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WORKING MEMORY TRAINING DECREASES HIPPOCAMPAL NEUROGENESIS

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Abstract—The relationship between adult hippocampal neurogenesis and cognition appears more complex than suggested by early reports. We aimed to determine if the duration and task demands of spatial memory training differentially affect hippocampal neurogenesis. Adult male rats were trained in the Morris water maze in a reference memory task for 4 days, or alternatively working memory for either 4 or 14 days. Four days of maze training did not impact neurogenesis regardless of whether reference or working memory paradigms were used. Interestingly, 2 weeks of working memory training using a hidden platform resulted in fewer newborn hippocampal neurons compared with controls that received either cue training or no maze exposure. Stress is a well-established negative regulator of hippocampal neurogenesis. We found that maze training in general, and a working memory task in particular, increased levels of circulating corticosterone after 4 days of training. Our study indicates that working memory training over a prolonged period of time reduces neurogenesis, and this reduction may partially be mediated by increased stress.

Key words: BrdU, Morris water maze, reference memory, working memory, corticosterone.

The discovery of adult hippocampal neurogenesis has stimulated efforts to understand the relationship between the newborn cells and hippocampus-dependent learning and memory. In a highly cited study, Gould et al. (1999) demonstrated increased survival of newborn hippocampal neurons in rats subjected to spatial learning in the Morris water maze. In contrast, van Praag et al. (1999) did not observe any changes in neurogenesis following water-maze training in mice. More recent studies have confirmed the complex nature of the phenomenon, since both decreases and increases in neurogenesis have been reported with water maze learning (Ambrogini et al., 2000, 2004; Van der Borght et al., 2005; Ehninger and Kempermann, 2006). These differences in results may depend on the temporal relationship between labeling of newborn neurons and exposure to the memory task (Dobrossy et al., 2003). The aim of the present study was to investigate whether training in different hippocampus-dependent spatial memory tasks for varying lengths of time differentially affects hippocampal neurogenesis in adult rats.

Given the controversy surrounding the impact of learning on hippocampal neurogenesis, we replicated one aspect of the experimental design of Gould and colleagues (1999) to determine whether 4 days of spatial reference memory training would increase hippocampal neurogenesis as suggested by their study. We found that all rats trained in the Morris water maze were able to learn the reference memory task over the 4-day training period, as revealed by the progressively shorter escape latencies to find the submerged (place) platform (Fig. 1A). The escape latency profiles were virtually identical to those reported in the study by Gould and coworkers (1999). In contrast to that study, which reported close to a threefold increase in the number of BrdU-positive cells in the hippocampus of rats who underwent training in the Morris water maze, we observed no difference between the groups (Table 1). In our study, approximately 90% of the BrdU-labeled cells co-expressed the mature neuronal marker NeuN, with no differences between rats that had experienced hippocampal learning (place) versus those that had a non-hippocampal learning experience (cue) in the maze. To make a more specific assessment of the effects of learning on cell proliferation, we studied an endogenous marker of cell division, Ki-67, and again found no significant differences between groups (Table 1). We were unable to replicate the finding of increased neurogenesis after spatial reference memory training made by Gould and collaborators (1999), despite the fact that we used the same strain, age and gender of rats and employed the same experimental design. Using slightly different designs, other research groups have also reported that there is no effect on neurogenesis of water maze training using a reference memory task (van Praag et al., 1999; Van der Borght et al., 2005). This further suggests that if reference memory can increase neurogenesis, it is not a very robust phenomenon and may be sensitive to very subtle environmental influences.

In parallel to the 4-day reference memory assignment, we also employed a spatial working memory task to determine whether other aspects of learning influence hippocampal neurogenesis. Note that a reference memory task assesses the ability to remember an event that remains constant, which is achieved by maintaining the position of the hidden platform in the same spatial location throughout maze training. A working memory task taps into a more short-term form of memory, as it requires the ability to remember a consistent response rule, but with a trial-

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Abbreviations: BrdU, bromodeoxyuridine; GCL, granule cell layer; S.E.M., standard error of the mean.

