Astrocytes in Molecular Layer of Cerebellum after Spatial Learning

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ABSTRACT

Introduction: Previous studies have suggested that the cerebellum is a primary site of motor learning. The cerebellar cortex has a particular glial architecture with large astroglial cells. In addition, more recent works have revealed that astrocytes play a more active role in neuronal activity. The aim of this study was to evaluate the number of astrocytes in the molecular layer of rat's cerebellum after spatial learning.

Methods: 21 male albino wistar rats were used in this study. Reference and working memory methods of Morris Water Maze (MWM) were used. Following behavioral testing, animals were decapitated under diethyl ether anesthesia. Brains were removed and fixed for 2 weeks for histological assessment. Finally, 7 µm thick coronal slices were cut and stained with PTAH staining for showing the astrocytes.

Results: Our results showed a significant difference in the number of astrocytes between the control, reference and working memory groups. On the other hand, the number of astrocytes in the working memory group was more than the other groups. There was no difference in density of astrocytes between the lateral and medial parts of the cerebellum in any group. It seems that the distribution of astrocytes in the lateral and medial parts of cerebellum is similar.

Discussion: We concluded that spatial learning such as reference or working memory methods, can increase the number of astrocytes in the cerebellum and this increase is similar in the cerebellar cortex.

1. Introduction

Previous studies about the plasticity of neurons have shown the ability of the nervous system to change its structure and function, which is a well-documented fact. Indeed, this ability is the basis of normal learning and memory (Crepel, Hemart, Jaillard, & Daniel, 1996; Diamond, 2001).

Some previous experimental studies (Brandeis, Brandys, & Yehuda, 1989; Ito, 1972) made the idea that the cerebellum is a primary site of motor learning, into one of the most appealing hypotheses of cerebellar function. This hypothesis has been supported by some

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experimental examinations such as lesion, electrical stimulation, and recording studies in a variety of movement systems (Lac, Raymond, Sejnowski, & Lisberger, 1995; Thach, 2007; Thompson & Krupa, 1994).

Recent experimental evidences demonstrated that the cerebellar networks are involved in the spatial learning (Leggio et al., 2000).

The cerebellar cortex has a particular glial architecture, with large astroglial cells that send radially-oriented branches to the molecular layer. These branches surround or intervene between neuronal elements. In the outer portions of the molecular layers, these branches, establish an external limiting membrane for the cerebellar cortex (Sabbatini, Barili, Bronzetti, Zaccheo, & Amenta, 1999).

Astrocytes have a major importance in the normal functioning of the CNS. Also, reactive astrocytes may act as a barrier, inhibiting neuroregeneration and neurite outgrowth. But the role of them in neuroprotection or in healing and recovery in various CNS pathologies remains mostly unclear (Silver & Miller, 2004).

Proliferation and/or differentiation of astrocytes as astrogenesis occur during normal development, as well as astrogenesis that occur in some diseases, damage or therapeutic intervention. Increase in the number of astrocytes also occurs in rat's hippocampus after spatial learning (Jahanshahi, Sadeghi, Hosseini, Naghdi, & Marjani, 2008).

As there are rare documents about the number of astrocytes after spatial learning test in cerebellum, the aim of this study was to evaluate the number of astrocytes in the molecular layer of rat's cerebellum after spatial learning by using the Morris Water Maze technique and reference and working memory methods.

2. Methods

21 male albino wistar rats (200-250g) were used in this study. Rats were obtained from the Pasteur institute of Iran. Animals were maintained under standard conditions with 12 h / 12 h light / dark cycle, and lights on at 7.00 a.m. Food and water were available. After adaptation to the environment, rats were divided into the control (without any testing), reference and working memory groups.

2.1. MWM Testing

As we have explained this technique in the previous studies (Jahanshahi et al., 2007; Jahanshahi et al., 2008) the rats were placed in a circular plastic pool, the pool was filled with water (24°C), which was 50 cm deep and made opaque by the addition of 2 liters of milk. A white, steel escape platform (10 cm in diameter) was placed in the middle of the 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.

