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Anatomical Gradients of Adult Neurogenesis and Activity: Young Neurons in the Ventral Dentate Gyrus Are Activated by Water Maze Training

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ABSTRACT: Hippocampal function varies in a subregion-specific fashion: spatial processing is thought to rely on the dorsal hippocampus, whereas anxiety-related behavior relies more on the ventral hippocampus. During development, neurogenesis in the dentate gyrus (DG) proceeds along ventral to dorsal as well as suprapyramidal to infrapyramidal gradients, but it is unclear whether regional differences in neurogenesis are maintained in adulthood. Moreover, it is unknown whether young neurons in the adult exhibit subregion-specific patterns of activation. We therefore examined the magnitude of neurogenesis and the activation of young and mature granule cells in DG subregions in adult rats that learned a spatial water maze task, swam with no platform, or were left untouched. We found that both adult neurogenesis and granule cell activation, as defined by c-fos expression in the granule cell population as a whole, were higher in the dorsal than the ventral DG. In contrast, c-fos expression in adult-born granule cells, identified by PSA-NCAM or location in the subgranular zone, occurred at a higher rate in the opposite subregion, the ventral DG. Interestingly, c-fos expression in the entire granule cell population was equivalent in water maze-trained rats and swim control rats, but was increased in the young granule cells only in the learning condition. These results provide new evidence that hippocampally-relevant experience activates young and mature neurons in different DG subregions and with different experiential specificity, and suggest that adult-born neurons may play a specific role in anxiety-related behavior or other nonspatial aspects of hippocampal function. © **2008 Wiley-Liss, Inc.**

KEY WORDS: dorsal; spatial memory; hippocampus; immediate-early gene; c-fos

INTRODUCTION

A large number of new neurons are added to the dentate gyrus (DG) of adult mammals (Cameron and McKay, 2001). There is considerable evidence that these adult-born granule neurons are physiologically functional (Wang et al., 2000; Snyder et al., 2001; van Praag et al., 2002; Esposito et al., 2005; Ge et al., 2006) and contribute to hippocampus-dependent behaviors (Shors et al., 2001, 2002; Santarelli et al., 2003; Snyder et al., 2005; Saxe et al., 2006, 2007; Winocur et al., 2006). Despite the wide range of behaviors that appear to require new neuron function, little is known about the specific contribution of new neurons to hippocampal function.

Functional gradients have been shown to exist within the hippocampus. Based on partial lesion and regional inactivation studies, the predominant view is that the dorsal hippocampus is particularly critical for spatial learning, whereas the ventral hippocampus is involved in regulating fear and anxiety (Moser et al., 1995; Kjelstrup et al., 2002; Bannerman et al., 2004; Pothuizen et al., 2004; Pentkowski et al., 2006). Other studies have suggested that the entire hippocampus is involved in processing spatial information but that the role of the ventral region is different from that of the dorsal region (Jung et al., 1994; de Hoz et al., 2003; McDonald et al., 2006). Thus, although their exact roles are not entirely agreed upon, it does seem clear that the dorsal and ventral regions contribute in different ways to hippocampus-dependent behaviors. The DG can also be divided into suprapyramidal and infrapyramidal "blades," which lie dorsolaterally and ventromedially, respectively. Blade-dependent differences in excitability, GABAergic inhibition and exploration-induced arc expression (Scharfman et al., 2002; Chawla et al., 2005; Ramirez-Amaya et al., 2005) indicate that cells in the two blades also contribute to different aspects of hippocampal function.

Comparisons across anatomically-defined regions of the hippocampus may provide an approach for understanding new neuron function (Sahay and Hen, 2007). Most studies of adult neurogenesis have either examined a small portion of the DG or have used stereological methods to quantify the total number of new neurons throughout the entire DG. Therefore, unlike developmental neurogenesis (Schlessinger et al., 1975), little is known about anatomical gradients of adult neurogenesis. In the present study, we examine anatomical gradients of adult neurogenesis and use immunohistochemistry for the immediate-early gene c-fos (Fos) to compare activation rates in different subregions, in young granule cells as well as in the

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overall granule cell population. Fos is expressed in an activity-dependent manner and has been used as a marker for activated neurons in several studies of granule cells in the DG (Worley et al., 1993; Vann et al., 2000; Countryman et al., 2005), including adult-born granule cells (Jessberger and Kempermann, 2003; Kee et al., 2007; Tashiro et al., 2007). Here we find that young and mature granule cells show different anatomical gradients of activation and different specificity for the experiences that increase Fos expression.

METHODS

Animals and Treatments

Fourteen adult (10 weeks old at the start of experiments) 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 two injections of 5-bromo-2′-deoxyuridine (BrdU; Sigma, 200 mg/kg/injection, dissolved at 20 mg/ml in saline, 0.007 N NaOH) spaced 10 h apart (Cameron and McKay, 2001; Snyder et al., 2005). Beginning 3 weeks after BrdU injections, all 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 (see 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.

Water Maze

On the final day of the experiment, eight 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 facility 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 nontoxic 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 trained in pairs for 16 trials. Each trial lasted 60 s, and rats were allowed to remain on the platform for 10 s after finding it. If a rat failed to locate the platform by the end of the trial, it was guided there by the experimenter. Trials were 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 hypothetical "platform" 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 (four 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.

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 and sectioned perpendicular to its long axis to enable comparable analyses along the entire axis (Gaarskjaer, 1978; Rapp and Amaral, 1988). This axis is most precisely described as septo-temporal, but it will be referred to here as the dorso-ventral axis, as this terminology is more commonly used in studies of hippocampal function. 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 or PSA-NCAM, on free floating sections. For BrdU/Fos double-immunolabeling, sections were first incubated with rabbit anti-Fos antibody (1:10,000; Calbiochem; 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 1 N 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; 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 done similarly, using rabbit anti-Fos (1:10,000; Calbiochem; 3 days at 4°C) then Alexa568 goat anti-rabbit followed by mouse anti-PSA-NCAM (1:200, Chemicon; 3 days at 4°C) then Alexa488 goat anti-mouse. All antibodies were diluted in phosphate-buffered saline containing 0.03% Triton X-100. One rat in the water maze-trained group had poor immunolabeling and was excluded from histological analysis.

