ELSEVIER

Neurobiology of Aging 34 (2013) 263–274

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Environmental enrichment restores neurogenesis and rapid acquisition in aged rats

Rachel B. Speisman[a], Ashok Kumar[b,c], Asha Rani[b,c], Jessica M. Pastoriza[a], Jamie E. Severance[a], Thomas C. Foster[b,c], Brandi K. Ormerod[a,b,c,*]

[a] *J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA*
[b] *Department of Neuroscience, University of Florida, Gainesville, FL, USA*
[c] *McKnight Brain Institute, University of Florida, Gainesville, FL, USA*

Received 20 October 2011; received in revised form 19 March 2012; accepted 19 May 2012

Abstract

Strategies combatting cognitive decline among the growing aging population are vital. We tested whether environmental enrichment could reverse age-impaired rapid spatial search strategy acquisition concomitantly with hippocampal neurogenesis in rats. Young (5–8 months) and aged (20–22 months) male Fischer 344 rats were pair-housed and exposed to environmental enrichment (n = 7 young, 9 aged) or housed individually (n = 7 young, 7 aged) for 10 weeks. After 5 weeks, hidden platform trials (5 blocks of 3 trials; 15 m inter-block interval), a probe trial, and then visible platform trials (5 blocks of 3 trials; 15 m inter-block interval) commenced in the water maze. One week after testing, rats were given 5 daily intraperitoneal bromodeoxyuridine (50 mg/kg) injections and perfused 4 weeks later to quantify neurogenesis. Although young rats outperformed aged rats, aged enriched rats outperformed aged individually housed rats on all behavioral measures. Neurogenesis decreased with age but enrichment enhanced new cell survival, regardless of age. The novel correlation between new neuron number and behavioral measures obtained in a rapid water maze task among aged rats, suggests that environmental enrichment increases their ability to rapidly acquire and flexibly use spatial information along with neurogenesis.

Keywords: Adult neurogenesis; Learning; Memory; Enrichment; Water maze; Age-related; Fisher 344; Neural progenitor cell; Hippocampus

1. Introduction

Altered hippocampal function likely contributes to age-related changes in cognitive ability because hippocampus-dependent tasks are sensitive to age-related cognitive decline (Foster, 1999). Decades ago, the standard Morris water maze task revealed impaired performances among some senescent rats (Gage et al., 1984; Rapp et al., 1987). More recent behavioral assessments have sought to increase task sensitivity to age-related cognitive decline (Kennard and Woodruff-Pak, 2011), so that the deficits and their underlying mechanisms can be better characterized and potentially manipulated. Here we employ a rapid water maze task sensitive to age-related cognitive decline to test whether daily exposure to an enriched environment can reverse the effects of age on hippocampal function concomitantly with hippocampal neurogenesis.

Neurogenesis is a striking form of neural plasticity that persists throughout life in the hippocampus and olfactory bulbs of all mammals investigated, including humans (Altman and Das, 1965; Cameron et al., 1993; Eriksson et al., 1998). Although the precise role that new neurons play in hippocampal integrity is debated, new neuron number in young animals generally correlates with their performance measures in hippocampus-dependent tasks (Deng et al., 2010; but see Epp and Galea, 2009). Manipulations that attenuate neurogenesis chronically associate with impaired performance (Madsen et al., 2003; Raber et al., 2004; Saxe

* Corresponding author at: J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL 32611, USA. Tel.: +1 352 273 8125; fax: +1 352 273 9221.
E-mail address: bormerod@bme.ufl.edu (B.K. Ormerod).

0197-4580/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2012.05.023

et al., 2006; Shors et al., 2002; Snyder et al., 2005; Winocur et al., 2006) while those that potentiate neurogenesis associate with better performance (Ormerod et al., 2004; van Praag et al., 2005; Dalla et al., 2009). Postmortem signs of hippocampal neurogenesis in human patients who exhibited profound memory impairments are scarce (Coras et al., 2010; Correa et al., 2004; Crossen et al., 1994; Monje et al., 2007; Roman and Sperduto, 1995; Siffert and Allen, 2000).

Hippocampal neurogenesis declines with age in rodents primarily because neural progenitor cells (NPCs) become increasingly quiescent and NPCs that do divide may be less likely to produce surviving neuronal progeny (Cameron and McKay, 1999; Hattiangady and Shetty, 2008; Kempermann et al., 1997; Kuhn et al., 1996; Lichtenwalner et al., 2001; Nacher et al., 2003). While several studies have related new neuron number and cognitive measures in aged rats (Drapeau et al., 2003, 2007; Driscoll et al., 2006; Lemaire et al., 2000), dogs (Siwak-Tapp et al., 2007), and nonhuman primates (Aizawa et al., 2009), the strength of this relationship among aged rats tested in the water maze varies. For example, new neuron number appears unrelated to the performance of aged rats in water maze tasks that distribute training across 8–10 days (Bizon and Gallagher, 2003; Bizon et al., 2004; Merrill et al., 2003) but related in protocols that mass training across 2–3 days (Drapeau et al., 2003; Driscoll et al., 2006). Moreover, new neuron survival in the hippocampi of aged rats is enhanced by their participation in early but not later trials of the distributed water maze protocol (Drapeau et al., 2007). These results suggest that the strength of the relationship between neurogenesis and water maze performance in aged rats may depend upon the speed of learning demanded by the task.