On each trial, 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 could climb onto the escape platform. If they failed to locate the platform within 60s, they were guided there. The rats were allowed to stay on the platform for 20s. After the final trial, the rats were towel dried and placed in a holding cage under a heating lamp before being returned to the home cage. The routs, used by rats, were recorded by infra-red digital camera. The rout and time of each trial was also recorded by a computer (Jahanshahi et al., 2007; Jahanshahi et al., 2008).

For the working memory test, two days after the pretraining phase, training on the navigation task was started. Only two trials were given per day, until performance steadied in the first trial (acquisition) and the animal had to find the platform in a new position (Fig. 1). The rats were allowed to stay there for 20s before they were returned to the home cage. On the second trial (retrieval), which was administrated 75 min later, the platform was in its previous position but the animals started from a different place to the preceding trial. The routs, used by rats, were recorded by infra-red digital camera. The rout and time of each trial was also recorded by a computer (Naghdi & Asadollahi, 2004; Sarihi, Motamedi, Naghdi, & Rashidy-Pour, 2000).

After learning the examinations, animals were decapitated after diethyl ether anesthesia and the brains were removed for histological verification. At first the brains were fixed in formaldehyde %10 and two weeks later impregnated with paraffin wax. After histological processing, sagittal serial slices of 7 m (from lateral to medial) of cerebellum were produced with Leitz rotary microtome (One section was selected form each 10 sections for staining, therefore, we had about 40 slides for morphometeric measurement). For astrocytes staining, we used PTAH (Phosphotanguestic Acid Haematoxy-

lin) staining method (Bancroft & Stevens, 1990) because it is a special staining method for astrocyte cells and their processes. In this method the astrocytes and the neurons appear blue and pink respectively.

Morphometeric measurements were carried out using an Olympus DP 12 digital camera and BX 51 microscope. We selected a field (20000 µm2) within the cerebellum and randomly selected, non-overlapping photographs was taken from the designated areas using a ×40 objective lens. Images were saved by the Bioreporter program. For cell counting, photographs at a magnification of ×40 (objective lens) were taken throughout the cerebellum and further processed as described above. All of the astrocytes on the field were counted and then the mean and SD of astrocytes number was measured (fig 2-B).

2.2. Statistical Analysis

The data expressed as Mean SD differences among areas were statistically evaluated using the one-way analysis of variance (ANOVA). Probability of < %5 ($P < 0.05$) was considered significant.

3. Results

Our data showed that after the first trials the rats in the experimental groups learned the site of hidden platform and in the next trials they found the platform faster than the first trials. The decrease in the time of all trials is shown in fig. 3.

There are significant differences in the number of astrocytes between the control and the reference memory groups. On the other hand, the number of astrocytes in the reference memory group was more than the control group. The mean and SD of the number of Astrocytes (per 20000 m2) in control, reference and working memory groups are shown in table 1.

Also there were significant differences in the number of astrocytes between the control and the working memory groups. The number of astrocytes in the working memory group was significantly more than that of the control group and this additional number was almost similar in lateral to medial parts of the cerebellum.

We found that the number of astrocytes in the working memory group was more than the other groups.