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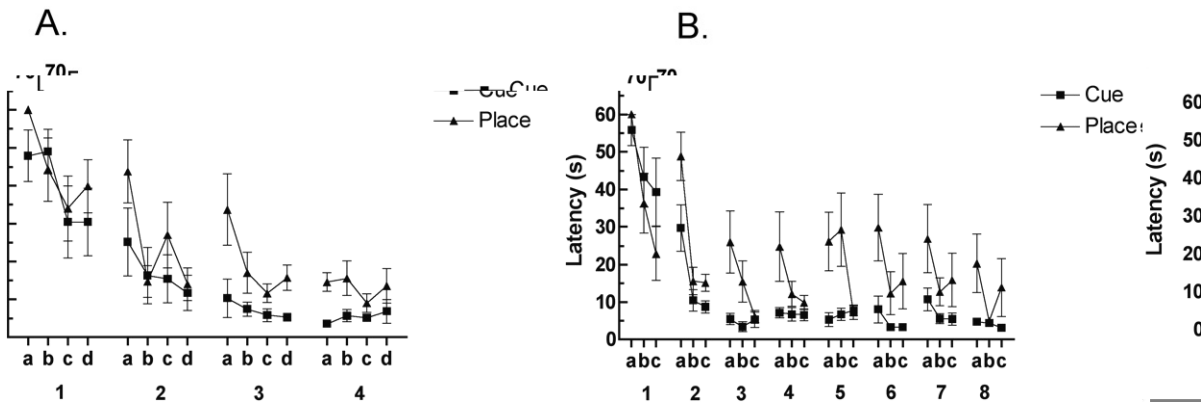

Fig. 1. Morris water maze escape latencies for cue (visible platform) and place (submerged platform) training. (A) Reference memory performance for 4-day learners. (B) Working memory performance for 4-day learners. Latencies (s) to find the platform are expressed as mean±S.E.M.

specific event exclusively determining the correct response for every particular session of training. We found that rats undergoing working memory training showed the characteristic relearning of the task each time the platform was moved, as demonstrated by the longer escape latencies on the first trial of each session (Fig. 1B).

With the 4-day working memory training, rats subjected to the hippocampus- dependent learning experience (place) exhibited a non-significant ($P=0.06$; Table 1) trend toward fewer BrdU-positive cells in the hippocampus as compared with no maze exposure control rats. Again, 90% of the BrdU-positive cells co-expressed NeuN. Rats subjected to cue or place training had fewer Ki-67-positive cells in the granule cell layer (GCL) compared with the no maze controls (Table 1, $F_{2,16}=4.1$, $P<0.05$; Fisher's PLSD, cue vs. no maze, $P<0.05$). Moreover, the apoptotic marker TUNEL revealed a significant increase in the number of dying cells in the hippocampus of maze learners compared with controls not exposed to the maze (0.67±0.22 vs. 1.9±0.58 vs. 2.6±0.2 TUNEL cells per section for no maze controls, cue learning, and place learning, respectively, $P<0.05$).

When we subjected rats to 14 days of working memory training, we observed significantly fewer BrdU-positive cells (Fig. 2) in those that had undergone hippocampus-dependent place learning compared with either cue training or no maze exposure (Table 1, $F_{2,20}=3.7$, $P<0.05$, Fisher's PLSD, place vs. cue or no maze, $P<0.05$). Again, 90% of the BrdU-labeled cells co-expressed NeuN in all groups. The place group had significantly fewer Ki-67-expressing cells than the no maze group ($P<0.05$) The place and the cue groups did not significantly differ regarding numbers of Ki-67-positive cells, although there was a trend for a reduction in newborn, Ki-67-positive neurons in the place group (Table 1, $F_{2,20}=2.8$, $P=0.08$). Note that Ki-67 is expressed in cells undergoing division at the time of perfusion, whereas BrdU is available for uptake and incorporation into cells in the S-phase of mitotic division at the time of injections.

Taken together, our data suggest that cell proliferation in the dentate gyrus is decreased after 4 and 14 days of place working memory training. One factor that could account for reductions in hippocampal neurogenesis is the acute stress associated with the training, which previously has been shown with maze training (Beiko et al., 2004), an effect that is attenuated after 5 days of intense reference memory training (Aguilar-Valles et al., 2005).

Table 1. Cell counts and corticosterone values

	Reference memory			Working memory				
	Control 4 d	Maze 4 d		Maze 4 d		Control 14 d	Maze 14 d	
	No maze	Cue	Place	Cue	Place	No maze	Cue	Place
N	7	7	7	7	7	8	7	8
BrdU	113±11	114±6.4	112±9.6	98±3.4	82±15	52±5.4	50±5.4	36±2.6*
Ki-67	60±4.8	64±5.6	61±5.1	46±2.0*	50±3.7	59±6.0	49±5.3	43±3.1*
Corticosterone	−67±82	240±76*	523±24**	581±61**	509±114**	ND	ND	ND

All cell numbers are expressed as numbers of positive cells per bilateral dentate GCL layer/section. Approximately 90% of all BrdU-labeled cells express the marker for mature neurons NeuN, regardless of training paradigm, with no significant group differences. Corticosterone values represent the difference in serum levels (ng/ml) after and before the event of water maze training. All values are presented as mean±S.E.M. Statistical analyses were made using ANOVA, Fisher's PLSD was applied for post hoc analyses. All 4 day experiments were performed simultaneously. The 14 d experiments were performed at a separate occasion. Abbreviations: Cue, maze training using a visible platform; ND, not determined; No maze, no maze training; Place, maze training using a hidden platform.

