ELSEVIER

Neurobiology of Aging 32 (2011) 1149–1156

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Septo-temporal gradients of neurogenesis and activity in 13-month-old rats

Jason S. Snyder [a,b,*], Preethi Ramchand [a], Sarah Rabbett [a], Ruvim Radik [b], J. Martin Wojtowicz [b], Heather A. Cameron [a]

[a] *Unit on Neuroplasticity, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, United States*
[b] *Department of Physiology, University of Toronto, 1 King's College Circle Rm. 3214, Toronto, ON, M5S 1A8, Canada*

Received 28 January 2009; received in revised form 17 April 2009; accepted 31 May 2009
Available online 25 July 2009

Abstract

Recent studies suggest that hippocampal function is partially dissociable along its septo-temporal axis: the septal hippocampus is more critical for spatial processing, while the temporal hippocampus may be more important for non-spatial-related behavior. In young adults, water maze training specifically activates new neurons in the temporal hippocampus, but it is unknown whether subregional differences are maintained in older animals, which have reduced neurogenesis levels. We therefore examined gradients of activity-related Fos expression and neurogenesis in 13-month-old rats and found that neurogenesis occurs relatively evenly throughout the dentate gyrus. Water maze experience significantly increased Fos expression in the suprapyramidal blade and Fos was highest in the septal pole of the dentate gyrus whether the animal learned a platform location, swam in the absence of a platform or remained in their cage. No Fos+ young neurons were found using typical markers of immature neurons. However, Fos expression in the subgranular zone, where adult-born neurons predominate, was disproportionally high in the temporal dentate gyrus. These findings indicate that adult-born neurons in the temporal hippocampus are preferentially activated compared with older neurons.
Published by Elsevier Inc.

Keywords: Adult neurogenesis; Dentate gyrus; Septal; Temporal; Dorsal; Ventral; Fos; Immediate-early gene; Aging; Spatial; Water maze; Stress; Anxiety

1. Introduction

While a role for the hippocampus in spatial memory is well established (Frankland and Bontempi, 2005), more recent evidence also points to a role for the hippocampus in regulating anxiety-related behavior. There is substantial evidence suggesting that these two functions of the hippocampus are at least partially subserved by different anatomical subregions: the septal hippocampus is particularly important for spatial learning and the temporal hippocampus regulates defensive, anxiety-related, and odor-mediated behaviors (Bannerman et al., 2004; Pentkowski et al., 2006; Hunsaker et al., 2008). Other studies suggest that the temporal hippocampus contributes to spatial learning as well, but to different aspects than the septal hippocampus, including processing larger spatial environments (Jung et al., 1994; Kjelstrup et al., 2008), learning over longer timescales (de Hoz et al., 2003), and mediating context-based inhibitory associations (McDonald et al., 2006). Thus, the function of the temporal hippocampus in regulating anxiety and spatial processing appears to differ from that of the septal hippocampus.

Given the septo-temporal functional dissociation, and findings suggesting that adult neurogenesis in the hippocampus is involved in both spatial learning and anxiety-related behavior (Leuner et al., 2006; Drew and Hen, 2007), we previously examined septo-temporal gradients (there referred to as "dorso-ventral" gradients) of neurogenesis and activity (i.e. Fos+ cells) in young neurons in young adult rats after learning in a spatial water maze task (Snyder et al., 2009). In that study, both neurogenesis and granule neuron Fos expression were higher in the septal dentate gyrus of the hippocampus.

* Corresponding author at: National Institutes of Health, Building 35/3C911, MSC3718, Bethesda, MD 20892, United States.
Tel.: +1 301 451 8281; fax: +1 301 480 4564.
E-mail address: snyderjason@mail.nih.gov (J.S. Snyder).

0197-4580/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.neurobiolaging.2009.05.022

However, expression of Fos by young neurons after water maze training was specific for the temporal dentate gyrus, suggesting that young granule neurons may play a different role than older granule neurons in water maze learning.

It is well documented that neurogenesis declines with age (Altman and Das, 1965; Seki and Arai, 1995; Kuhn et al., 1996; McDonald and Wojtowicz, 2005), and there are also reports that the expression of activity-dependent immediate early genes (IEGs) is reduced with age (Small et al., 2004). However, it is unclear whether there are changes in septo-temporal gradients of neurogenesis and activity with aging, which could alter distinct aspects of hippocampus-dependent learning in old age. Therefore, in the current study, we examined neurogenesis and water maze-induced Fos expression in septo-temporal and infrapyramidal-suprapyramidal axes of the dentate gyrus in 13-month-old rats. We found weak subregional differences in levels of neurogenesis but strong biases for Fos expression in the septal dentate gyrus and in the suprapyramidal blade after water maze experience. In the temporal dentate gyrus, but not septal dentate gyrus, water maze training activated adult-born neurons (in the subgranular zone) more than older neurons (in the outer rows).

