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The Similarity of Astrocytes Number in Dentate Gyrus and CA3 Subfield of Rats Hippocampus

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Abstract: The dentate gyrus is a part of hippocampal formation that it contains granule cells, which project to the pyramidal cells and interneurons of the CA3 subfield of the hippocampus. Astrocytes play a more active role in neuronal activity, including regulating ion flux currents, energy production, neurotransmitter release and synaptogenesis. Astrocytes are the only cells in the brain that contain the energy molecule glycogen. The close relationship between dentate gyrus and CA3 area can cause the similarity of the number of astrocytes in these areas. In this study 5 male albino wistar rats were used. Rats were housed in large plastic cage in animal house and were maintained under standard conditions, after histological processing, The 7 μ m slides of the brains were stained with PTAH staining for showing the astrocytes. This staining is specialized for astrocytes. We showed that the number of astrocytes in different (ant., mid., post) parts of dentate gyrus and CA3 of hippocampus is the same. For example, the anterior parts of two area have the most number of astrocytes and the middle parts of two area have the least number of astrocytes. We concluded that dentate gyrus and CA3 area of hippocampus have the same group of astrocytes.

Key words: Dentate gyrus, CA3 hippocampus, astrocyte, PTAH staining

INTRODUCTION

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 demonstrated interactions between neurons and glia (Antanitus, 2006).

Recent studies have shown that astrocytes are necessary for the formation, function and stability of CNS synapses *in vitro* and have provided increasingly provocative evidence that astrocytes also actively participate in synaptic plasticity within the developing brain (Ullian *et al.*, 2004).

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 (Williams *et al.*, 1995).

The hippocampal formation consist of the subiculum, the hippocampus and the dentate gyrus (Knowles, 1992). The hippocampus can be subdivided into three subfields; the CA1, CA2 and CA3 areas (Amaral and Witter, 1995).

Glial cells in the hippocampal formation contains the Astrocytes, Microglia and Oligodendrocytes (Williams *et al.*, 1995). In this study, because of the important role of astrocytes, we focused it's number in dentate gyrus and CA3 area of hippocampus.

Astrocytes, strategically positioned between the capillaries and neurons, are thout to play a role in neuronal energy metabolism (Pellerin and Magistretti, 2003; Forsyth *et al.*, 1996). Glycogen is localized in the brain almost exclusively in astrocytes (Gruetter, 2003; Tsacopoulos and Magistretti, 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 enheatment [known as glia swelling] (Laming *et al.*, 2000; Teter and Ashford, 2002).

The close relationship between dentate gyrus and CA3 area can cause the similarity of the number of astrocytes in these areas.

MATERIALS AND METHODS

Subjects: Five male albino wistar rats (200-250 g) obtained from Pasteur institute of Iran were used. Rats were housed in large plastic cage in animal house of Cellular and Molecular Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran, food and water were available. Animals were maintained under standard conditions and 12/12 h light/dark cycle with lights on at 7.00 am.

Histology: After two weeks, 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 parafine. After embedding, we prepared serial section with 7 μm thickness for each slide. For staining of astrocytes, we used PTAH staining (Bancroft and Stevens, 1990) because it is the special staining for astrocyte cells and their processes (Fig. 1).

In this method the astrocytes become blue and the neurons become pink (Bancroft and Stevens, 1990) (Fig. 1).

Morphometric measurement were carried out using on olympus DP 12 digital camera and BX 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 \pm SD differences among areas were statistically evaluated using the one-way analysis of variance (ANOVA). Probabilites of <5% ($p < 0.05$) were considered significant.

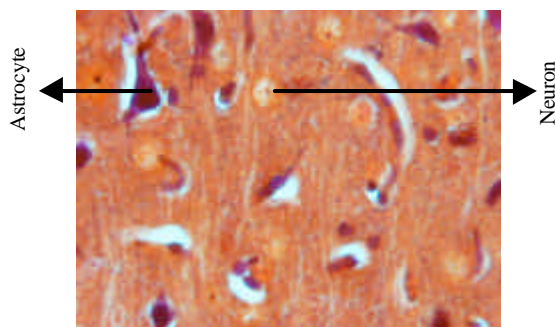


Fig. 1: Astrocyte with PTAH staining x100

Table 1: The mean of astrocytes number in DG and CA3

Compartment	Mean	Area (μm^2)	SD	SE
DG	73.73	36000	22.610	1.707
CA3	41.95	75000	11.227	0.846

Table 2: The astrocytes number in different (anterior, middl, posterior) parts of dentate gyrus and CA3 of hippocampus

Compartment	Mean	Area (μm^2)	SD	SE
DGa	80.25	36000	29.552	3.815
DGm	65.67	36000	16.342	2.110
DGp	74.40	36000	16.783	2.167
CA3a	45.45	75000	14.623	1.888
CA3m	39.12	75000	9.175	1.184
CA3p	41.13	75000	7.918	1.022

RESULTS

The hillus of the dentate gyrus have more astrocytes than the other area of hippocampus, but with usage of our results, the ratio of the number of astrocytes in different parts of dentate gyrus and CA3 area of hippocampus is similar.

The mean and SD of the number of Astrocytes in dentate gyrus (per 36000 μm^2) were 73.73 \pm 22.61. The mean and SD of the number of Astrocytes in CA3 area of hippocampus (per 75000 μm^2) were 41.95 \pm 1.22 (Table 1).

Then divided CA3 area of hippocampus and the dentate gyrus to three third: Anterior one-third, middle one-third and posterior one-third due to the differ function between anterior and posterior hippocampus (Moser and Moser, 1998; Greicius *et al.*, 2003; Pothuizen *et al.*, 2004). Then we analysed differences in astrocytes number between these parts. The mean and SD of astrocytes number in different parts (ant., mid., post.) of dentate gyrus and CA3 depicted on Table 2.

There is significant differences between anterior and middle parts of dentate gyrus and also there is significant differences between posterior and middle parts of it, is significant. However no significant difference were observed between anterior and posterior parts of dentate gyrus.

Also, there is significant differences between anterior and middle parts, anterior and posterior parts of CA3. However no significant difference were observed between middle and posterior parts of CA3.

DISCUSSION

We divided dentate gyrus and hippocampus to: Anterior one-third, middle one-third and posterior one-third due to the differ function between anterior and posterior hippocampus (Moser and Moser 1998; Greicius *et al.*, 2003; Pothuizen *et al.*, 2004).

Unfortunately, we have not several document to compare these to our results, therefore, we explain our results.

In this study, the differences between anterior and middle parts of dentate gyrus and CA3 area of hippocampus were significant and also the difference between posterior and middle parts of dentate gyrus was significant. Whereas the difference between anterior one-third and posterior one-third was not significant. The difference between middle and posterior parts of CA3 was not significant.

The number of astrocytes in different (ant., mid., post.) parts of CA3 is the same of different parts of dentate gyrus. For example, the anterior parts of two area have the most number of astrocytes and the middle parts of two area have the least number of astrocytes. As the astrocytes play an important role in synaptic connection between two partner neurons (Araque, 2001; Castonguay, 2001; Fields and Stevens-Graham, 2002; Oliet, 2002; Volterra, 2002), the same number of astrocytes in dentate gyrus and CA3 of hippocampus can be reason of relationship between them.

Present results and resemble with the Pilegaard's study, that works in 3-month male wistar rats and the mean and SD of astrocytes number in dentate gyrus in his study were 88.00 ± 15.00 (Pilegaard and Ladefoged, 1996).

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