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Hippocampus-dependent strategy choice predicts low levels of cell proliferation in the dentate gyrus

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ABSTRACT

Neurogenesis continues to occur throughout life in the mammalian hippocampus. Previous research has suggested that the production of new neurons in the hippocampus during adulthood may be related to hippocampus-dependent learning and memory. However, the exact relationship between adult neurogenesis and learning and memory remains unclear. Here we investigated whether learning strategy selection is related to cell proliferation or to survival of new neurons in the hippocampus of adult male rats. We trained rats on alternating blocks of hippocampus-dependent (hidden platform) and hippocampus-independent (visible platform) versions of the Morris water task with the platform always in the same position. Following training, rats were given a probe session during which the platform was visible and in a novel location. Preferred strategy was determined by observing the initial swim path. Rats were classified as place strategy (hippocampus-dependent) users if they swam to the old platform location. Cue strategy (hippocampus-independent) users were classified as those rats that swam initially to the visible platform. Our results indicate that rats that preferentially used a place strategy had significantly lower cell proliferation than cue strategy users. However, there was no significant difference in cell survival or number of immature neurons between strategy user groups. These results suggest that low levels of cell proliferation in the dentate gyrus may be conducive or coincident with more efficient memory processing in the hippocampus.

1. Introduction

Different types of learning and memory are facilitated by the presence of multiple memory systems in the brain. It is widely acknowledged that the hippocampus plays an important role in spatial learning (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Whishaw, & Kolb, 1983) and that the dorsal striatum and substantia nigra pars-compacta are important for response learning (Cook & Kesner, 1988; Da Cunha et al., 2006). Double dissociations between these structures and types of learning indicate that these are parallel learning systems (McDonald & White, 1993; Packard & McGaugh, 1992, 1996).

McDonald and White (1993) demonstrated that a triple dissociation exists between the functions of the hippocampus, amygdala and dorsal striatum. In that study rats were tested on a win-shift task, a win-stay task and a conditioned cue preference task. Hippocampus damage disrupted performance only on the win-shift task, while striatal damage impaired performance only on the win-stay task and amygdala damage impaired acquisition only on the conditioned cue preference. This study elegantly demonstrated that different neural systems can act independently in the acquisition of various types of information.

Cue competition paradigms in which rodents are trained in the same task to perform both spatial and response learning have allowed further insight into the relative contribution of each system (Devan & White, 1999; McDonald & White, 1994). Research of this type illustrates that in the intact brain, the various memory systems do not always operate completely independently. Instead the memory systems interact through both cooperative and competitive mechanisms so that for a given memory type, the most efficient system (or combination of systems) is used (Epp et al., 2008; Maren, Aharonov, & Fanselow, 1997). When spatial information is required for a task solution the hippocampus is more active whereas when stimulus-response learning is required the striatum is more active (Miranda, Blanco, Begega, Rubio, & Arias, 2006). In tasks where either spatial or response strategies can be utilized there is evidence that approximately 50% of rats will use a spatial strategy while the others will preferentially use the response strategy (Devan & White, 1999; McDonald & White, 1994). Given that response learning is mediated by the dorsal striatum and spatial learning is mediated by the hippocampus it is reasonable to assume that damage to one of these structures would shift the percentage of rats that use each strategy. Previous research has shown that hippocampus (Packard & McGaugh, 1996) or fornix le-

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sions (Devan & White, 1999; McDonald & White, 1994) result in nearly all rats using a response strategy and dorsal striatum lesions produce the opposite effect with nearly all rats using a spatial strategy (Devan & White, 1999; McDonald & White, 1994). The shift in strategy use associated with damage to one of the memory systems is theoretically straightforward. However, what is less clear is why certain intact animals preferentially utilize the response versus place strategy. It may be the case that the contribution of each structure is an indicator of the relative efficiency of each system. For example, response strategy users may have a dorsal striatum memory system that functions more efficiently than the hippocampus memory system with the opposite being true in place strategy users.