Cell Quantification

For regional comparison, sections were assigned to one of four equal bins along the dorso-ventral length of the DG (D1 = dorsal, D2 = mid-dorsal, V3 = mid-ventral, V4 = ventral; see Fig. 2 insets). Within each section, attention was paid to

TABLE 1.

Dorso-Ventral Gradients in Populations of Adult-Born Granule Cells Identified Using Different Methods

		Granule cells born in adulthood		
Method	All granule cells	BrdU	PSA-NCAM	SGZ
Age of population	0–15 weeks	4 weeks	<4 weeks	≈0–6 weeks
Size of population (cells/mm^3)	400,000	3,400	15,000	66,000
Relative size of population		1×	4.4×	19×
More neurogenesis		Dorsal	Dorsal	
Higher Fos density	Dorsal		Ventral	Ventral
Greater increase in Fos	Equal		Ventral	
Activated by water maze	Yes		Yes	
Activated by swim control	Yes		No	

whether cells were located within the infrapyramidal or suprapyramidal blade of the DG.

Three different methods of identifying adult-born granule neurons were used in this study, in order to optimize the population size for each analysis and provide independent confirmation of findings with different detection methods (summarized in Table 1).

Quantification of Fos+ and BrdU+ cells was performed on every 10th section throughout the dorso-ventral extent of the DG (19–22 sections per rat). Nuclei were counted as positive for Fos expression if the Fos signal stood out clearly when compared with the surrounding tissue and if the staining evenly filled the entire nucleus. BrdU and Fos were quantified in the same sections. Analysis of Fos staining in PSA-NCAM+ cells was performed on every 5th section in the D1 and V4 quartiles. Every BrdU+ and PSA-NCAM+ cell was examined for Fos expression with an epifluorescence microscope (Olympus BX51) and a 60× oil immersion lens (N.A. 1.25). Potential double-labeled cells were then further examined using confocal microscopy (Olympus FV300) to confirm or reject colocalization of the two labels. To enable the objective characterization of a PSA-NCAM+ cell as Fos-positive or Fos-negative, the brightness of the Fos signal was measured as follows. At the middle focal plane of the cell, where Fos staining was brightest, the intensity of the nuclear Fos signal was measured and compared with the background intensity, measured in a random larger region of the hilus in the same optical field that appeared devoid of Fos staining. Cells with Fos staining brighter than 1.2× background were considered positive. This cutoff was chosen to approximate the staining intensity judged positive by eye. PSA-NCAM+ cell density (irrespective of Fos staining), which is labor-intensive due to the large number of cells with intertwined processes, was measured in two sections per animal—one from the middle of D1 (dorsal pole) and one from the middle of V4 (ventral pole). Total granule cell density was calculated using the optical fractionator method (West, 1993) in three Hoechst33258-counterstained sections (spaced 240 μm apart) from the middle of D1 and of V4. Total granule cell number per section was estimated by counting cells at 60× with a 15 μm × 15 μm counting frame and 175 μm × 80 μm sampling grid, using Stereoinvestigator software (Microbrightfield). For all analyses, cross-sectional area of the granule cell layer was measured at 4× and multiplied by the section thickness (40 μm) to yield region volumes. Cell densities were then determined by dividing the number of cells in each region of the granule cell layer by its respective volume.

To characterize activity in a broader population of adult-born cells, we calculated the proportion of Fos+ cells that were located in the subgranular zone (SGZ), defined for this purpose as the deepest row of granule cells in the granule cell layer, bordering the hilus. The granule cell layer follows an outside-in gradient of formation that does not produce strict layering of granule cells by age but nonetheless results in the deepest portion of the granule cell layer containing a high proportion of granule cells that are young (Crespo et al., 1986; Dayer et al., 2003; Kempermann et al., 2003; Seri et al., 2004) and possess immature electrophysiological properties (Wang et al., 2000). The number of Fos+ cells in the subgranular zone and the total number of Fos+ cells in the granule cell layer (subgranular zone plus other layers) were counted in two sections per animal, one from the middle of D1 and one from the middle of V4 and the percentage of Fos+ cells in the subgranular zone was calculated ($100 \times Fos_{SGZ}/Fos_{total}$). The percentage of Fos+ cells in the subgranular zone was compared across subregions (D1 vs. V4) and was also compared, within each subregion, to the chance value expected if Fos+ was distributed equally in all layers (= 100/No. of layers).

Statistical Analysis

Statistical analyses were performed using Systat software (http://www.systat.com). Comparisons were made using 2-way or 3-way analysis of variance (ANOVA) and Tukey's HSD for post hoc analyses, with significance set at $P < 0.05$.

RESULTS

Water Maze

Trained rats showed a decreased latency to find the hidden platform over 16 trials in the water maze (four blocks of four