In aged rodents, daily exposure to environmental enrichment primarily stimulates neurogenesis by increasing the probability that new neurons survive to maturity (Kempermann et al., 1997, 1998, 2002; Leal-Galicia et al., 2008; Segovia et al., 2006) and improves the rapid acquisition of spatial information in a condensed water maze task (Kumar et al., 2012). Here we tested the hypothesis that daily exposure to environmental enrichment would reverse age-related impairments in rats' abilities to rapidly acquire a spatial search strategy concomitantly with ongoing rates of neurogenesis.

2. Methods

2.1. Subjects

Young (5–8 months old) and aged (20–22 months old) sexually naive male F344 rats obtained from the National Institute of Aging colony at Harlan Sprague Dawley (Indianapolis, IN, USA) were treated in accordance with University of Florida and federal policies regarding the ethical use of animals for experimentation. Rats exhibiting signs of aggression (bites and scratches) or age-related health problems (poor grooming, hunching, excessive porphyrin secretion, weight loss, and tumors) were euthanized humanely.

2.2. Differential experience: environmental enrichment and individual housing

For the 10-week experiment, the rats were housed in a 12:12 hour light cycle with access to food and water *ad libitum* either individually ($n = 7$ young [YI] and $n = 7$ aged [AI]) or pair housed with 2–3 hours of access daily to an enriched environment ($n = 7$ young [YE] and $n = 9$ aged [AE]). The goal of the differential experience protocol was to provide opportunities for the enriched group to engage in a variety of hippocampus-dependent behaviors while limiting them for the individually housed group. The enriched environment consisted of a large wooden box, empty water maze tank, or large wire cage containing assorted 3-dimensional toys (e.g., plastic tubes, balls, and various objects), food, and water. The environment and toys were randomly rotated daily to maintain novelty. Daily exposure to this environment modifies hippocampal electrophysiology and facilitates the rapid acquisition of a spatial search strategy in aged rats (Foster and Dumas, 2001; Kumar et al., 2007, 2012). Behavioral testing commenced in the 4th week of differential experience and bromodeoxyuridine (BrdU) injections commenced 1 week after behavioral testing was completed. The rats were perfused 4 weeks after the final BrdU injection to quantify neurogenesis (Fig. 1).

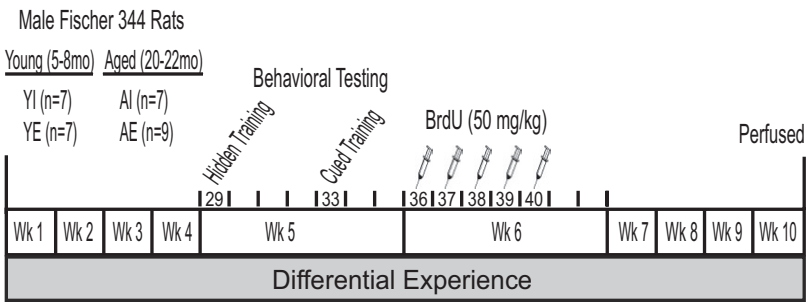

Fig. 1. Experiment timeline. Rats were housed individually ($n = 7$ young, $n = 7$ aged) or in pairs and exposed to an enriched environment daily ($n = 7$ young, $n = 9$ aged) for 10 weeks. In the 5th week, rats were trained and tested on hidden platform trials and then visible platform trials 3 days later. Beginning 1 week after testing, rats were injected daily with bromodeoxyuridine (BrdU; 50 mg/kg) over 5 days and then perfused 4 weeks later to quantify neurogenesis.

2.3. Water maze training and testing

A black water maze tank (1.7 m diameter) filled with water (27 ± 2 °C) to a depth of 8 cm below its rim was housed in a well-lit room. A Columbus Instruments (Columbus, OH, USA) tracking system recorded escape latencies (seconds) and path lengths (cm; see Fig. 2). Hidden and cued platform training consisted of 5 blocks (15 minute inter-block interval) of 3 60-second trials (20 second inter-trial interval) administered in a single session. This massed protocol is sensitive to both age-related cognitive decline (Carter et al., 2009; Foster and Kumar, 2007; Foster et al., 2003) and the effects of differential experience on cognition in aged rats (Kumar et al., 2012). Rats were dried between blocks.

2.3.1. Hidden platform trials

After 4 weeks of differential experience, rats were trained over a single session to locate a platform (29 cm diameter) hidden approximately 1 cm below the water surface in the north-east quadrant of the pool in the presence of highly visible extramaze cues. Rats were first habituated to the pool by being given 3 opportunities to climb onto the platform from different directions. On the subsequent hidden platform trials, the rats were released randomly from north, south, west, or east start locations and given 60 seconds to locate the hidden platform before being guided.

2.3.2. Probe trial

A 60-second free swim probe trial during which the platform was removed from the pool was conducted 15 minutes after the last hidden platform training block. The rats were released from the quadrant opposite the goal quadrant and discrimination scores [t(G) − t(O)]/[t(G) + t(O)], where t(O) is time spent in the opposite quadrant and t(G) is time spent in the goal quadrant, served as our strength of learning measure.

2.3.3. Cued trials

Three days after the hidden platform training session, rats were trained to locate the now flagged platform that protruded approximately 1.5 cm above the water in water maze tank now surrounded by a black curtain to mask distal cues. The rats were guided to the flagged platform if they failed to escape the maze within 60 seconds. The north, south east, and west release points and the location of the flagged platform were changed on each trial.

