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Activation and survival of immature neurons in the dentate gyrus with spatial memory is dependent on time of exposure to spatial learning and age of cells at examination

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ABSTRACT

Neurogenesis continues to occur throughout life in the dentate gyrus of the hippocampus and may be related to hippocampus-dependent learning. We have recently reported that there is an enhancement of neurogenesis in the hippocampus only when BrdU is administered 6 days prior to starting spatial training but not when training started either 1 day or 11 days following BrdU administration. In that study, all rats were perfused on day 16 after BrdU injection in order to compare cells of the same age (i.e. 16 day old cells) and thus the survival time after learning was different between groups. This study was designed to address whether the amount of time that passed following training could also contribute to the effects of spatial learning on hippocampal neurogenesis and whether there was differential new neuron activation in response to spatial learning that depended on the age of new cells at the time of spatial learning. Here we tested whether a survival period of 5 days following spatial learning at either 1–5, 6–10 or 11–15 days following BrdU administration would alter cell survival and/or activation of new neurons. Our results indicate that 5 days after training in the Morris water task cell survival is unaltered by training on days 1–5, increased by training at days 6–10 and decreased when training occurs on days 11–15. Furthermore spatial learners trained on days 6–10 or 11–15 show greater activation of new neurons compared to cue-trained rats during a probe trial 5 days after training. In addition, rats trained on the spatial task on days 11–15 had a greater number of activated new neurons compared to rats trained on the spatial task on days 6–10. These results suggest there is a gradual removal of older BrdU-labeled new neurons following spatial learning perhaps due to a competitive interaction with a population of younger BrdU-labeled new neurons.

1. Introduction

It is now widely accepted that in the adult mammalian brain, neurogenesis occurs throughout life in the dentate gyrus of the hippocampus (Altman & Das, 1965; Cameron, Woolley, McEwen, & Gould, 1993; Eriksson et al., 1998; Kaplan & Hinds, 1977). The function of these new cells remains uncertain and controversial. However, mounting evidence points towards a role in hippocampus-dependent learning and memory (for review see Leuner, Gould, and Shors (2006)). New neurons produced in the subgranular zone of the dentate gyrus migrate into the granule cell layer where they integrate and extend axons into the CA3 region, (Hastings & Gould, 1999; Markakis & Gage, 1999; Stanfield & Trice, 1988), become electrophysiologically active and mature into functional granule cells (van Praag et al., 2002).

Numerous studies have demonstrated that training rats in certain hippocampus-dependent tasks alters the number of cells that survive to maturity (Ambrogini et al., 2000; Epp, Haack, & Galea, 2009; Epp, Spritzer, & Galea, 2007; Gould, Beylin, Tanapat, Reeves, & Shors, 1999; Hairston et al., 2005; Leuner et al., 2004; Olariu, Cleaver, Shore, Brewer, & Cameron, 2005). Gould and colleagues (1999) first showed that rats trained in a spatial navigation version of the Morris water task (MWT) had a greater number of new surviving cells compared to rats trained in a non-spatial (hippocampus-independent) version of the MWT. However, several studies have reported that hippocampus-dependent learning does not alter cell survival (Dobrossy et al., 2003; Mohapel, Mundt-Petersen, Brundin, & Frielingsdorf, 2006; Van der Borght, Wallinga, Luiten, Eggen, & Van der Zee, 2005) or decreases cell survival following spatial learning (Ambrogini, Orisini et al., 2004; Epp et al., 2009). We and others have shown that the effect of spatial learning on neurogenesis in the hippocampus is dependent on a variety of factors such as timing, quality of learning and task difficulty (Epp et al., 2007, 2009; Sisti, Glass, & Shors, 2007).

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Recent evidence suggests that timing may be critical in explaining why only some studies have found enhanced cell survival in response to spatial learning. We have recently demonstrated that spatial learning increases cell survival specifically when training occurs 6–10 days following bromodeoxyuridine (BrdU) administration but there was no change in cell survival when training occurred either 1–5 days or 11–15 days after BrdU administration (Epp et al., 2007). However, another study described a decrease in cell survival following spatial learning that occurred 11–15 days following BrdU administration (Ambrogini, Orisini et al., 2004). One possible reason for this discrepancy may be the length of time between training and examination of new cells in the dentate gyrus. In the Ambrogini (2004) study, BrdU-labeled cells were examined three days after training on days 11–15 while in our study BrdU-labeled cells were examined one day after training (Epp et al., 2007). This difference in the time after training may have allowed for a delayed decrease in cell survival in the Ambrogini study that had not yet occurred one day following training in our earlier study.