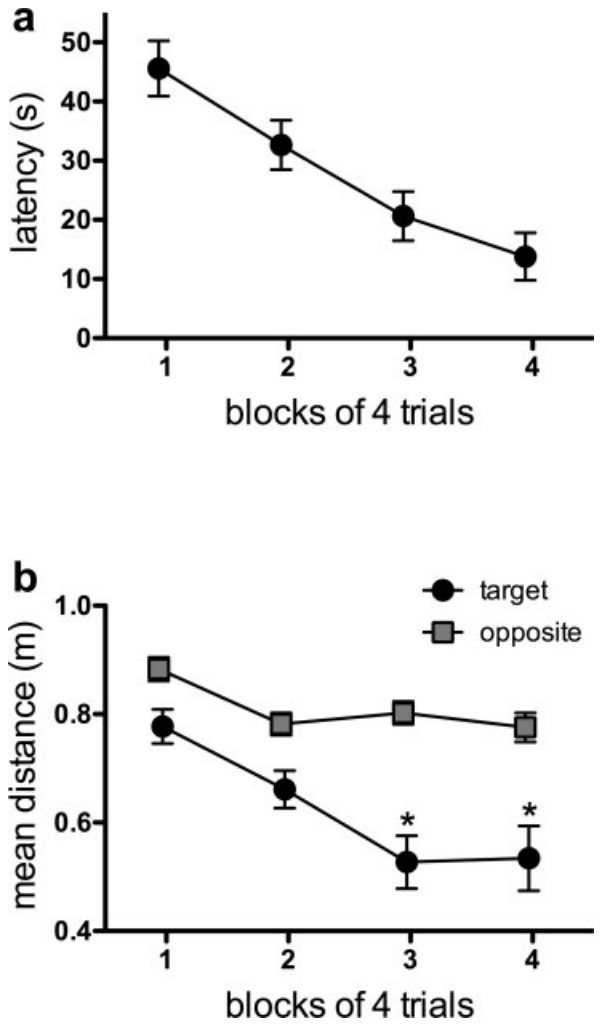

FIGURE 1. Water maze training. (a) In a single 16-trial training session, rats learned to quickly locate the hidden platform (repeated measures ANOVA, main effect of block, $P < 0.0001$). (b) Rats developed a spatial bias, as seen by significant main effects of block and distance from platform (2-way repeated measures ANOVA, both $P < 0.001$). There was also a block × distance interaction such that, in blocks three and four, the mean distance to the target (correct) platform was significantly shorter than the distance to the equivalent location in the opposite quadrant of the pool (*, $P < 0.0001$).

trials) (Fig. 1a; One-way repeated measures ANOVA, main effect of block, $F_{3,31} = 22$, $P < 0.0001$). There were significant differences between all blocks in the latency to find the platform (post hoc, $P < 0.05$) except for blocks three and four. To verify that decreases in latency reflected spatial search strategies, the mean distance to the platform location, a measure of spatial bias (Gallagher et al., 1993), was also compared across the trials. Two-way repeated measures ANOVA, with block and platform location as factors, showed significant main effects of block ($F_{3,42} = 16$, $P < 0.0001$), platform location ($F_{1,42} = 26$, $P = 0.0002$) and a block × platform location interaction ($F_{3,42} = 4.5$, $P < 0.01$; Fig. 1b). Rats had a significant spatial bias towards the correct, target platform location on blocks three and four: the mean distance to the platform location was significantly less than the distance to the equivalent location in the opposite quadrant of the pool ($P < 0.001$; Fig. 1b).

Regional Differences in Neurogenesis

The density of 4-week-old neurons, labeled with BrdU, was compared across dorso-ventral position (see Methods) and blade. BrdU+ cell counts were done only in water maze-trained rats rather than comparing across treatment groups, because BrdU+ cells were 4 weeks old, an age at which young granule cells are no longer susceptible to cell death (Dayer et al., 2003; Kempermann et al., 2003). In addition, rats were treated identically until 2 h before perfusion, and this interval is almost certainly too short to see a differential survival effect (Olariu et al., 2005). Two-way ANOVA showed a significant main effect of dorso-ventral position on the density of BrdU+ cells ($F_{3,40} = 6.4$, $P = 0.001$; Figs. 2 and 3). Post hoc analysis showed that the dorsal DG had significantly more neurogenesis than the ventral DG: both dorsal quartiles (D1&D2) had ~70% higher BrdU+ cell density than the ventral-most quartile (V4; $P < 0.01$ in both cases). There was also a significant main effect of blade, with BrdU+ cell density significantly higher in the infrapyramidal blade than in the suprapyramidal blade ($F_{1,40} = 8.1$, $P < 0.01$; Fig. 3). There was no blade × dorso-ventral interaction ($F_{3,40} = 0.7$, $P = 0.6$).

Because differences in BrdU+ cell density were most pronounced between the dorsal-most and ventral-most quartiles, we focused on these two regions for our analysis of the endogenous marker of young neurons, PSA-NCAM. As for BrdU+ cell analysis, only water maze-trained rats were examined, because the post-treatment survival interval was too short to expect treatment effects on the number of young neurons. A two-way ANOVA on PSA-NCAM+ young neuron density in regions D1 and V4 found no significant effect of blade but found a significant effect of dorso-ventral position on PSA-NCAM+ cell density; PSA-NCAM+ cell density was higher in the dorsal DG than in the ventral DG ($F_{1,24} = 6.5$, $P = 0.02$; Fig. 3b). Thus, the dorso-ventral gradient of neurogenesis is similar whether measured in four-week-old BrdU+ cells or in immature PSA-NCAM+ cells.

Equivalent Overall Granule Cell Density in Different Subregions

Greater BrdU+ and PSA-NCAM+ cell density in the dorsal DG could reflect a higher ratio of young:mature granule cells or it could be caused by a more general increase in the density of granule cells of all ages in the dorsal compared to ventral DG. To distinguish between these possibilities a two-way ANOVA on total granule cell density in regions D1 and V4 was performed. We found no significant effect of blade ($F_{1,12} = 0.4$, $P = 0.5$) or dorso-ventral position on total granule cell density ($F_{1,12} = 1$, $P = 0.3$; dorsal 433,913 ± 28,911 cells/mm^3, ventral 396,650 ± 16,677 cells/mm^3, mean ± se; Fig. 3c). Thus, because the overall granule cell density is constant,