2.4. BrdU injections and histology

BrdU was dissolved in fresh 0.9% sterile saline (20 mg/mL wt/vol) and injected intraperitoneally (2.5 mL/kg or 50 mg/kg) once per day over 5 days, starting 1 week after behavioral testing to minimize the well-known effects of learning on neurogenesis (Epp et al., 2010; Gould et al., 1999). This BrdU dose safely and effectively labels dividing NPCs in the hippocampus of young and aged adult rodents (Cameron and McKay, 2001; Drapeau et al., 2003; Kolb et al., 1999).

Approximately 4 weeks after the final BrdU injection, the rats were anesthetized with 90 mg/kg ketamine and 10 mg/kg xylazine (Webster Veterinary, Sterling, MA, USA) and perfused transcardially with ice-cold isotonic saline and 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA). By 4 weeks, many new cells express mature neuronal proteins and are relatively permanent (Cameron and McKay, 2001; van Praag et al., 2002). Extracted brains were stored overnight in perfusate, equilibrated in 30% sucrose (appproximately 4 days) at 4 °C and then sectioned coronally through the rostral caudal extent of the hippocampal dentate gyrus at 40 μm intervals using a freezing stage sledge microtome (American Optical Corp., Buffalo, NY, USA). Sections were stored at −20 °C in 30% ethylene glycol, 25% glycerin, and 45% 0.1 M sodium phosphate buffer (vol/vol/vol) until immunostained.

2.5. Immunohistochemistry

Free-floating sections were stained immunohistochemically to quantify 28-32 day-old $BrdU^+$ cells and confirm their neuronal or glial phenotypes as described previously (Ormerod et al., 2004; Palmer et al., 2000). Sections were washed repeatedly between steps in Tris-buffered saline (TBS; pH 7.4).

2.5.1. Enzyme substrate immunostaining

$BrdU^+$ cells were revealed enzymatically on every 12th section through the dentate gyrus of each rat and counted under light microscopy to estimate total new cell numbers (Fig. 3). Sections were incubated in 0.3% H_2O_2 for 10 minutes to quench endogenous peroxidase, rinsed in 0.9% NaCl and then incubated in 2M HCl for 20 minutes at 37 °C to denature DNA. The sections were then blocked in a solution of 3% normal donkey serum and 0.1% Triton-X in TBS and incubated overnight in blocking solution containing rat anti-BrdU (1:500; AbD Serotec, Raleigh, NC, USA) at 4 °C and then for 4 hours in biotinylated secondary anti-rat IgG (Jackson ImmunoResearch, West Grove, PA, USA; 1:500) at room temperature (RT). Next, the sections were incubated in avidin-biotin horseradish peroxidase (Vector Laboratories, Burlingame, CA, USA) and then reacted in a solution of 0.02% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma Aldrich, St. Louis, MO, USA) and 0.5% H_2O_2. Sections were mounted on glass slides, dried overnight, dehydrated in an alcohol series and then coverslipped under Permount (Fisher Scientific, Pittsburgh, PA, USA).

2.5.2. Fluorescent immunostaining

The percentage of $BrdU^+$ cells expressing neuronal or glial protein was quantified on sections that were immunostained using fluorescent secondary antibodies under confocal microscopy (Fig. 4). The sections were blocked in a

solution of 3% normal donkey serum and 0.1% Triton-X in TBS and then incubated overnight at 4 °C in blocking solution containing the mature neuronal marker mouse anti-Neuronal Nucleii (NeuN, 1:500; Chemicon, Temecula, CA, USA) and the immature neuronal marker goat anti-doublecortin (DCX, 1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA) or the oligodendrocyte precursor marker rabbit anti-chondroitin sulfate proteoglycan (NG2, 1:500; Chemicon) and the astrocyte marker chicken anti-glial fibrillary acidic protein (GFAP, 1:750; EnCor Biotech, Alachua, FL, USA). The next day, sections were incubated in maximally cross-adsorbed fluorescein isothiocyanate (FITC)-conjugated anti-mouse and cyanine 5 (Cy5)-conjugated anti-goat secondary antibodies to reveal neurons or FITC-conjugated anti-rabbit and Cy5-conjugated anti-chicken secondary antibodies to reveal glia for 4 hours at RT (all secondary antibodies 1:500; Jackson Immunoresearch, Westgrove, PA, USA). Sections were then fixed in 4% paraformaldehyde, rinsed in 0.9% NaCl, incubated in 2 M HCl and then incubated overnight at 4 °C in rat anti-BrdU (1:500; AbD Serotec, Raleigh, NC, USA) and then Cy3-conjugated anti-rat secondary for 4 hours at RT the next day before being incubated in 4',6-diamidino-2-phenylindole (DAPI; 1:10,000; Calbiochem, San Diego, CA, USA) for 10 minutes and then mounted on glass slides under 2.5% diazobicyclooctane (in TBS with 10% polyvinyl alcohol and 20% glycerol).

