Pakistan Journal of Biological Sciences 10 (21): 3964-3966, 2007
ISSN 1028-8880
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The Astrocytes Number in Different Subfield of Rat's Hippocampus in Reference Memory Learning Method

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Abstract: In this study with usage of morris water maze and reference memory technique, we used 10 male albino wistar rats. Five rats in control group and 5 rats in Reference memory group. After histological preparation, the slides were stained with PTAH staining for showing the Astrocytes. Present results showed significant difference in astrocytes number in CA1, CA2 and CA3 area of hippocampus between control and reference memory group. The number of astrocytes is increased in reference memory group. Then we divided the hippocampus to three parts: Anterior, middle and posterior and with compare of different area (CA1, CA2 and CA3) of hippocampus, we found that the increase of astrocytes number in posterior two-third of CA2 and CA3 is more than of it's number in the anterior one-third.

Key words: Hippocampus, astrocytes, reference memory, spatial learning

INTRODUCTION

The hippocampal formation plays an important role in memory and learning. The Morris Water Maze (MWM) is a test of spatial learning for rodents that 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 (Vorhees and Williams, 2006). Learning needs some instrument for information storage and information maintenances mechanisms resemble to memory. In the other hand, the memory always accompany with learning (Markowitsch; 1995).

The principal cell type in the subiculum and hippocampus is pyramidal neurons. The main cell type in the dentate gyrus is the granule cell. Apart from principal neurons, the hippocampal formation contains different types of glial cells, especially astrocytes (Williams *et al*., 1995).

Astrocytes, strategically positioned between the capillaries and neurons, are though to play a role in neuronal energy metabolism (Pellerin and Magisterettip, 2003; Forsyth *et al*., 1996). Glycogen is localized in the brain almost exclusively in astrocytes (Gruetter, 2003, Tsacopolos and Magistrattip 1996).

Astrocytes and microglia play critical roles in CNS response to and recovery from injury (Rabcheusky, 2002; Bechmann and Nitsch 1997, Teter and Ashford, 2002). 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 ensheatment known as glia swelling (Laming *et al*., 2000; Teter and Ashford, 2002).

Recently the researches showed that the astrocytes, not only receive the information from environment, but also send the signals to neurons (Caudle, 2006).

According of our hypothesis, the number of astrocytes after spatial learning must increase, because astrocytes have a closely relationship to synapses.

MATERIALS AND METHODS

Between 2005-2006 year 10 male albino wistar rats (200-250 g) obtained from pasteur institute of Iran were used. Rats were housed in large plastic cage, food and water were available. Animals were maintained under standard conditions and 12/12 h light/dark cycle with lights on at 7.00 a.m.

After accommodation with environment, we divided rats to control and reference memory groups. we used of Morris Water Maze technique for spatial learning in Reference memory group.

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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 (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 sec, they were guided there. The rats were allowed to stay on the platform for 20 sec. 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 rout of rats was recorded by Infra-red digital camera and also rout and time of each trial were recorded by computer (Naghdi 2004; Sarihi *et al*., 2000).

After learning examinations, animals were decapitated after ether anesthesia and the brains were removed for histological verification, at first the brains fixed in 10% formalin and two week later, we processed them for embedding with paraffine. After embedding, we prepared serial section with 7 μm thickness for each slide. For staining of astrocytes, we used PTAH staining (Bancroft, 1990) because it is the special staining for astrocyte cells and their processes. In this method the astrocytes become blue and the neurons become pink (Fig. 1).

Morphometric measurement were carried out using on Olympus DP 12 digital camera and B×51 microscope, selecting a field within the specified cell layer and counting all of the astrocytes shown on the monitor.

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

RESULTS

There is significant differences in astrocytes number between control and reference memory group in CA1 and CA3 subfields, but difference in CA2 between control and reference memory groups.