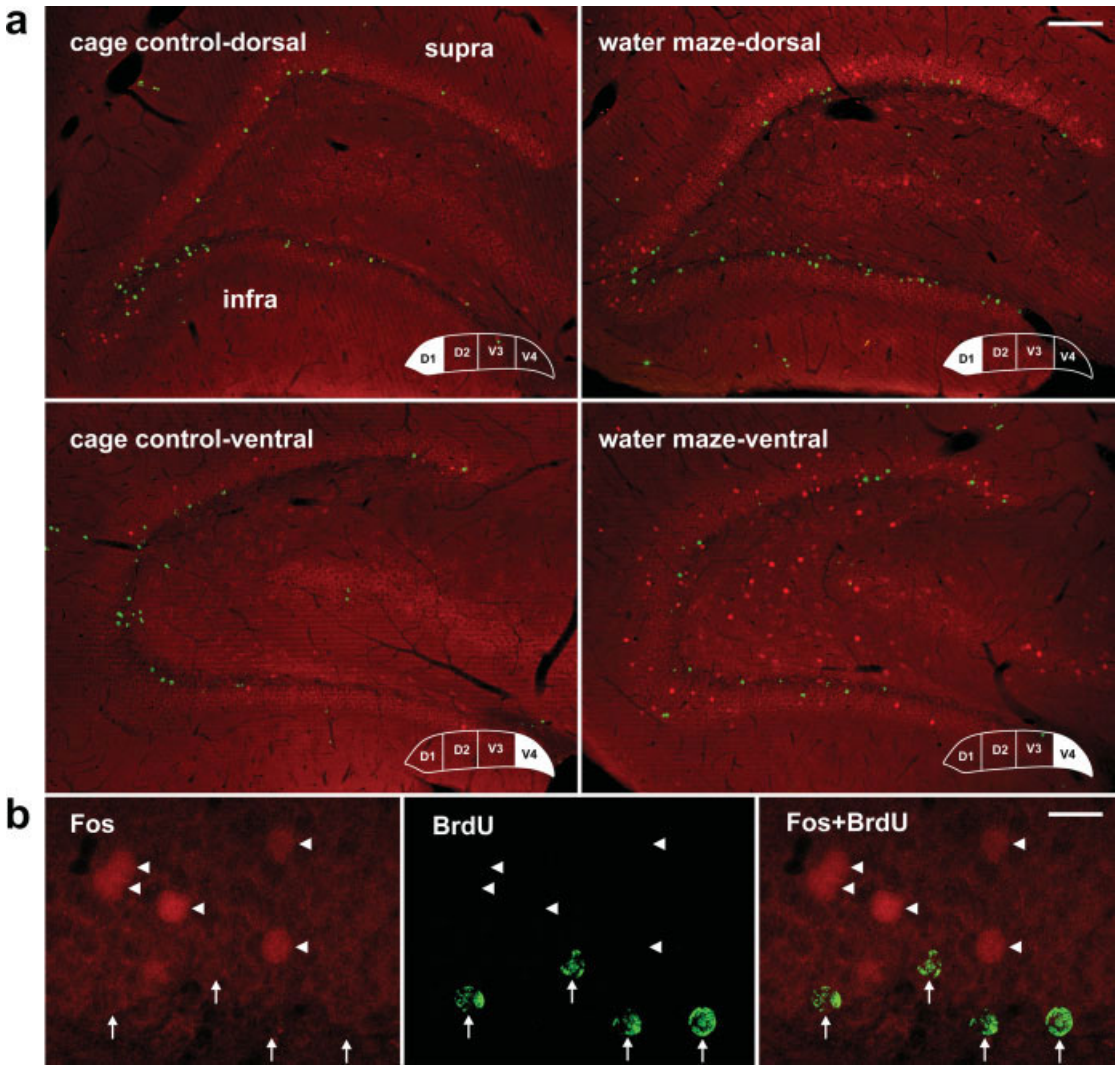

FIGURE 2. Photographs showing BrdU immunostaining (green) and c-fos (Fos) immunostaining (red). (a) Examples taken from dorsal (D1) and ventral (V4) poles of the dentate gyrus (see inset drawings) in control and water maze-trained rats. (b) Higher magnification photos show BrdU and Fos staining in different cells. Arrows indicate BrdU+ cells, arrowheads indicate Fos+ cells. Supra, suprapyramidal blade; infra, infrapyramidal blade. Scale bars: a: 200 µm, b: 20 µm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

we conclude that regional differences in BrdU+ and PSA-NCAM+ (and Fos+, below) cell density reflect differences in the proportion of granule cells expressing these markers.

Effects of Subregion and Experience on Overall Fos Expression within the Entire Granule Cell Layer

Immediate-early genes contribute to signaling cascades required for induction of long-term synaptic plasticity and consolidation of long-term memory (Kubik et al., 2007), and their expression patterns parallel those seen physiologically in "place cells" (Guzowski et al., 1999; Leutgeb et al., 2005). Together, these features make immediate-early genes such as Fos suitable for identifying neurons acutely involved in hippocampal functioning. A three-way ANOVA was used to compare Fos expression, with treatment, dorso-ventral position and blade as factors. There was a significant main effect of treatment on the density of Fos+ cells ($F_{2,80} = 21$, $P < 0.001$; Figs. 2 and 4). Maze-trained and swim control rats had approximately twice as many Fos+ cells as cage controls (post hoc tests, $P < 0.001$), confirming the previously observed activity-dependence of Fos staining in the DG (Vann et al., 2000; Countryman et al., 2005; Kee et al., 2007). Interestingly, Fos+ cell density was not different between water maze-trained rats and swim controls ($P = 0.99$). There was a significant main effect of dorso-ventral position ($F_{3,80} = 22$, $P < 0.001$), with the dorsal-most quartile (D1) having greater Fos density than all other quartiles (post hoc tests, $P < 0.001$), consistent with place cell data showing that, during exploration, the proportion of neurons displaying place fields is greater in dorsal CA1 than in ventral

