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Sex differences in neurogenesis and activation of new neurons in response to spatial learning and memory

Carmen Chow, Jonathan R. Epp [1], Stephanie E. Lieblich, Cindy K. Barha, Liisa A.M. Galea *

Program in Neuroscience, Department of Psychology, Brain Research Centre, University of British Columbia, Vancouver, BC, Canada

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Summary Adult hippocampal neurogenesis is often associated with hippocampus-dependent learning and memory. Throughout a new neuron's development, it is differentially sensitive to factors that can influence its survival and functionality. Previous research shows that spatial training that occurred 6—10 days after an injection of the DNA synthesis marker, bromodeoxyuridine (BrdU), increased cell survival in male rats. Because sex differences in spatial cognition and hippocampal neurogenesis have been reported, it is unclear whether spatial training would influence hippocampal neurogenesis in the same way in males and females. Therefore, this study examined sex differences in hippocampal neurogenesis following training in a spatial task. Male and female rats were trained in the spatial or cued version of the Morris water maze 6—10 days after one injection of BrdU (200 mg/kg). Twenty days following BrdU injection, all animals were given a probe trial and perfused. Males performed better in the spatial, but not cue, task than females. Spatial training increased BrdU-labeled cells relative to cue training only in males, but both males and females showed greater activation of new cells (BrdU co-labeled with immediate early gene product zif268) after spatial training compared to cue training. Furthermore, performance during spatial training was positively correlated with cell activation in females but not males. This study shows that while spatial training differentially regulates hippocampal neurogenesis in males and females, the activity of new neurons in response to spatial memory retrieval is similar. These findings highlight the importance of sex on neural plasticity and cognition.

1. Introduction

The hippocampus is one of two main areas that harbors continual neurogenesis in adulthood (Altman and Das, 1965). Adult neurogenesis exists in most mammalian species,

* Corresponding author. Tel.: +1 604 822 6536; fax: +1 604 822 6923.
E-mail address: lgalea@psych.ubc.ca (L.A.M. Galea).
[1] JRE is now located at the Hospital for Sick Children, Toronto, ON, Canada.

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including humans (Eriksson et al., 1998) and can be subdivided into cell proliferation, migration, differentiation, and maturation. Regulation of adult neurogenesis at any of these stages can lead to changes in the number of new neurons that are produced.

The function of these new neurons in the hippocampus has been linked to hippocampus-dependent learning and memory (Leuner et al., 2006). Training on hippocampus-dependent tasks such as the spatial Morris water maze increases hippocampal neurogenesis (Gould et al., 1999). Furthermore exercise, which increases hippocampal neurogenesis, also facilitated spatial learning (van Praag et al., 1999), while irradiation, which decreases hippocampal neurogenesis, impaired long-term spatial memory (Snyder et al., 2005). Furthermore a partial reduction in hippocampal neurogenesis, via genetic knockdown, was sufficient to disrupt spatial learning and discrimination (Zhang et al., 2008; Clelland et al., 2009). Additionally, greater reductions in hippocampal neurogenesis impaired spatial memory and novel object recognition memory, whereas lesser reductions in hippocampal neurogenesis did not significantly affect performance compared to controls (Jessberger et al., 2009). These studies show that reducing neurogenesis to certain levels can impair hippocampus-dependent learning and memory.

Exposure to hippocampus-dependent learning can also differentially regulate the survival of new hippocampal cells depending on the type of task (Leuner et al., 2006), quality of learning (Sisti et al., 2007; Epp et al., 2007), task difficulty (Epp et al., 2010), and/or the age of cells at the time of exposure and perfusion (Epp et al., 2007, 2011). The ability of spatial training to promote hippocampal neurogenesis depends on the stage of development during which immature neurons are exposed to spatial training (Epp et al., 2007). Spatial training 6–10 days after an injection of the DNA synthesis marker, bromodeoxyuridine (BrdU) increased cell survival, but spatial training on days 1–5 or 11–15 after BrdU injection either did not change (Epp et al., 2007) or decreased cell survival depending on the age of new neurons at the time of examination (Epp et al., 2011). Because the time window for incorporation of BrdU into dividing cells is just 2 h (Nowakowski et al., 1989), this method allows the tracking of a group of similarly aged cells. Therefore, these data suggest that there is a critical period in new neuron development during which cell survival is most affected by spatial training.