* $P<0.05$.

** $P<0.005$.

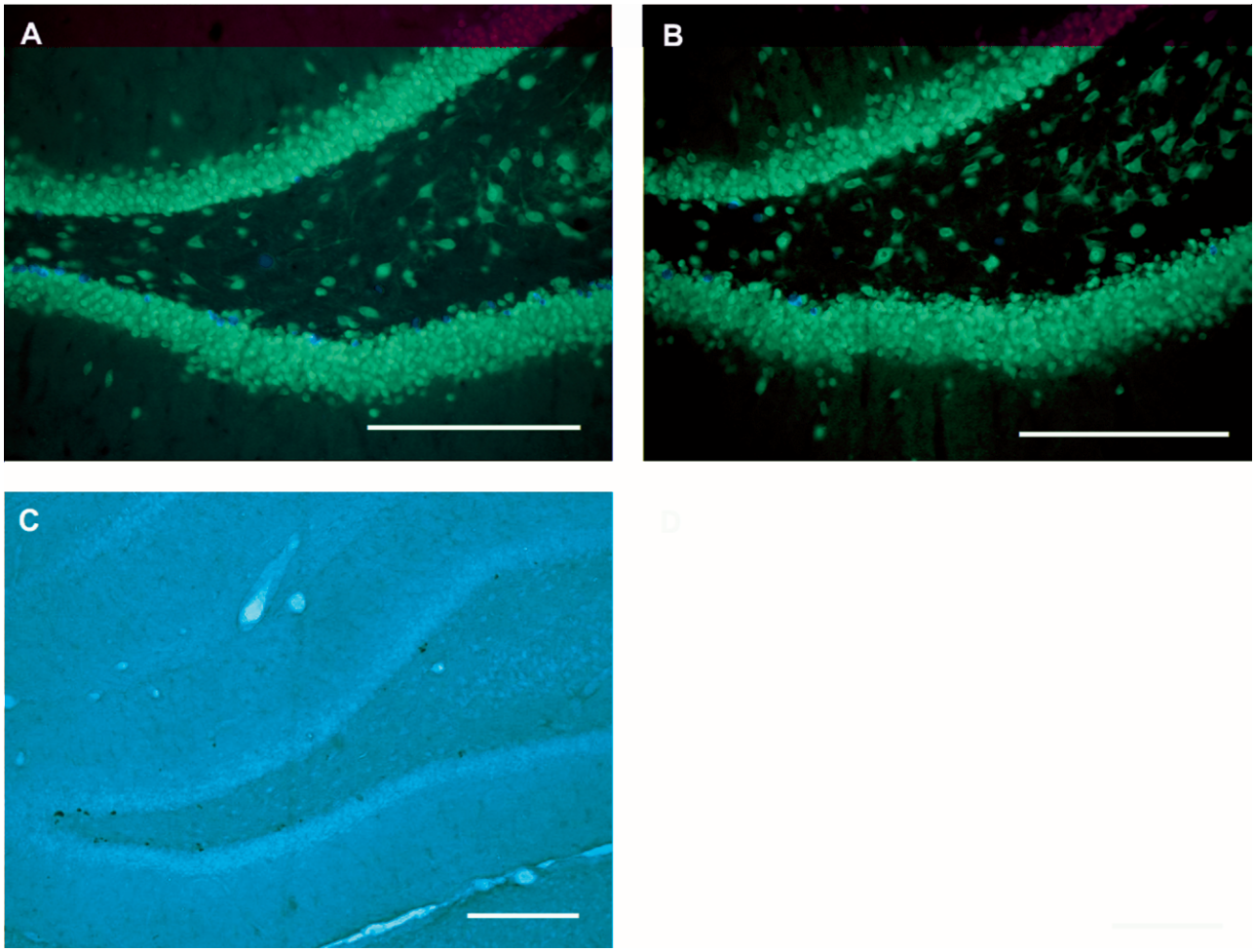

Fig. 2. Working memory training decreases neurogenesis after 14 days of training. BrdU (red) and NeuN (green) double immunohistochemistry of a rat subjected to no maze (A) or working memory training (B). Representative micrograph of Ki-67 (C). Scale bars=400 μm.