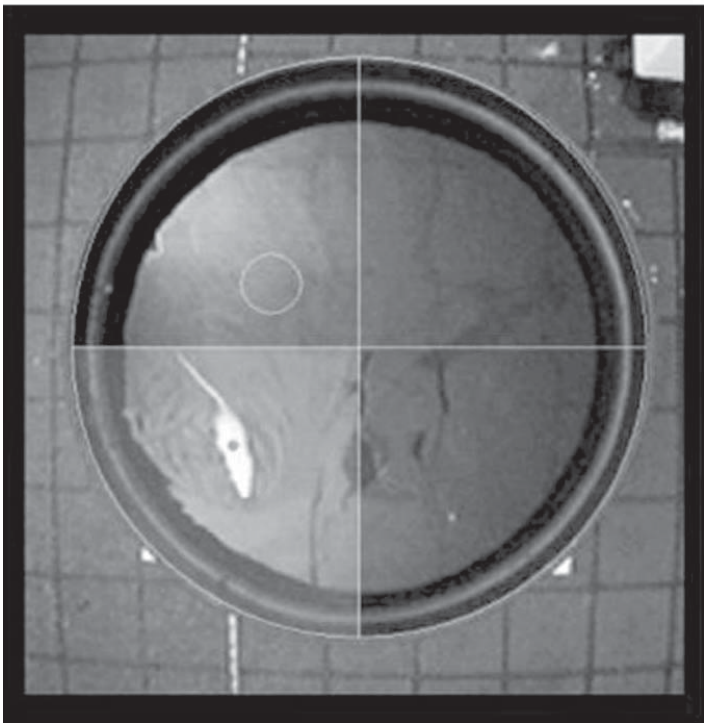

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Figure 1. the platform in a new position, animal swims in previous position

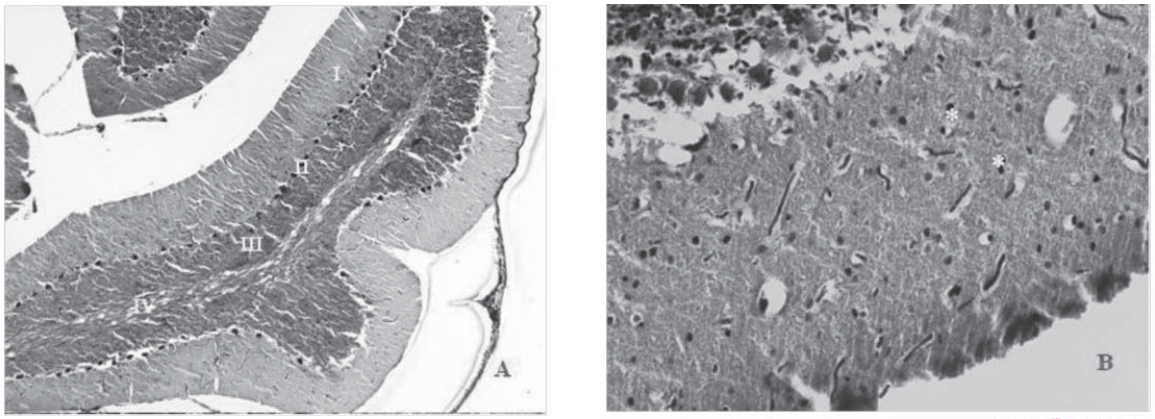

Figure 2. molecular layer of Cerebellum. A = ×10 and B = ×40 with PTAH staining.
Yellow asterisks are astrocytes and Red asterisks are purkinje cells
I=molecular layer, II=purkinje layer, III=granular layer, IV=white mater

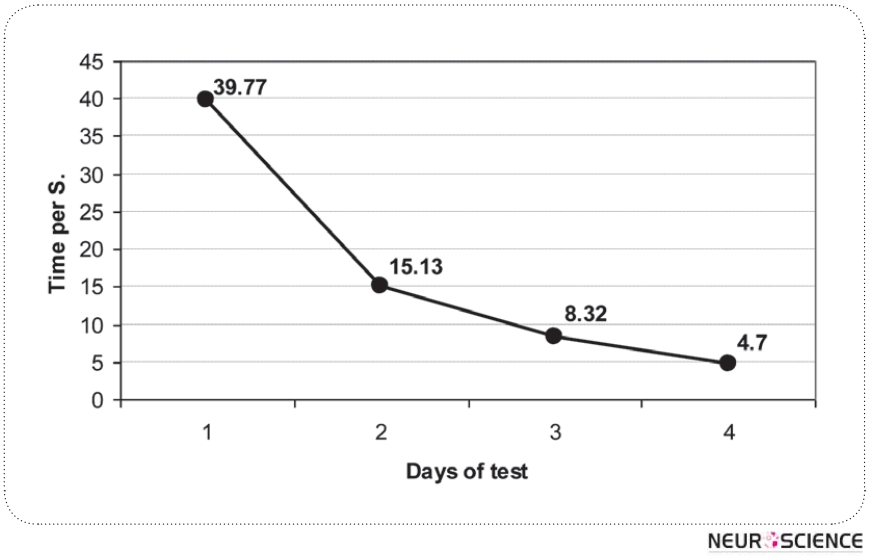

Figure 3. The mean time of finding platform from North of Maze in trial days

In survey of molecular layer slices from lateral to medial of cerebellum in the control group, the density of astrocytes had no significant difference; this finding was also observed in the reference and working memory groups (table 2).