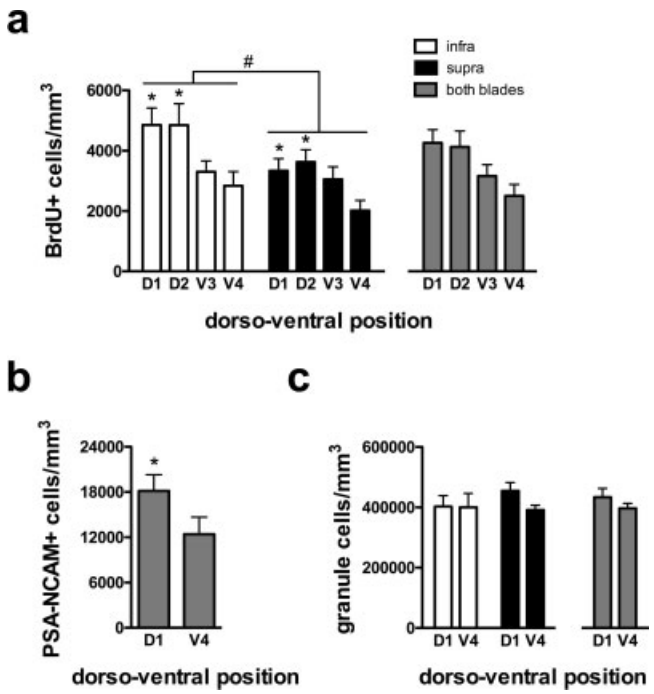

FIGURE 3. Dorso-ventral and infrapyramidal-suprapyramidal blade neurogenesis gradients. (a) By two-way ANOVA (dorso-ventral × blade) there was a significant main effect of dorso-ventral position ($P = 0.001$) on BrdU+ cell density. Post hoc tests showed significantly higher density of 4w old BrdU+ cells in the dorsal-dentate gyrus (regions D1 and D2) than in the ventral dentate gyrus (region V4; *, $P < 0.01$). BrdU+ cell density was also higher in the infrapyramidal (infra) blade than in the suprapyramidal (supra) blade (#, main effect of blade, $P < 0.01$). Note that the graph of both blades (both) represents the combined density in the different dorso-ventral regions, rather than the average of the two blade values, and was not included in statistical analyses. (b) The density of cells immunostaining for PSA-NCAM, an endogenous marker of young neurons, was significantly higher in dorsal than in ventral DG (*, $P < 0.05$), matching the gradient seen in 4-week old BrdU+ cells. (c) Total granule cell density was not different between blades or between the dorsal and ventral poles of the DG ($P \geq 0.3$ for both).

CA1 (Jung et al., 1994). A significant main effect of blade was also found, with the suprapyramidal blade having a higher Fos+ cell density than the infrapyramidal blade ($F_{1,80} = 68$, $P < 0.001$). Additionally, there was a significant treatment x blade interaction ($F_{2,80} = 17$, $P < 0.001$), with post hoc tests showing that both water maze training and swimming increased Fos+ cell density only in the suprapyramidal blade ($P < 0.001$ vs. suprapyramidal blade of cage control animals and vs. infrapyramidal blades in all groups; Figs. 2 and 4). This restriction of the treatment effect to the suprapyramidal blade is consistent with the expression pattern previously seen for the immediate-early gene Arc (Chawla et al., 2005). Finally, there was no significant interaction between treatment and dorso-ventral position on Fos+ cell density ($F_{6,80} = 2$, $P = 0.08$), indicating that granule cells in all dorso-ventral subregions were equally recruited.

Effects of Subregion and Experience on Fos Expression in Young Neurons

Low numbers of BrdU+/Fos+ cells, as expected based on the small population of BrdU+ cells and low frequency of Fos expression throughout the DG, prevented statistical comparisons using BrdU to identify young neurons. PSA-NCAM is a reliable marker of young neurons expressing characteristic enhancement of long-term potentiation (LTP) (Schmidt-Hieber et al., 2004). Because it is expressed in new neurons for 3–4 weeks (Seki, 2002), it identifies a larger population of young neurons than two BrdU injections (4.4× larger in the current study), while following the same dorso-ventral gradient (Fig. 3).

We therefore examined Fos expression in this larger population of PSA-NCAM+ young neurons in the dorsal (D1) and ventral (V4) poles of the DG. A two-way ANOVA (treatment x dorso-ventral position) showed that treatment affected PSA-NCAM+/Fos+ cell density in a subregion-specific manner (Fig. 5). There was a significant main effect of treatment ($F_{2,20} = 9.1$, $P < 0.01$) with water maze-trained rats having greater PSA-NCAM+/Fos+ cell density than both swim control and cage control rats (both post hocs $P < 0.01$; Figs. 5d-f). There was no difference between swim control and cage control animals (post hoc $P = 0.98$). We found no main effect of dorso-ventral position on PSA-NCAM+/Fos+ cell density but there was a significant interaction between treatment and dorso-ventral position ($F_{2,20} = 3.9$, $P < 0.05$). This was attributable to a significant elevation in PSA-NCAM+/Fos+ cell density in the ventral DG of water maze-trained rats relative to the dorsal DG in the same rats and relative to dorsal and ventral regions of swim control and cage control rats (all post hocs $P < 0.01$). There were no overall or subregion-specific differences in PSA-NCAM+/Fos+ cell density between cage controls and swim controls (all post hocs $P > 0.6$). The elevated ventral PSA-NCAM+/Fos+ cell density in water maze-trained rats appeared to be greater in the suprapyramidal blade than the infrapyramidal blade (Fig. 5f), but data were collapsed across blade for the statistical analysis in order to achieve a normal distribution with small numbers of cells. Taken together, these findings indicate that young neurons are activated by platform location training in a water maze but are not activated by swimming in the same spatial arena in the absence of a platform. In addition, these data show that greater activation of young neurons occurs in the ventral region of the DG, even though this region has fewer young neurons and less overall Fos activation.

To obtain a complementary measure of regional activity in a broader population of adult-born neurons, we also analyzed the proportion of Fos+ cells that were located in the SGZ in water maze-trained rats (Figs. 5b,c, and g). The granule cell layer follows an outside-in gradient of formation that continues through adulthood, resulting in a high proportion of young neurons in the subgranular zone (Crespo et al., 1986; Dayer et al., 2003; Kempermann et al., 2003). By two-way ANOVA we found no difference between the suprapyramidal and infra-

pyramidal blades in the proportion of Fos+ cells in the subgranular zone but observed a significant effect of dorso-ventral location ($F_{1,24} = 47$, $P < 0.001$; Fig. 5g). The proportion of Fos+ cells found in the SGZ was more than three times higher in the ventral DG than in the dorsal DG. Moreover, in the dorsal DG the proportion of Fos+ cells found in the SGZ was below chance levels based on the number of rows in the granule cell layer (8.1%; chance = 14.6% based on 6.8 layers; $T_6 = 4.2$, $P < 0.01$), whereas in the ventral DG the proportion was above significantly higher than chance levels (28.8%; chance = 18.2% based on 5.5 layers; $T_6 = 3.9$, $P < 0.01$). These findings are consistent with the increased activation of PSA-NCAM+ cells seen in the ventral DG. Additionally, they suggest that in the ventral region younger neurons are more likely to be activated than older granule cells, while in the dorsal region the opposite is true.