2.6. Cell quantification

2.6.1. Total new cell number

$BrdU^+$ cells distributed through the subgranular zones and granule cell layers (GCL; Fig. 3A) were counted on sets of every 12th section (9–10 sections per rat) through the rostral-caudal extent of the hippocampal dentate gyrus under a 40× objective on a Zeiss AxioObserver Z1 inverted microscope (Thornwood, NY, USA) and optical fractionator principles (Kempermann et al., 2002; West et al., 1991). The first section of each rat's set was randomly selected from the 1st–11th section of dentate gyrus. Because $BrdU^+$ cells are typically distributed irregularly, we counted all new cells (mean ± standard error of the mean [SEM] total $BrdU^+$ cells: YI = 297 ± 66; YE = 493 ± 75; AI = 183 ± 25; AE = 370 ± 51), excluding obvious cell "caps" that could represent cells in adjacent cell sections and multiplied that number by 12 (the section interval) to generate a stereological estimate of total cell number (see Fig. 3) without fractionating section thickness (Kempermann et al., 2002). Because age- or enrichment-related changes in $BrdU^+$ cell nucleus diameter could affect cell estimates estimated this way, we confirmed that nuclear diameters of approximately 10–20 $BrdU^+$ cells completely contained within 1 of these sections in 3–4 rats per group were consistent (YI = 8.48 ± 0.40 μm, YE = 8.19 ± 0.13 μm, AI = 8.79 ± 0.33 μm and AE = 8.79 ± 0.22 μm; age effect [$F(1,9) = 2.82$], enrichment effect [$F(1,9) = 0.29$], interaction effect [$F(1,9) = 0.28$]). Because exposure to enriched environments can increase hippocampal volumes, we measured GCL and subgranular zone areas (in mm^2) under a 20× objective using AxioVision software (version 4.8, Zeiss, Thornwood, NY, USA) and then calculated volumes using a truncated cone formula that accurately predicts the volume of many biological regions (Galea et al., 2000; Seifert et al., 2010; Uylings et al., 1986): Volume = $\frac{1}{3}I(h_1 + \sqrt{h_1} \times \sqrt{h_2} + h_2)$, where I is the distance between sections (480 μm) and the 2 section areas for which volumes between are calculated are h_1 and h_2. Although neither age nor differential experience affected $BrdU^+$ cell nuclei diameters, $BrdU^+$ cells per mm^2 as well as total cell estimates are reported because of expected effects of enrichment on dentate volumes.

2.6.2. New cell phenotypes

To determine whether new cells differentiated into neurons or glia, we examined at least 100 $BrdU^+$ cells on quadruple fluorescent-stained sections (2–4 in young rats and 4–6 in aged rats) randomly selected from a set of every 12th section through the dentate gyrus for the coexpression of neuronal and glial proteins using a Zeiss meta LSM 710 fully spectral laser scanning confocal microscope with 405, 488, 543 and 633nm laser lines (Thornwood, NY, USA) under a 40× objective (and 2.3× digital zoom). $BrdU^+$ cells were considered colabeled when a full "z-dimension" scan revealed its BrdU/$DAPI^+$ nucleus was unambiguously associated with DCX and/or NeuN, NG2, or GFAP. The percentage of $BrdU^+$ cells expressing each protein was calculated (Fig. 4).

2.7. Statistical analyses

Statistical analyses were performed using Statistica software (Version 10; Statsoft, Tulsa, OK, USA). Analyses of variance (ANOVA) explored the effects age (young, aged) and experience (individually housed, enriched group-housed), on cognitive (latencies, path lengths, and probe trial discrimination index scores), health (body mass, swim speeds), and neurogenesis (new cell numbers, percentage and total new neurons and glia) measures and Newman–Keuls post hoc tests revealed group differences. χ^2 tests revealed the number of animals that performed at or above chance on probe trials and Pearson product moment correlations (r) tested relationships between neurogenesis and behavioral measures. The α level for all statistical tests was set at 0.05.

3. Results

3.1. Daily enrichment partially reverses the effect of age on spatial ability

3.1.1. Enrichment enhances spatial learning in aged rats

Because measures of path length and latency over trials were correlated positively ($r(29) = 0.82$; $p < 0.0001$), we

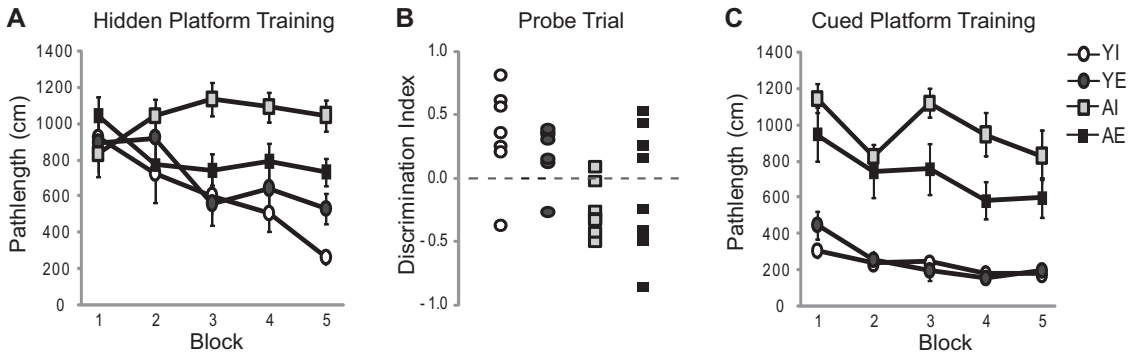

Fig. 2. Exposure to an enriched environment subdues age-dependent impairments on hidden and visible platform trials. The rats were trained on hidden platform trials (A), tested on a probe trial administered 15 minutes after the last hidden platform trial (B) and cued platform trials 3 days later (C). Line graphs depict group means (± standard error of the mean) of measures obtained from the young rats housed individually (YI; white circles), young rats housed in an enriched environment (YE; dark gray circles), aged rats housed individually (AI; light gray squares) and aged rats housed in an enriched environment (AE; black squares) groups. (A) Enrichment enhanced the ability of aged rats to rapidly acquire a spatial search strategy. On all training blocks combined, young rats swam more directly to the hidden platform than aged rats. AI rats swam more circuitous routes to the hidden platform than either AE rats or young rats in either group. (B) Probe trial discrimination index (DI) scores varied by age and experience. χ^2 tests confirmed that the percentage of rats that performed above or below chance (discrimination index (DI) score = 0, dashed line) decreased with age but increased with exposure to enrichment. Specifically, 69% of enriched rats performed above chance whereas only 50% of individually housed rats performed above chance. (C) Previous experience influences performance on visible platform trials. Young rats outperformed aged rats on all training blocks, including the initial training block, likely because they retained procedural information from the spatial task and AE rats outperformed AI rats.