The first aim of the current study was to determine whether the time windows between labeling new cells with BrdU, training and examination of cells is important for detecting changes in cell survival. The second aim of the current study was to examine whether changes in cell survival that result from spatial learning at different times after BrdU administration cause a change in the number of new cells that are activated by memory retrieval. Several studies have used immediate early gene (IEG) activation as a marker of cellular activation in new neurons in response to hippocampus-dependent learning and memory (Kee, Teixeira, Wang, & Frankland, 2007; Ramirez-Amaya, Marrone, Gage, Worley, & Barnes, 2006; Snyder, Choe et al., 2009; Snyder, Radik, Wojtowicz, & Cameron, 2009; Tashiro, Makino, & Gage, 2007). In the current study we sought to determine whether the critical period for enhancing cell survival (6–10 days after BrdU administration) has a corresponding increase in activation of these new cells compared to training on days 1–5 or 11–15.

To address these aims, we trained rats in the MWT at one of three time points following BrdU administration (1–5, 6–10 or 11–15 days) and examined all groups five days following training in order to allow sufficient time for any delayed changes in cell survival to occur after the completion of training. Two hours prior to perfusion all rats were given a single probe trial in the MWT to assess spatial memory and to determine whether memory retrieval differentially activates new neurons dependent on the age of the cells at the time of training.

2. Materials and methods

2.1. Subjects

Subjects were 75 male Sprague Dawley rats (Charles River; Saint-Constant, Quebec, Canada) 70–75 days old and weighed between 300 and 350 g at the beginning of testing. Rats were housed individually in standard cages with a polyvinylchloride tube, paper towels, cedar bedding and free access to food and water. Rats were habituated to their housing conditions for 1 week, and then were handled five minutes per day for 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

A circular pool, 180 cm in diameter, was filled with approximately 21 °C water to a depth of 30 cm. Nontoxic white tempura paint was added to the water to render it opaque. Numerous large cues were placed around the room on all sides of the pool (see Epp et al., 2009). A camera mounted above the pool was connected to *Anymaze* tracking software (Stoelting Co; Wood Dale, IL, USA) which was used to record the distance to reach the platform during training, as well as the percentage of time spent in each quadrant and in the vicinity of the platform location during the probe trial.

2.3. Procedure

All rats were given a single intraperitoneal injection of BrdU (200 mg/kg; Sigma, Oakville, ON, Canada) on day 0 of the experiment. Then, rats were divided into three groups to be tested at three different time points, days 1–5 (Cue $n = 16$; Place $n = 16$), 6–10 (Cue $n = 8$ Place $n = 13$) or 11–15 (Cue $n = 12$; Place $n = 10$) after BrdU administration (see Table 1 for a timeline of the training procedure). Half of the rats at each time point were trained on the place (hippocampus-dependent) version of the task and half were trained on the cue (hippocampus-independent) version of the task. In the place version of the task, a hidden platform was located in the center of one quadrant of the pool, 2 cm below the surface of the water. In the cue version of the task, the top of the platform extended 2 cm above the surface of the water and moved randomly to the center of a new quadrant following each trial so that a spatial strategy could not be used. In either case, all rats were given one daily training session for 5 days with four trials per session starting from a different one of the four cardinal compass points on each trial.

Five days following the final training session all rats were given a single probe trial. During this trial the rats swam for 30 s in the same pool without an escape platform to assess retention of the platform location. We compared the amount of time spent in the quadrant of the pool that had previously contained the hidden platform (the target quadrant) as a measure of probe trial performance. We also compared the amount of time spent in a smaller area comprising 10% of the pool area centered over the platform location (the platform zone).

Two hours following the probe trial the rats were deeply anesthetized with sodium pentobarbital and then were perfused transcardially with 60 ml of 0.9% saline followed by 120 ml of 4% formaldehyde (Sigma) in 0.1 M phosphate buffered saline (PBS; Sigma). The brains were extracted and placed in 4% formaldehyde solution for 24 h and then were transferred into a solution containing 30% sucrose (Sigma) in 0.1 M PBS for at least four days. A Leica vibratome (VT1000S; Richmond Hill, ON, Canada) was used to section the brains. Throughout the rostral–caudal extent of the hippocampus 10 series of 40 μm sections were collected. The sections were then stored in antifreeze (ethylene glycol/glycerol; Sigma) at −20 °C until processing.

2.4. Immunohistochemistry

2.4.1. BrdU labeling

The tissue was rinsed three times and left overnight in 0.1 M PBS to remove the antifreeze solution. Tissue sections were incubated at room temperature in 0.6% H_2O_2 (Sigma) for 30 min followed by three rinses in 0.1 M PBS prior to a 30 min incubation

Table 1
Timeline of training and perfusion.

BrdU	MWT training	Probe trial/perfusion
Day 0	Days 1–5	Day 10
Day 0	Days 6–10	Day 15
Day 0	Days 11–15	Day 20