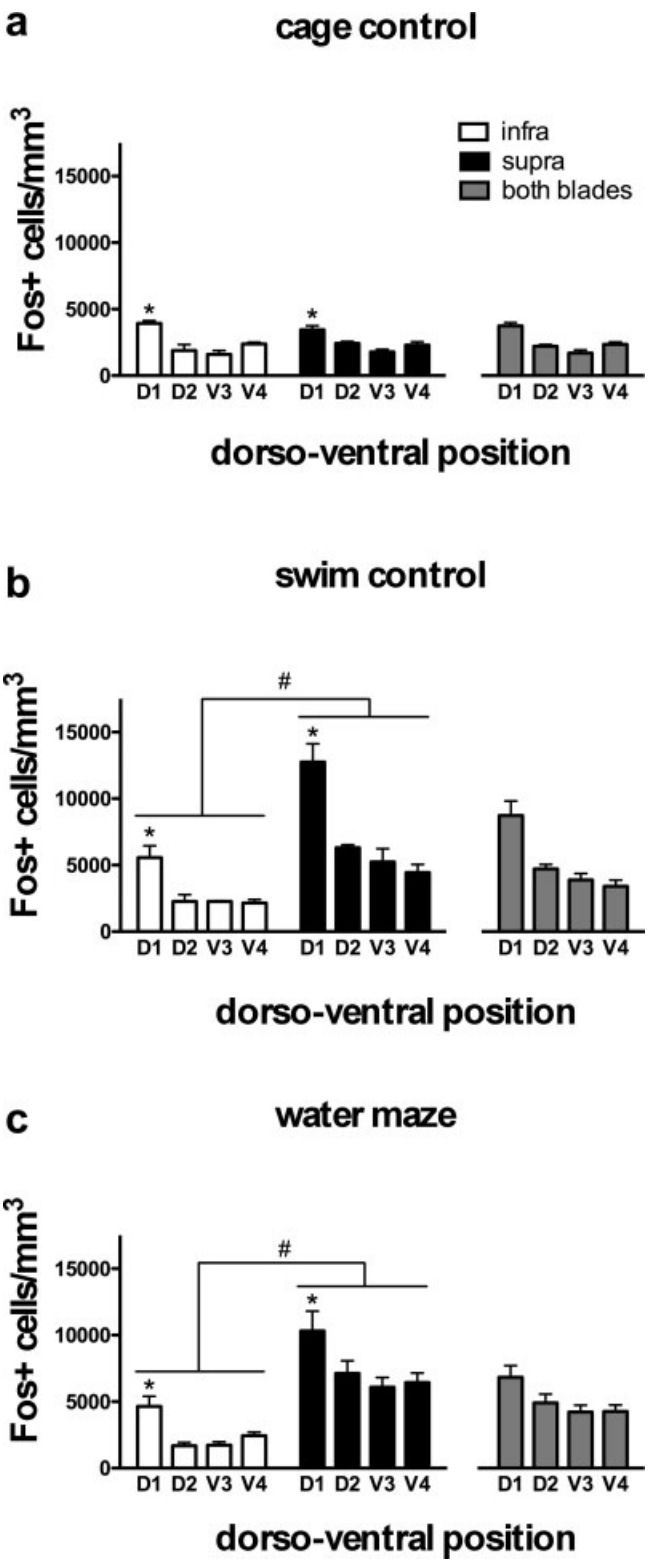

FIGURE 4. Dorso-ventral and blade gradients of overall Fos expression in the DG granule cell layer. A three-way ANOVA (experience × dorso-ventral position × blade) revealed significant main effects of experience ($P < 0.001$); post hoc tests showed that swim control rats (b) and water maze rats (c) had higher Fos+ cell densities than cage controls (a; $P < 0.001$). Significant main effects of blade ($P < 0.001$) and dorso-ventral position ($P < 0.001$) were also seen. Post hoc tests revealed that the dorsal-most quartile (D1) had greater Fos+ density than all other quartiles (*, post hocs all $P < 0.001$). There was a significant interaction between experience and blade ($P < 0.001$); post hoc tests showed that Fos+ cell density in swim control and water maze rats was increased specifically in the suprapyramidal blade (#, $P < 0.001$). There was no significant interaction between experience and dorso-ventral position ($P > 0.05$).

DISCUSSION

Regional Patterns of Neurogenesis in Development and Adulthood

Anatomical subregions are apparent during development of the DG, when the ventral DG forms prior to the dorsal DG and the suprapyramidal blade forms before the infrapyramidal blade (Schlessinger et al., 1975). The higher density of new neurons in the infrapyramidal blade and dorsal DG observed in the current study matches gradients of neurogenesis observed late in development (Schlessinger et al., 1975), suggesting that regional differences in adult neurogenesis are remnants of developmental gradients.

A handful of studies have previously looked for dorso-ventral gradients of adult neurogenesis, with conflicting results showing either no significant difference (Banasr et al., 2006; Olariu et al., 2007), more new neurons in the dorsal DG (Dawirs et al., 1998; Ferland et al., 2002), or more neurogenesis at both poles with less in the middle (Silva et al., 2006). The current study differed from all previous studies because sectioning was done perpendicular to the dorso-ventral axis which divides the DG into subregions that map closely onto the known anatomical segregation of inputs to the DG (Dolorfo and Amaral, 1998; Pitkanen et al., 2000). Sectioning along this axis also

produces sections with a similar shape and thickness throughout the dorso-ventral extent, minimizing the changes that can complicate comparative analyses within the DG (Gaarskjaer, 1978; Rapp and Amaral, 1988). By normalizing cell counts to the volume of tissue analyzed (i.e., density), we eliminated the inevitable problem of dividing the DG, which has no visible dorso-ventral boundaries, into pieces that are not exactly the same size. Because the overall granule cell density was equivalent across subregions, gradients in densities of cells expressing the different markers reflect similar gradients in the proportions of the total granule cells expressing these markers.