report only path lengths to avoid redundancy. An ANOVA exploring the effects of age (young vs. aged), training block (blocks 1–5), and differential experience (individually housed vs. enriched) on path length (Fig. 2A) revealed significant effects of age ($F(1,26) = 15.65$; $p < 0.001$) and training block ($F(4,104) = 5.85$; $p < 0.001$) and significant age × environment ($F(1,26) = 5.57$; $p < 0.05$), age × training block ($F(4,104) = 4.94$; $p < 0.001$) and age × environment × training block ($F(4,104) = 3.24$; $p < 0.05$) interaction effects. All rats improved their performance across blocks (block 1 > 3–5 and 3 > 5; p values < 0.005) but, as expected, young rats outperformed aged rats. Across all blocks, AI rats performed more poorly than AE rats ($p < 0.05$) and young rats in either group (p values < 0.001) while YI and YE rats' performance improved equally rapidly across training blocks (p values > 0.1). Specifically, AI rats performed significantly more poorly on training blocks 3, 4, and 5 (p values < 0.01) and AE rats performed significantly more poorly only on training block 5 relative to YI and YE rats (p values < 0.01).

An ANOVA on swim speeds (mean ± [SEM] cm/second = 26.06 ±1.07 [YI], 29.92 ± 2.07 [YE], 19.45 ± 0.94 [AI], and 18.68 ± 1.64 [AE]) revealed a significant effect of age ($F(1,26) = 33.49$; $p < 0.001$) and a significant environment × training block interaction ($F(4,104) = 2.68$; $p < 0.05$). Young rats swam significantly faster than aged rats across all training blocks and enriched rats swam significantly faster during the first training block relative to all other training blocks (p values < 0.05). The effects of age on swim speed are unsurprising because aged rats were heavier than young rats (mean [± SEM] g = 336.83 ± 6.38 [YI], 347.37 ± 4.44 [YE], 419.21 ± 10.78 [AI], and 406.74 ± 8.21 [AE]; $F(1,26) = 78.87$; $p < 0.001$). The effect of enrichment on the performance of aged rats on hidden trials is likely related to cognition because enrichment neither affected swim speeds nor body mass in aged rats.

An ANOVA exploring the effects of age, training block, and environment on the percent of time spent in the outer annulus during hidden platform trials (mean [± SEM] % = 56.07 ± 4.47 [YI], 43.00 ± 4.15 [YE], 81.98 ± 0.92 [AI], and 64.32 ± 3.13 [AE]) revealed significant effects of age ($F(1,26) = 46.63$; $p < 0.0001$), environment ($F(1,26) = 19.76$; $p < 0.0001$), training block ($F(4,104) = 3.32$; $p < 0.05$), and a significant age × training block interaction effect ($F(4,104) = 3.95$; $p < 0.01$). Overall, the percentage of time spent in the outer annulus significantly decreased in young versus aged rats, in enriched versus individually housed rats and on later versus earlier training blocks block 1 and 2 > 5; p values < 0.05). While all young rats decreased their time spent in the outer annulus across blocks (block 1 and 2 > 3–5; p values < 0.01), aged rats maintained their time across blocks (p values > 0.78).

An ANOVA revealed a significant effect of age on probe trial discrimination index scores ($F(1,26) = 8.40$, p values < 0.01) but no effect of environment (Fig. 2B). Because 1 YI and 1 YE rat performed at chance (i.e., discrimination index = 0) and only 1 AI rat performed above chance, χ^2 tests on the percentage of rats performing above or below chance were employed to confirm effects of age ($\chi^2 = 78.55$, $p < 0.0001$) and differential experience ($\chi^2 = 14.44$, $p < 0.0005$), with 69% of enriched rats performing above chance and only 50% of individually housed rats performing above chance. The effect of differential experience was mainly due to an effect of environmental enrichment ob-

served in aged rats (χ^2 = 71.59, $p < 0.0005$). Taken together, these data confirm that although young rats outperformed aged rats, enrichment enhanced the ability of aged rats to rapidly acquire a spatial search strategy.

3.1.2. Enrichment enhances cue discrimination learning in aged rats

An ANOVA exploring the effects of age, training block, and environment on path length for the cued discrimination task revealed significant effects of age ($F(1,26) = 62.37$; $p < 0.001$) and training block ($F(4,104) = 9.92$; $p < 0.001$) but not enrichment. Post hoc tests confirmed that young rats swam more directly to the visible platform than aged rats but that all groups exhibited improved performance across training blocks (block 1 > 2 > 3, 4, and 5; p values ≤ 0.05). An ANOVA across blocks within each age and treatment group indicated a significant effect of training in 3 groups (YE: $F(4,24) = 5.68$; $p < 0.005$; AE: $F(4,32) = 3.66$; $p < 0.05$; AI: $F(4,24) = 3.28$; $p < 0.05$), with a tendency ($p = 0.07$) for a training effect in YI animals. This tendency was due, in part, to near asymptotic performance on the first training block. Indeed, young rats exhibited shorter path lengths on the first block of cue training relative to the first block of spatial training (Fig. 2A and C) indicating a carryover effect of prior training on the spatial task. Finally, age tended to interact with environment ($p < 0.10$) and post hoc tests indicated that AE rats swam shorter path lengths than AI rats ($p = 0.05$; Fig. 2C).