4. Discussion

Behavioral finding of our study was similar to the others (Naghdi & Asadollahi, 2004; Sarihi et al., 2000). As we focused on the astrocytes density, we showed that the difference in the number of astrocytes between the control and the two experimental groups was statistically significant. On the other hand, the density of astrocytes in rats with learning test was more than the control group.

These results indicated that the working memory method of spatial learning probably has increasing effect on astrocytogenesis in cerebellum.

It seems that there is no significant difference in the number of astrocytes from lateral to medial of the cerebellar cortex in the control group or in the other groups, and it seems that there is no cytostructural difference in the distribution of astrocytes in different parts of the cerebellum.

Table 1. the mean of astrocytes number in Cerebellum in control, reference and working memory groups

	Mean	Std. Deviation	P value
Control	30.55	7.39	
Reference	37.53	7.37	P<0.01
Working	48.61	6.68	P<0.01

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Table 2. The mean of astrocytes number from lateral to medial of molecular layer in Cerebellum in control, reference and working memory groups

	Mean Control	Mean Reference	Mean Working	Area
1	32	35	60	20000 µm2
2	29	35	48	20000 µm2
3	33	37	52	20000 µm2
4	31	36	47	20000 µm2
5	32	42	42	20000 µm2
6	30	36	53	20000 µm2
7	32	37	51	20000 µm2
8	34	37	46	20000 µm2
9	33	38	48	20000 µm2
10	34	39	49	20000 µm2
11	32	37	49	20000 µm2
12	33	36	52	20000 µm2
13	27	41	47	20000 µm2
14	31	38	51	20000 µm2
15	31	41	52	20000 µm2
16	28	37	46	20000 µm2
17	32	40	45	20000 µm2
18	27	40	52	20000 µm2
19	29	45	45	20000 µm2
20	30	36	50	20000 µm2

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To identify the origin of these astrocytes, in 2001 Mao reported that the following three possibilities can be considered. Differentiation is the first possibility; astrocytes may be generated via local progenitors. Migration is the second; they may be the consequence of migration of matured or newborn astrocytes from elsewhere to this site. And the last possibility, they may be local astrocytes that are triggered to express (Mao, Lau, Petroske, & Wang, 2001).

When we analyzed the physiological data of rat's behavior, we found that our results resemble many researches that have worked on the spatial learning. For example, all of the rats in the reference group find the hidden platform faster than in the first trial, on the other hand, the time of platform finding decreased in next trials (Brandeis et al., 1989; Isgor & Sengelaub, 1998; Naghdi & Asadollahi, 2004; Redish & Touretzky, 1998; Sarihi et al., 2000).

Similar to our data, Kleim's findings in 2007 demonstrated that just like their neuronal partners, astrocytes are morphologically sensitive to experience. Kleim showed that motor learning increased the volume of astrocytes and the thickness of molecular layer per Purkinje cell in the paramedian lobule of the cerebellum. This finding demonstrated that some activities such as motor learning, but not mere motor activity, is associated with an increase in volume of astrocytes per Purkinje cell in the cortex of cerebellum (Kleim, Vij, Ballard, & Greenough, 1997).

Also, other documents have explained the relationship between learning activity and astrogenesis. For example, the synaptic activity and changes in the morphology of Bergmann glial processes suggests that the learning activities induce an increase in the astrocytic processes. This increased volume, that was observed here, is related to the learning-induced synaptogenesis (Jahanshahi et al., 2007; Jahanshahi et al., 2008).

5. Conclusion

According to our data, we concluded that the spatial learning techniques such as reference and working memory method in Morris Water Maze can increase the number of astrocytes in the molecular layer of cerebellar cortex.

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