Ehninger and Kempermann (2006) report that training mice in the Morris water maze before exposing them to a reference memory test can abolish the reduction in hippocampal neurogenesis otherwise seen after the reference memory task. Whether this is due to less stress following pre-training is unclear, as indicators of stress were not assessed. Another possible confounding variable is that physical activity might directly impact hippocampal neurogenesis. However, earlier studies suggest no effect on neurogenesis of involuntary activity, such as swimming in a water maze (van Praag et al., 1999; Ehninger and Kempermann, 2006). Whereas the aforementioned studies focused on how performing tasks requiring learning and memory impact neurogenesis, other experiments have examined how experimental manipulations of hippocampal neurogenesis alter performance in spatial memory tasks. Thus, several studies indicate that decreased hippocampal neurogenesis leads to impaired spatial reference memory (Madsen et al., 2003; Rola et al., 2004) and one study even suggests that new neurons are required for long-term spatial memory (Snyder et al., 2005). It is not clear whether the same is true for spatial working memory.

As working memory training is a more challenging task than reference memory, it might be more stressful. To test for this hypothesis, we examined serum levels of the stress hormone corticosterone after the 4-day reference and working memory training. When comparing the change in corticosterone levels before and after water maze training we found that all training experiences in the maze resulted in significant elevations of corticosterone levels (Table 1). The working memory tasks tended to produce significantly greater elevations of circulating corticosterone. However, these elevations were seen also with visual cue training rather than just the hippocampus-dependent place training, suggesting that training in general but not learning per se is stressful. Therefore, the selective decrease in hippocampal neurogenesis in the group subjected to working memory training using the hippocampus-dependent place task cannot be fully accounted for by increased stress hormone production. One might speculate that new neurons interfere with the formation of short-term memories, and therefore they are not recruited to the mature hippocampal circuitry. That could explain why we found fewer surviving new neurons in animals that had been trained to

Fig. 3. Experimental design. Abbreviations: B, retroorbital blood collection; I, BrdU injections (50 mg/kg×4); P, perfusion; P/B, perfusion and blood collection; RM, reference memory; WM, working memory.

repeatedly form new short-term memories. A recent study shows that increased numbers of new hippocampal neurons after enriched environment may not be necessary for improved spatial learning (Meshi et al., 2006).

We conclude that repeated hippocampus-dependent spatial working memory training induces a response, partly stress-mediated, which reduces neurogenesis in the adult rat hippocampus.

EXPERIMENTAL PROCEDURES

Adult male Sprague–Dawley rats weighing 300–350 g at the beginning of the study were used. Animal handling and surgical procedures were carried out according to the regulations of the animal ethical committee at Lund University. All experiments followed the Public Health Service Guide for the Care and Use of Laboratory Animals. The study was designed to minimize the number of animals used and their suffering.

Bromodeoxyuridine (BrdU; Sigma-Aldrich, St. Louis, MO, USA) administration and immunohistochemical techniques have been described in detail elsewhere (Mohapel et al., 2004, 2005). All microscopic analyses were performed on coded slides, to which the investigator was blinded. All BrdU-positive cells in the granule cell layer (GCL), including the subgranular zone, of the dentate gyrus were counted in five equally spaced sections per animal throughout the dorsal hippocampus (−3.14 to −4.5 mm relative to bregma) using epifluorescence microscopy with a 40× objective. TUNEL- and Ki-67-positive cells were counted in the same manner using light microscopy. Cell numbers are expressed as average per section±standard error of the mean (S.E.M.).

Laser scanning confocal microscopy (Leica TCS, SL; Leica, Wetzlar, Germany) was applied to determine whether a BrdU-positive cell co-expressed NeuN or not, using orthogonal reconstructions derived from 1 μm z series at 63× magnification. Fifty BrdU-positive cells per animal were scanned in the GCL of the dentate gyrus. Statistical analyses were made using analysis of variance (ANOVA).

Water maze training was conducted in a cylindrical (140 cm diameter) tank filled with 22 °C water, made opaque using milk powder. For each experimental paradigm either a visible platform (cue test) or a hidden platform (place test), submerged 3 cm below the water surface, was used. For the 4-day training experiment, groups of rats were trained on either the reference memory (Gould et al., 1999) or working memory task. The working memory training employed a new platform location and release point for every block of trials, where two blocks of three trials were given daily. The swim path and latency to find the platform were recorded for each trial. A group of matched control rats did not receive any maze exposure. Maze training was initiated 1 week after BrdU injections (Fig. 3). The 14-day experiment was performed at a different occasion and maze testing was initiated 1 day following BrdU injections (Fig. 3). All groups were perfused directly after the last training session (N=6–8 per group).

Blood was sampled from half of the rats in each group exposed to water maze training for 4 days. First, blood was drawn retroorbitally under brief isoflurane anesthesia to obtain a baseline value for corticosteroid levels 1 day before water maze testing. Second, a sample was taken from the right auricle of the heart, immediately after the last water maze session was completed, in conjunction with perfusions. All blood samples were drawn simultaneously in the water maze and "no maze" groups, using the same method, at approximately the same time point in the afternoon. Serum was analyzed for corticosterone using a radioimmunoassay.

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