that of many researches that studied on spatial learning [14,15,19-21].

In some studies, researchers used water maze technique and counted the number of neurons in different areas of hippocampus. For example, Rapp and Gallagher studied on the young (6 month) and aged (27-28 month) mice in Morris water maze spatial learning method, and then counted the number of neurons in the hippocampi in a stereological way. Although their study was on the neurons, the behavioral results of their study resemble to ours [22].

Also Pilegaard and Ladefoged stereologically counted the number of astrocytes in the molecular layer of dentate gyrus in different ages. In their study the mean number of astrocytes in the youngest group was 88.00 ± 15.00; it is similar to our results [23].

We concluded that the number of astrocytes in rat's dentate gyrus increased due to both method of spatial learning (reference memory and working memory) and this increase can be affected by the duration of the learning period.

Table 1. The mean of number of astrocytes in the hilus of the dentate gyrus in control, reference memory and working memory groups.

Group	Area (μm²)	Mean	Mean Std. Error	Std. Deviation
Control	36000	73.73	1.704	22.61
RMG	36000	300.57	5.987	79.429
WMG	36000	375.77	4.112	54.555

(RMG: reference memory group; WMG: working memory group)

Table 2. The mean number of astrocytes in the hilus of the dentate gyrus according to the anterior, middle and posterior one-thirds.

Group/Region	Area (μm²)	Mean	Mean Std. Error	Std. Deviation
Control/Ant.	36000	80.25	3.815	29.552
Control/Mid.	36000	65.67	2.11	16.342
Control/Post.	36000	74.4	2.167	16.783
RMG/Ant.	36000	309.92	10.011	77.545
RMG/Mid.	36000	303.77	8.895	68.9
RMG/Post.	36000	291.78	11.571	89.627
WMG/Ant.	36000	379.95	7.237	56.056
WMG/Mid.	36000	368.53	6.521	50.508
WMG/Post.	36000	380.05	7.254	56.192

(RMG: reference memory group; WMG: working memory group; Ant.: anterior; Mid.: middle; Post.: posterior)

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