An ANOVA exploring the effects of age, training block, and environment on average swim speed across visible platform trials (YI = 27.88 ± 1.04 cm/second, YE = 26.28 ± 1.33 cm/second, AI = 20.69 ± 1.10 cm/second, and AE = 22.74 ± 1.31 cm/second) confirmed that young rats swam significantly faster than aged rats ($F(1,26) = 18.82$; $p < 0.001$), but there was no effect of differential experience. Age tended to interact with training block ($F(4,104) = 2.32$; $p = 0.062$) such that young rats increased their swim speeds (block 1 < 4–5; p values < 0.05, while aged rats maintained their slower swim speeds ($p > 0.74$) across all blocks.

An ANOVA exploring the effects of age, training block, and environment on the percentage of time spent in the outer annulus during cued platform training (YI = 36.00 ± 2.94%, YE = 28.90 ± 2.94%, AI = 75.14 ± 4.08%, and AE = 64.15 ± 4.69%) revealed significant effects of age ($F(1,26) = 88.64$; $p < 0.0001$) and differential experience ($F(1,26) = 5.24$; $p < 0.05$). Less time was spent in the outer annulus by young versus aged rats and by enriched versus individually housed rats. While all rats decreased the time they spent in the outer annulus across blocks ($F(4,104) = 12.57$; $p < 0.0001$; training blocks 1 and 2 > 3–5; $p < 0.05$), young rats ventured from the maze wall in early trials (block 1 > 2 > 3–5; p values < 0.001) whereas aged rats ventured from the wall only in later training blocks (block 3 > 4 and 5; p values < 0.05; age × block interaction effect: $F(4,104) = 8.18$; $p < 0.001$).

3.2. The effect of enrichment overcomes the effect of age on neurogenesis

3.2.1. Enriched environment reverses the effect of age on total new cell number

An ANOVA revealed significant effects of age ($F(1,26) = 4.26$; $p < 0.05$) and environment ($F(1,26) = 11.14$; $p < 0.01$) on the total number of new ($BrdU^+$) cells produced and/or surviving 4 weeks in young and aged rats (Fig. 3). More new cells were found in the dentate gyri of young versus aged rats and in enriched versus individually housed rats (Fig. 3C). Enrichment similarly increased the number of new cells in the dentate gyri of both young and aged rats. This effect of enrichment appears robust because we found

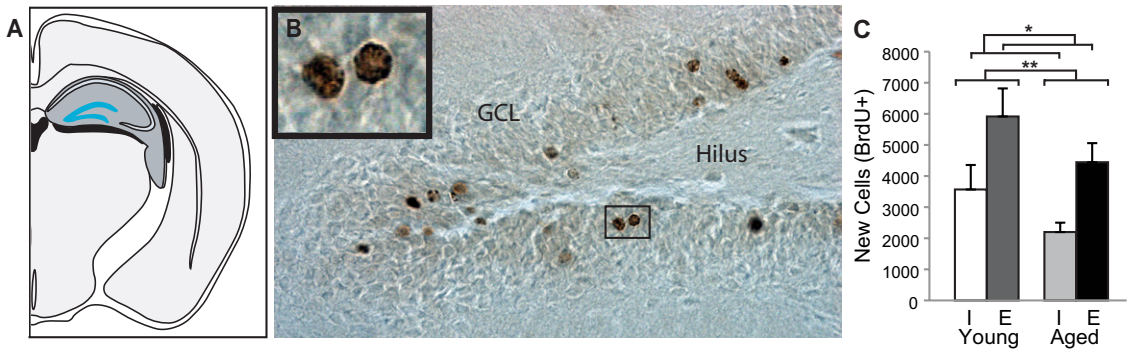

Fig. 3. Exposure to an enriched environment reversed the effects of age on neurogenesis. Rats were given 5 daily injections of bromodeoxyuridine (BrdU) beginning 1 week after behavioral testing and perfused 4 weeks later. The total number of new cells surviving 4 weeks was estimated stereologically using $BrdU^+$ cell counts obtained under light microscopy from every 12th section through the dentate gyrus (DG). The bar graph depicts group means (± standard error of the mean) of total new cell number in the dentate gyri of young rats housed individually (YI; white bars), young rats housed in an enriched environment (YE; dark gray bars), aged rats housed individually (AI; light gray bars) and aged rats housed in an enriched environment (AE; black bars). (A) Coronal view of the rat brain. The dentate granule cell layer (GCL) is highlighted in turquoise. (B) Photomicrograph of new ($BrdU^+$) cells in the DG of an aged rat. Representative examples of 4-5 week-old cells labeled with BrdU (in brown) revealed enzymatically with 3,3'-diaminobenzidine tetrahydrochloride (DAB). (C) Total new cell numbers declined with age but were potentiated by enrichment, regardless of age. More new cells survived approximately 4 weeks in the dentate gyri of young vehrsus aged and enriched versus individually housed rats. * $p \leq 0.05$ and ** $p \leq 0.01$.