2. Methods

2.1. Animals and treatments

Fourteen 13-month-old male Long Evans rats (Charles River, Quebec) were used in the following experiments. All animals were individually housed, and all treatments conformed to animal health and welfare guidelines of the University of Toronto. To label adult-born DG granule cells, all rats were given 2 intraperitoneal injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma, 50 mg/kg/injection, dissolved at 20 mg/ml in saline, 0.007N NaOH) spaced 10 h apart, for 5 consecutive days. Beginning 3 weeks after BrdU injections, all 14 rats were handled 5 min per day for 5 days to minimize stress associated with behavioral procedures. Four weeks after BrdU injections, rats were divided into three groups that were either trained in the Morris water maze (as described below), put in the water maze with no platform (swim controls), or left untouched (cage controls). All rats were perfused exactly 2 h after their first water maze trial (∼90 min after their last trial) or at the same time of day (cage controls) to assess activity-dependent Fos expression.

2.2. Water maze

On the final day of the experiment, 8 rats were trained in the Morris water maze, a hippocampus-dependent task (Morris et al., 1982) known to induce Fos expression in adult-born granule neurons (Jessberger and Kempermann, 2003; Kee et al., 2007; Tashiro et al., 2007). The testing apparatus was as previously described (Snyder et al., 2005). Briefly, the pool was 180 cm in diameter and filled with water. Water was kept at 25 °C and non-toxic white paint was added to hide a 10 cm wide platform present in the SE quadrant. Distal cues were present on the walls of the room to allow rats to develop a spatial strategy for escaping the water by mounting the platform. Rats were allowed to remain on the platform for 10 s after finding it. If a rat failed to locate the platform within 60 s, it was guided to the correct location by the experimenter. Rats were trained in pairs for 16 trials, 1–2 min apart, allowing initially naïve rats to quickly develop a spatial memory for the platform location. Latency to find the platform and mean proximity to the platform were calculated for each trial using HVS Image software (Buckingham, UK). For the proximity measure (Gallagher et al., 1993), the distance of the rat from the platform location was calculated at 0.1 s intervals. The mean of these values was then calculated for each trial. A rat that searches in the correct area of the pool will have a low mean distance value, providing a measure of spatial bias for the platform location that should decrease across trials if animals learn the location of the platform. In addition, if the search pattern is spatially selective, the difference between the mean distance from the platform location and from a similarly sized location at the opposite side of the pool should increase across blocks. Following training, rats were returned to their home cages until being perfused exactly 2 h after their first training trial. Swim control rats ($n = 3$) were placed in the pool in the absence of a platform and allowed to swim for 60 s, 45 s, 30 s and 15 s (4 trials of each) to approximate the swim times of the trained rats. They were perfused 2 h after the first swim trial. Cage control rats ($n = 3$) were perfused directly from their home cages with no behavioral manipulation.

2.3. Immunohistochemistry

Animals were perfused with phosphate-buffered saline followed by 8% paraformaldehyde. Brains were fixed in paraformaldehyde for an additional 24 h. The right hippocampus was extracted, cut into 4 approximately equal-length pieces and sectioned essentially perpendicular to its long (septo-temporal) axis. Since hippocampi were not forcibly straightened some curvature remained, but this procedure generally enabled comparable analyses along the entire axis (Gaarskjaer, 1978; Rapp and Amaral, 1988) (Fig. 3A). Sections were cut at 40 μm using a vibratome for a total of ∼200 sections.

Sequential fluorescent double labeling was performed for Fos, followed by BrdU, on free floating sections. For BrdU/Fos double-immunolabeling, sections were first incubated with rabbit anti-Fos antibody (1:10,000; Calbiochem #PC38; 3 days at 4 °C) followed by Alexa568 goat anti-rabbit secondary antibody (1:200; Molecular Probes; 2 h at room temperature). Sections were then treated with 1N HCl at 45 °C for 40 min to denature DNA and expose BrdU. Sections were then incubated with rat anti-BrdU antibody (1:200; Accurate #OBT0030; 1 day at 4 °C) followed by Alexa488 goat anti-rat secondary antibody (1:200; Molecular Probes; 2 h at room temperature). PSA-NCAM/Fos staining was