Studies have also investigated whether training on hippocampus-dependent tasks activates new neurons. Cell activation can be quantified using immediate early genes (IEG) such as *c-Fos*, *arc*, or *zif268*, which are transiently expressed in response to neuronal activation and have a role in neural plasticity and memory consolidation (Guzowski et al., 2001; Jones et al., 2001). IEG expression in adult-born neurons is increased in response to exploration of a new environment (Ramirez-Amaya et al., 2006), re-exposure to a familiar environment (Tashiro et al., 2007), spatial learning (Jessberger and Kempermann, 2003; Kee et al., 2007; Snyder et al., 2009), and memory retrieval (Snyder et al., 2005; Epp et al., 2011).

Thus far the majority of research in this area has been conducted in male animals. However there are significant sex differences in the regulation of neurogenesis in the adult dentate gyrus by factors such as stress, breeding season, and gonadal hormones (Falconer and Galea, 2003; Westenbroek et al., 2004; Galea and McEwen, 1999; Barker and Galea, 2008). Furthermore, sex differences in spatial performance exist across a wide variety of species, with males typically outperforming females (Galea et al., 1996; Gaulin and Fitz-Gerald, 1986). To our knowledge only one study has examined sex differences in the effects of training on a hippocampus-dependent task on hippocampal neurogenesis (Dalla et al., 2009). This study showed that faster acquisition of trace eyeblink conditioning was correlated with a greater percent increase in cell survival in females compared to males. Intriguingly, sex differences in performance of the trace eyeblink conditioning task favors females, unlike performance in the Morris water maze which typically favors males (Galea et al., 1996). To our knowledge, no study has examined the effect of spatial training on hippocampal neurogenesis and activation of new neurons in males and females.

Therefore, the current study aimed to determine whether there are sex differences in the survival of new neurons after Morris water maze training and whether there is differential activation in response to spatial memory retrieval. Adult male and female rats were trained in the Morris water maze 6–10 days after BrdU injection and were given a probe trial on day 20 to examine new cell activation via the IEG product zif268. We hypothesized that males would outperform females in acquisition of the Morris water maze, would have higher levels of hippocampal neurogenesis in response to training and show greater activation of new neurons in response to memory retrieval compared to females.

2. Methods

2.1. Subjects

Sixty-three Sprague Dawley rats (males: $n = 29$; females: $n = 34$) between 58 and 62 days old that were bred and raised in the Department of Psychology at the University of British Columbia were used in this study. All animals were pair-housed in polycarbonate bins (48 cm × 27 cm × 20 cm) with a polyvinylchloride tube, paper towels, aspen chip bedding, and free access to food and water. The colony room was kept at a temperature of 20 °C with 50–70% humidity, and maintained on a 12/12 h light–dark cycle (lights on at 0700). Animals were left undisturbed until handling began 5 days prior to the start of the experiment. All testing was carried out in accordance with the Canadian Council for Animal Care guidelines and was approved by the animal care committee at the University of British Columbia. All efforts were made to reduce the number of animals used and to minimize their suffering.

2.2. Apparatus

The Morris water maze was a white circular pool that was 180 cm in diameter and filled with water mixed with white tempura (non-toxic) paint to render it opaque. Large distal cues were placed on all four walls of the room surrounding the pool and remained constant throughout the study. A camera installed above the center of the pool was connected to a computer running ANY-maze (Stoelting, Wood Dale, IL, USA) in order to record measures of performance such as