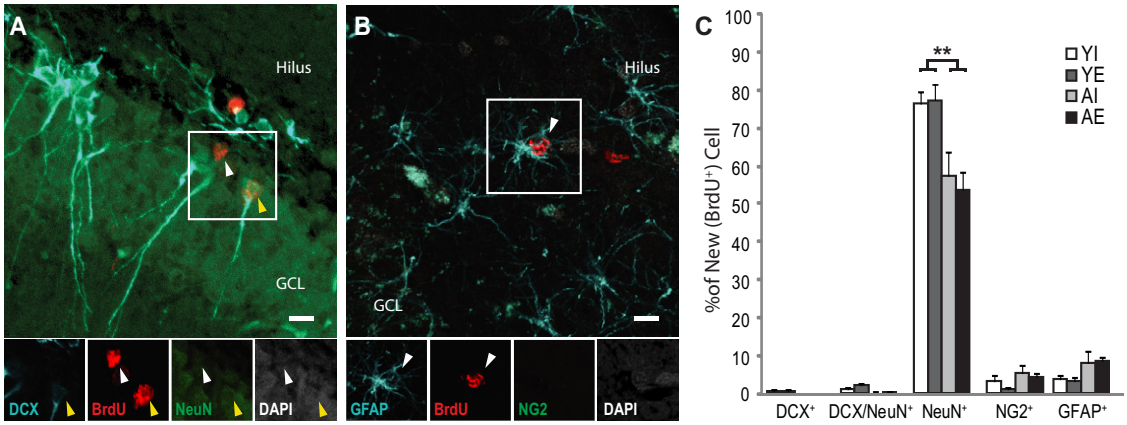

Fig. 4. Fewer new cells expressed mature neuronal phenotypes in the dentate gyri of aged rats. At least 100 bromodeoxyuridine (BrdU)$^+$ cells per rat were examined under confocal microscopy (40× objective with 2.3× digital zoom) to calculate proportions expressing markers of immature (doublecortin [DCX]$^+$), transitioning (DCX/NeuN$^+$), or mature (Neuronal Nucleii [NeuN]$^+$) neurons, as well as glial fibrillary acidic protein (GFAP)$^+$ astrocytes or chondroitin sulfate proteoglycan (NG2)$^+$ oligodendrocyte precursors, which were revealed using fluorescent immunohistochemistry. All BrdU$^+$ cells stained with the nuclear marker 4'6-Diamidino-2-Phenylindole Dihydrochloride (DAPI). (A and B) Confocal images of new neurons and astrocytes in the dentate gyrus of an adult rat. Representative images of approximately 4-week-old BrdU$^+$ cells (in red) that express the neuronal markers DCX (in blue) and/or NeuN (in green; A) or the glial markers GFAP (in blue) or NG2 (in green; B). (C) The proportion of new cells expressing neuronal phenotypes decreased with age and was unaffected by enrichment regardless of age. Mean (± standard error of the mean) percentage of new cells in the dentate gyri of young rats housed individually (YI; white bars), young rats housed in enriched environment (YE; dark gray bars), aged rats housed individually (AI; light gray bars), and aged rats housed in an enriched environment (AE; black bars) expressing neuronal and glial phenotypes are shown. In all rats, the majority of approximately 4-week-old cells expressed mature neuronal phenotypes. However, a lower percentage of new cells expressing mature neuronal phenotypes and a higher percentage of new cells expressing glial phenotypes was detected in aged versus young rats. No effect of differential experience on the percentage of new cells expressing either phenotype was detected in young or aged rats. * $p \leq 0.05$ and ** $p \leq 0.01$.

similarly increased (mean ± [SEM] new cell densities enriched: 1345.13 ± 204.12 cells/mm^3 vs. individually housed: 881.10 ± 156.82 cells/mm^3, $F(1,26) = 5.83$; $p < 0.05$) despite increased GCL volumes (enriched: 3.97 ± 0.31 mm^3 vs. individually housed: 3.25 ± 0.17 mm^3; $F(1,26) = 7.81$; $p < 0.01$). Neither cell density nor GCL volume was affected by age or the interaction between age and environment.

3.2.2. Enriched environment does not reverse the effect of age on neuronal differentiation

We calculated the proportion of BrdU$^+$ cells that coexpressed markers for immature (DCX$^+$), transitioning (DCX/NeuN$^+$), or mature (NeuN$^+$) neurons, or GFAP$^+$ astrocytes, or NG2$^+$ oligodendrocyte precursors (Fig. 4A–C). An ANOVA exploring the effects of age and environment on the percentage of new cells expressing each phenotype revealed significant effects of age ($F(1,26) = 7.99$; $p < 0.01$) and phenotype ($F(1,104) = 532.30$; $p < 0.001$) and a significant age × phenotype interaction effect ($F(1,104) = 17.18$; $p < 0.001$; Fig. 4D). Consistent with the extended survival period of the study, the majority of new cells expressed a mature neuronal phenotype ($p < 0.0001$ vs. all other phenotypes). Of the < 10% of BrdU$^+$ cells expressing glial or immature neuronal phenotypes, astrocyte phenotypes were expressed most frequently ($p < 0.01$ vs. immature and transitioning neurons). Significantly fewer BrdU$^+$ cells expressed a mature neuronal phenotype in aged versus young rats ($p < 0.0001$) and this effect was not reversed by enrichment. In fact, a higher proportion of BrdU$^+$ cells in aged versus young rats did not express the markers of differentiation employed in this study (YI = 13.80 ± 3.79, YE = 15.94 ± 5.07, AI = 27.85 ± 8.10, AE = 33.05 ± 4.39% BrdU$^+$ cells; $F(1,26) = 8.00$; $p < 0.01$).