Regional Differences in Activation and Function of the Hippocampus

Numerous behavioral studies have suggested that the dorsal and ventral hippocampus have dissociable roles in spatial memory and anxious/fearful behavior: the dorsal hippocampus contributes to spatial water maze memory, but not fear-related behavior in an elevated plus maze, whereas the ventral hippocampus contributes to fear-related behavior but not spatial learning (Bannerman et al., 2004). These distinct behavioral roles are supported by separation of anatomical inputs to the hippocampus. The ventral hippocampus receives strong direct projections from the hypothalamus and amygdala, structures involved in responding to anxiogenic, fearful and stressful stimuli (Swanson and Cowan, 1977; Risold and Swanson, 1996; Pitkanen et al., 2000; Petrovich et al., 2001), whereas the dorsal hippocampus receives input from the dorsolateral portion of the entorhinal cortex (EC) (Dolorfo and Amaral, 1998), which is specifically required for spatial learning (Steffenach et al., 2005).

In the current study, we observed higher Fos+ cell density in the dorsal DG and suprapyramidal blade, consistent with previous studies showing dorso-ventral and suprapyramidal-

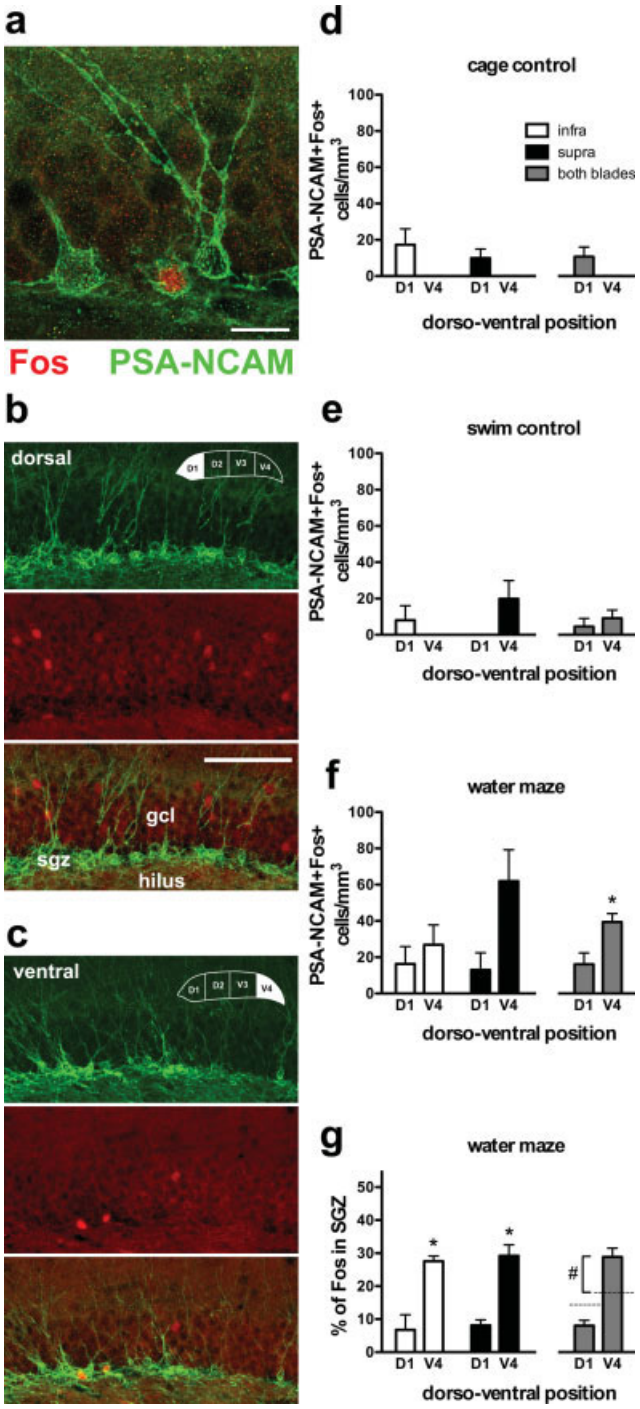

FIGURE 5. Water maze training increases activation of young neurons in the ventral DG. (a) Confocal image of a PSA-NCAM+ young granule neuron expressing Fos after training in the water maze. (b, c) Confocal images of PSA-NCAM+ and Fos+ neurons in the dorsal (b) and ventral (c) DG after water maze training. In b, several Fos+ cells can be seen in the granule cell layer (GCL). In c, one Fos+ cell is seen in the middle of the granule cell layer, and two Fos+ cells are seen in the subgranular zone (SGZ), where immature PSA-NCAM-expressing neurons and other adult-born neurons reside. (d–f) The density of cells double-labeled with Fos and PSA-NCAM in the dorsal and ventral poles (D1 and V4, respectively) of the dentate gyrus in cage control rats (d), swim control rats (e) and water maze-trained rats (f). Two-way ANOVA (experience × dorso-ventral position) showed that water maze training significantly increased PSA-NCAM+/Fos+ cell density relative to both cage control and swim control conditions (main effect and post hoc tests, $P < 0.01$). A significant interaction between dorso-ventral region and experience and significant post hoc tests showed that PSA-NCAM+/Fos+ cell density was increased specifically in the ventral dentate gyrus in water maze animals (*, interaction and post hoc tests all $P < 0.05$). (g) The proportion of Fos+ granule cells located in the SGZ as opposed to the remainder of the GCL was significantly higher in the ventral dentate gyrus than in the dorsal dentate gyrus (*, main effect of dorso-ventral position in two-way blade x dorso-ventral position ANOVA, $P < 0.001$). In the dorsal dentate gyrus, the proportion of Fos+ cells in the SGZ was below chance levels (one sample *t*-test, $P < 0.01$), while in the ventral dentate gyrus, Fos-activity in the SGZ was significantly above chance (#, one sample *t*-test, $P < 0.01$). Dotted lines shows chance levels of Fos expression (100/number of layers). Scale bars: $A = 20$ µm; $B = 100$ µm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