The mean and SD of the number of astrocytes in different area of hippocampus (per 75000 μm ,) is depicted on Table 1. In control group, the mean of astrocytes number in CA1 and CA2 was similar and more than CA3 subfield (Jahanshahi *et al*., 2006). In reference memory group, the similarity of astrocytes number was between CA1 and CA3.

Table 1: The mean of astrocytes number in hippocampal areas in control and reference memory groups

SEM	SD	Area (μm,)	Mean	Compartment
1.303	17.292	75000	49.00	CA1 c
1.9	25.21	75000	118.57	CA1 r
1.901	25.214	75000	48.82	CA2 c
1.778	23.593	75000	58.91	CA2 r
0.846	11.227	75000	41.95	CA3 c
2.348	31.143	75000	116.60	CA3 r

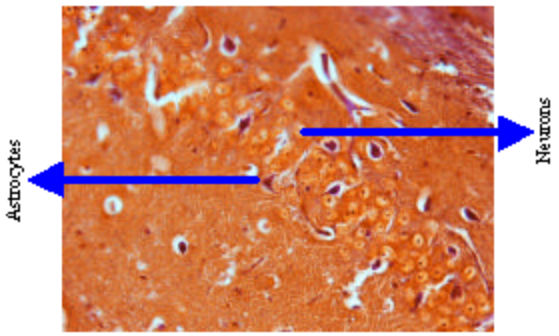

Fig. 1: Pyramidal layer of hippocampus with PTAH staining×40

DISCUSSION

The differences of astrocytes number between all areas (CA1, CA2 and CA3) of hippocampus in control and reference memory groups were significant. In all area, the number of astrocytes increased. Also, after the deviation of hippocampus to three parts anterior, middle and posterior one-thirds, because their functional differences (Maser, 1998), we showed that in CA1 area of reference memory group, the differences between anterior, middle and posterior. Parts were not significant, whether these differences in CA2 and CA3 areas were significant. The most number of astrocytes in CA2 area of reference memory group, was in post. One-third of hippocampus and in CA3 area was in middle one-third.

Present results indicated that the Reference memory method of spatial learning can cause increasing of astrocytes number in posterior two-third of hippocampus. Physiologically, present results similar and resemble to many researches that worked on the spatial learning (Sarihi *et al*., 2000; Naghdi and Sadollahi, 2004; Redish and Touretzkey, 1998; Isgor and Sengelawo, 1998; Bronders *et al*., 1989).

Many studies provided the relationship between exercise and neurogenesis in hippocampus and specially in dentate gyrus (Van Praag *et al*., 1999). Physical exercise increase the neurogenesis in hippocampus as well as genetic factors (Madsen *et al*., 2005; Van Praag *et al*., 1999). One of the exercise and learning method is the Morris Water Maze, that it can increase neurogenesis in dentate gyrus (Rosenzweig *et al*., 2003).

Keuker *et al*., reported that working memory in aged animals significantly differ from the young animals, wereath the reference memory don't changes with ages (Keuker *et al*., 2003).

Rusakov *et al*., in (1997) reported 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 correlates 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 CA1, but not dentate, thus indicating alterations in local neural circuitry. This finding indicates subtle changes in synaptic organization 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 (Rusakov *et al*., 1997).

These researches almost are resemble to present this study and showed that spatial learning can increase the synaptic location and indirectly we showed that the increase of synaptic number, can increase the number of astrocytes.

REFERENCES

Bancroft, J.B. and A. Stevens, 1990, Theory and Practice of Histological Techniques. Churchil Livinsgstone, Edinburgh.

Bechmann, I. and R. Nitsch, 1997. Astrocytes and microglia cells in corporate degenerating fibers following enthorhinal lesion. Glia, 20: 145-154.

Bronders, R., S.Y. Brandy and S. Yehuda, 1989. The use of the MWM in the study of memory and learning. J. Neurosci., 48: 29-62.

Caudle, R.M., 2006. Memory in astrocytes. A hypothesis. Theoretical Biology and Medical Modelling, pp: 3-2.