One possible modulator of hippocampal efficiency is the rate of neurogenesis in the hippocampus of adults. It is now well accepted that neurogenesis continues to occur throughout life in the mammalian hippocampus (Altman & Das, 1965; Cameron, Woolley, McEwen, & Gould, 1993; Eriksson et al., 1998; Kaplan & Hinds, 1977). It has been demonstrated that adult generated neurons develop mature neuronal phenotypes and exhibit similar functional properties compared to the existing mature neurons (Cameron et al.,1993; Hastings & Gould, 1999; Markakis & Gage, 1999; van Praag et al., 2002). The exact function that new neurons play in the adult hippocampus remains unclear and controversial but there is mounting evidence for some type of role in hippocampus-dependent learning and memory (Epp, Spritzer, & Galea, 2007; Gould, Beylin, Tanapat, Reeves, & Shors, 1999). Adult neurogenesis is comprised of at least two different aspects, cell proliferation and cell survival, both of which have been implicated in hippocampus-dependent learning and memory. Evidence of an interaction between learning and adult neurogenesis has been provided by a number of studies that indicate spatial learning increases the number of adult generated neurons that survive to maturity (Ambrogini et al., 2000; Epp et al., 2007; Gould et al., 1999; Leuner et al., 2004), although this has not been universally shown. Ambrogini and colleagues (2004) found that spatial learning decreased the survival of new neurons relative to non-spatial learning or naïve rats. Several recent papers have indicated that not only task training but specifically the quality of learning determines in part the number of surviving cells (Dobrossy et al., 2003; Epp et al., 2007; Sisti, Glass, & Shors, 2007).

Considerably less work has focused on cell proliferation and learning interactions but there is some evidence of a relationship. Recently, Pham, McEwen, Ledoux, and Nader (2005) showed that acquisition of an association between a shock and context results in a decrease in cell proliferation in the hippocampus compared to control animals. There is little evidence however, that proliferation is altered by or required for spatial learning, an important function of the hippocampus. A recent study has shown that spatial learning results in an increase in Ki67, an endogenous marker of cell proliferation, once asymptotic performance is reached (Dupret et al., 2007). However, this was in comparison to a naïve control group and thus the differences may be attributed to factors other than learning such as exercise.

It is difficult to draw conclusions regarding the importance of neurogenesis for hippocampus-dependent learning due to the number of conflicting findings. There are likely numerous methodological differences that may account for some of the equivocal results in the literature concerning adult neurogenesis and spatial learning (Epp et al., 2007; Taupin, 2007). However, one possible explanation may be a failure to account for individual differences in the way that an animal learns. Past studies have not addressed whether there is a difference in cell proliferation or survival in animals that prefer a place strategy versus a cue strategy. Here we examine whether the efficiency of the hippocampus, as determined by whether rats choose a place strategy in a cue competition task, is related to the level of adult neurogenesis, both cell proliferation and cell survival, in the hippocampus. To do so, rats were trained to find both a visible and hidden platform in a fixed location in a swimming pool and then given a probe trial to determine the preferred strategy. We examined whether measures of cell proliferation and cell survival in the hippocampus related to strategy use to determine whether these neurogenic measures were related to the use of different behavioral strategies.

2. Materials and methods

2.1. Subjects

Subjects were 29 male Long-Evans hooded rats (Charles River, Saint-Constant Quebec), 70 days of age and weighing between 300–350 g at the start of testing. All procedures were carried out in accordance with the Canadian Council for Animal Care guidelines and were approved by the University of British Columbia animal care committee. Rats were housed individually in standard cages with *ad libitum* access to food and water. Upon arrival rats were allowed to habituate to the colony for one week. Each rat was then handled for 5 min per day for 5 days prior to behavioral testing.

2.1.1. Apparatus

A circular swimming pool, 180 cm in diameter was filled to a depth of 30 cm with room temperature water (approximately 21 °C). White non-toxic paint was added to make the water opaque. A camera centered above the pool was connected to a computer running ANY-maze video tracking system (Stoelting; Wood Dale IL) and was used to analyze the movement of rats in the pool. The pool was surrounded by distinctive distal cues that could be used for place navigation.

2.1.2. Procedure

Twenty of the rats were exposed to the training procedure. A platform 10 cm in diameter was placed in the center of the northeast quadrant of the pool. The platform extended 2 cm above the surface of the water during the first and second daily sessions. On the third day of training the platform was submerged 2 cm below the surface of the water but remained in the same position. This procedure was repeated twice more so that each rat received 6 days of training with the visible platform and 3 days with the hidden platform. Each session was comprised of four trials, one starting from each of the four cardinal compass points in a pseudo-random order each day. On day 10, the platform was moved to the middle of the southwest quadrant and was extended above the surface of the water. Two probe trials were conducted during which, the rats were released from the two points equidistant from the original platform location and the new platform location. Rats were classified as preferring the cue strategy or place strategy based on their swim path during the probe trials. Rats that swam within 10 cm of the original platform location on one of the two probe trials were considered place strategy users. Those that made a direct path towards the visible platform in its new location were considered cue strategy users. A third group was also classified as ambiguous because they first entered the original training quadrant but did not approach the platform area. An additional nine cage control rats were housed and handled in the same fashion but did not receive any behavioral training.

2.1.3. Immunohistochemistry

On day 11, rats were given a lethal overdose of sodium pentobarbital and were then perfused transcardially with 60 ml of 0.1 M phosphate buffered saline (PBS) followed by 120 ml of 4%