infrapyramidal gradients of activity during spatial navigation (Jung et al., 1994; Chawla et al., 2005; Ramirez-Amaya et al., 2005). Additionally, we found that the dorsal DG had higher Fos+ activity, regardless of treatment, suggesting that the dorsal DG is generally more active than the ventral DG and that experience recruited granule cells from all dorso-ventral DG subregions equally. Interestingly, we found that activity levels were equivalent in rats learning a platform location and those swimming in the absence of reinforcement, suggesting that the granule cell population responds similarly during active learning of a spatial location and undirected exploration. This is perhaps not surprising because rats can learn about their environment regardless of whether or not learning is being guided and measured by a researcher. This activation in swim control animals is also consistent with place cell activation seen during free exploration (Leutgeb et al., 2007) and the proposed role for the hippocampus in "automatic recording of attended experience" (Morris and Frey, 1997).

Roles for Young Neurons in Learning and Behavior

We found two major distinctions between the activation of young granule cells and older granule cells that suggest that young granule cells in the ventral DG play a different role in water maze learning than mature granule cells. The first difference was that Fos expression in PSA-NCAM+ young granule cells showed a ventral over dorsal gradient of Fos expression, opposite to the gradient seen in the overall granule cell population. Importantly, the larger number of activated young granule cells in the ventral DG does not simply reflect a larger number of new neurons in this region, because neurogenesis showed the opposite gradient. This gradient difference was replicated in an analysis of Fos expression in adult-born neurons in the subgranular zone. This subgranular zone analysis also allowed us to directly compare Fos expression in younger and older neurons across dorso-ventral subregions. In the ventral region, the younger granule cells of the subgranular zone were more likely to be activated by water maze training than granule cells in the more superficial layers, whereas in the dorsal area, the older granule cells were more likely to be activated than the younger subgranular neurons. No previous studies have compared immediate-early gene expression in young neurons in different DG subregions. However, a few studies have examined activation of adult-born neurons and found either enhanced activation (Ramirez-Amaya et al., 2006; Kee et al., 2007) or no difference in activation (Jessberger and Kempermann, 2003; Tashiro et al., 2007) of new cells relative to older cells. These studies are difficult to directly compare because of differences in stimuli used for activation (water maze vs. unreinforced exploration) and prior experience of the animals (previous water maze training, enriched housing conditions, etc.).

The second difference between young and old granule cells was related to the specific experiences that activated the young and older granule cells. Mature granule cell activation, as seen through Fos expression throughout the granule cell layer, occurred to the same degree in water maze trained rats as in swim control animals. However, young granule cells did not show this expected pattern. Unlike the overall granule cell population, PSA-NCAM+ young granule cells showed a large, 4-fold, increase in activation in the water maze learning condition but no change in activation in the swim control condition relative to the home cage condition. Interestingly, Gould et al. (1999) found that survival of young granule neurons increases after spatial training in the water maze but not in the swim control condition, further supporting the idea that hippocampus-dependent learning experiences have special significance for young granule cells.

The fact that water maze training induces Fos expression in young granule cells suggests that these young neurons may play a role in spatial learning and memory. Although spatial tasks such as the hidden-platform water maze used in this study are often considered to be more dependent on the dorsal hippocampus, studies have demonstrated a role for the ventral hippocampus in spatial memory as well (de Hoz et al., 2003; Ferbinteanu et al., 2003; McDonald et al., 2006). Studies looking for impairment after loss of adult neurogenesis have not found evidence that young granule neurons are needed for acquisition in the water maze (Shors et al., 2002; Madsen et al., 2003; Snyder et al., 2005; Saxe et al., 2006). However, inhibition of adult neurogenesis does impair long-term retention of spatial platform location (Snyder et al., 2005), suggesting that the Fos+ young neurons in the ventral DG in the current study may be involved in long-term spatial memory formation. Consistent with a role in long-term memory, the ventral hippocampus requires more time than the dorsal hippocampus to consolidate water maze memories (de Hoz et al., 2003), and it is primarily the ventral DG that is activated by the retrieval of remote water maze memory (Gusev et al., 2005).

Alternatively, it is possible that the young neurons activated in the ventral DG are responding to nonspatial aspects of experience in the water maze. Water maze learning has strong anxiogenic and stressful, in addition to spatial, components (Beiko et al., 2004), suggesting that the new neurons in the ventral DG may contribute to the expression of the fear- or anxiety-related behaviors that are believed to be mediated by the ventral hippocampus (Bannerman et al., 2004). This role for young neurons would be consistent with the idea that young neurons are involved in depression and anxiety-related behavior and/or the effects of antidepressants on those behaviors (Santarelli et al., 2003; Duman, 2004; Sapolsky, 2004; Drew and Hen, 2007). However, the lack of increase in Fos expression in young neurons in the very stressful swim control condition, argues that new neurons are not simply activated by all fearful or stressful experiences. It may be the case, though, that young granule neurons in the ventral DG are important for learning that occurs in a fearful context. The impairment in contextual fear conditioning observed after loss of new neurons is consistent with this possibility (Saxe et al., 2006; Winocur et al., 2006; Wojtowicz et al., 2008), as are findings that the ventral, as well as dorsal, hippocampus is important for consolidation of contextual fear memory (Rudy and Matus-Amat, 2005;

Sutherland et al., 2008). More broadly, it may be that the nonspatial functions proposed for the hippocampus, and in some cases for the ventral hippocampus in particular, activate young neurons during learning. These functions include resolving conflicts between two possible responses (McNaughton and Wickens, 2003), inhibiting locomotor activity (Bast and Feldon, 2003), or inhibiting affectively positive associations or memories (Davidson and Jarrard, 2004). It will be important in the future to determine which types of experience, spatial or nonstressful or otherwise, activate young neurons in the dorsal DG and to discover whether mnemonic and anxiety-related roles are dissociable or are in fact part of a common function for these new neurons.

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