Forsyth, R., A. Fray and M. Boutelle *et al*., 1996. A role for astrocytes in glucose delivery to neurons? Dev. Neurosci., 18: 360-370

Gruetter, R., 2001. Glycogen: The forgotten cerebral energy store. J. Neurosci Res., 74: 179-183.

Isgor, C. and D.R. Sengelaub, 1998. Prenatal gonadal steroids affect adult spatial behavior, CA1 and CA3 pyramidal cell morphology in rats. Horm. Behav., 34: 183-198.

Jahanshahi, M., Y. Sadeghi and A. Hosseini, 2006. Estimation of Astrocyte number in different subfield of rat hippocampus. Pak. J. Biol. Sci., 9: 1595-1597.

Keuker, J.I.H. and G. Biurrun *et al*., 2004. Preservation of hippocampal neuron numbers and hippocampal subfield volumes in behaviorally characterized aged tree shrews. J. Comparative Neurol., 468: 509-517.

Laming, P.R. *et al*., 2000 Neuronal-glial interactions and behaviour. Neurosci. Biobehav. Rev., 24: 295-340.

Madsen, T.M. and D.D. Yeh *et al.,* 2005. Electroconvulsive seizure treatment increases cell proliferation in rat frontal cortex. Neuropsychopharmacologt, 30: 27-34.

Markowitsch, H.J., 1995 Anatomical Basis of Memory Disorders. In: The Cognitive Neurosci. M.S. Gazzamiga (Ed.), Cambridge, MA Mit Press, pp: 665-679.

Maser, M.B. and E.I. Maser, 1998. Functional differentiation in he hippocampus. J. Hippocampus, 8: 608-619.

Naghdi N. and A. Asadollahi, 2004. Genomic and nongenomic effects of intra hippocampal micro injection of testosterone on long-term memory in male adult rats. Behav. Brain Res., 153: 1-6.

Pellerin, L. and J. Magistrettip, 2003. Food for thought challenging the dogmas. J. Cereb B1F1 Meta., 23: 1282-1286

Rabcheusky, A.G., 2002. Influences of Activated Microglia/Brain Macrophages on Spinal Cord Injury and Regeneration. In: Microglia in the Regeneration and Degenerating Cerebral Nervous System. Streit, W.J. (Ed.), New York: Springer-verlag, pp: 209-226.

Redish, A.D. and D. Touretzky, 1998. The role of the hippocampus in solving the morris water maze. Neural. Comp., 10: 39-73.

Rosenzweig, E.S., A.D. Redish, B.L. McNaughton and C.A. Barnes, 2003. Hippocampal map realignment and spatial learning. Nature Neuroscience, 6: 609-615.

Rusakov, D.A., H.A. Davies, E. Harrison and G. Diana *et al*., 1997. Ultrastractural synaptic correlates of spatial learning in rat hippocampus. Neurocscience, 80: 69-77.

Sarihi A., F. Motamedi, N. Naghdi and A. Rashidy, 2000. Lidocaine reversible inactivation of the median raphe nucleus has no effect on reference memory but enhances working memory versions of the Morris water maze task. Behavioural Brain Res., 114: 1-9.

Teter, B. and J.W. Ashford, 2002. Neuroplasticity in alzheimer's disease. J. Neurosci Res., 70: 402-437.

Tsacopolos, M. and P.J. Magistretti, 1996. Metabolic coupling between glia and neurons. J. Neurosci., 16: 877-885.

Van Praag, H., B.R. Christic, T.J. Sejnowski and F.H. Gage, 1999. Runing enhances neurogenesis, learning and long term potentiation in mice. Proceedings of the National Academy of Sciences, USA., 96: 13427-13431.

Vorhees, C.V. and M.T. Williams, 2006. Morris water maze procedures for assessing spatial and related forms of learning and memory. Nature Protocols 1, pp: 848-858.

Williams, P.L., L.H. Bannister and M.M. Berry *et al*., 1995. Gray's Anatomy. In: Nervous system. 38th Edn. London. Churchill Livingston, pp: 1123-1129.