3.2.3. Enriched environment increases net neurogenesis

We next determined the total number of new neurons (immature, transitioning, and mature neurons combined) and new glia (oligodendrocytes and astrocytes) by multiplying the estimated total number of BrdU$^+$ cells by the proportion of BrdU$^+$ cells coexpressing each phenotype (Fig. 5). An ANOVA exploring the effects of age and environment on total new neuron number revealed statistically significant effects of age ($F(1,26) = 10.32$; $p < 0.01$) and environment ($F(1,26) = 7.18$; $p < 0.05$). More new neurons were found in the dentate gyri of young versus aged rats and in enriched versus individually housed rats (Fig. 5A). Importantly, no age × environment interaction was observed indicating that enrichment increased net neurogenesis similarly in young and aged rats. An ANOVA exploring the effects of age and environment on total new glia revealed a statistically significant effect only for age ($F(1,26) = 4.26$; $p = 0.05$), such that more new glia (primarily astrocytes) were found in the dentate gyri of aged versus young rats (Fig. 5B). However, the reliability of this effect requires replication in future work because of the low frequency in which new glia were observed.

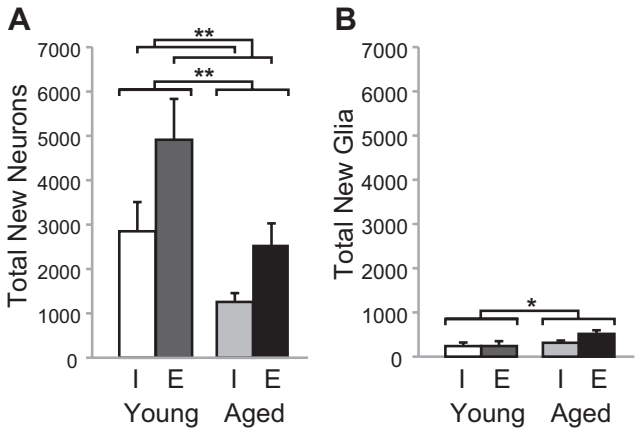

Fig. 5. Net neurogenesis declines with age but is increased by exposure to enrichment whereas age-dependent increases in gliogenesis are unaffected by enrichment. Net neurogenesis and gliogenesis was calculated by multiplying total new cell numbers (Fig. 3) by percentage of new cells expressing neuronal and glial phenotypes (Fig. 4), respectively. The bar graphs depict group mean (± standard error of the mean) numbers of neurons (A) or glia (B) in the dentate gyri of young rats housed individually (YI; white bars), young rats housed in an enriched environment (YE; dark gray bars), aged rats housed individually (AI; light gray bars), and aged rats housed in an enriched environment (AE; black bars) rats. (A) Net neurogenesis declines with age but increases with enrichment, independent of age. Neurogenesis declined with age and was potentiated by exposure to an enriched environment regardless of age. A few weeks of exposure to an enriched environment, therefore, returned levels of hippocampal neurogenesis in aged rats to those observed in young individually housed rats. (B) Age-dependent increases in gliogenesis are unaffected by exposure to enrichment. We detected a small but significant increase in gliogenesis in aged versus young rats that was unaffected by differential experience. * $p \leq 0.05$ and ** $p \leq 0.01$.

3.3. Higher rates of neurogenesis relate to better water maze performance in aged rats

Pearson product-moment correlations were employed to measure the relationships between ongoing neurogenesis and measures of water maze performance (mean path length and discrimination index) in each age group. Note that longer path lengths on hidden platform trials are indicative of more circuitous routes and therefore poorer performance whereas higher discrimination index scores indicate better discrimination between the target and opposite quadrants on probe trials and therefore better performance. New neuron number correlated significantly with average path length across hidden platform trials ($r = -0.56$; $p < 0.05$; Fig. 6B) and probe trial discrimination index scores ($r = 0.59$; $p < 0.05$; Fig. 6D), in aged but not young rats.

4. Discussion

In the current study, we confirmed that hippocampal neurogenesis and spatial learning are compromised by age and that exposure to environmental enrichment potentiates neurogenesis, regardless of age. We found that environmental enrichment improves the performance of aged but not young rats on a water maze task in which the hidden platform location is learned in a single day. We propose that this task requires the ability to rapidly acquire and flexibly use spatial information that appears intact and therefore unaffected by enrichment in young rats but compromised and improved by enrichment in aged rats. We also reveal a novel age-specific relationship between total new neuron number and indexes of ability in a rapid water maze task.

Decreased hippocampal neurogenesis is characteristic of aging (Cameron and McKay, 1999; Kuhn et al., 1996; Nacher et al., 2003). Although our single experiment end point cannot disentangle the effects of age on NPC proliferation versus new cell survival, our effect is consistent with the well-known effects of age on NPC proliferation. Fewer $BrdU^+$ cells expressed neuronal markers and more $BrdU^+$ cells were devoid of differentiation markers in the hippocampi of aged versus young rats, which is consistent with some reports that neuronal differentiation is compromised by age (Kempermann et al., 1998) Our finding that gliogenesis increased with age has been noted by others (Bizon et al., 2004) but because so few new glia were detected in the dentate gyri of either young or aged rats, the reliability of this effect should be tested in future work. Overall, our findings support published work showing age-related decreases in neurogenesis are mediated by increasing NPC quiescence across life and because fewer NPC progeny adopt neuronal fates.

Environmental enrichment increases neurogenesis in aged rodents by potentiating neuronal differentiation and new cell survival (Kempermann et al., 1998, 2002; Leal-Galicia et al., 2008; Segovia et al., 2006). Indeed, we found similar enrichment-induced increases in the number of new cells surviving 4–5 weeks in the dentate gyri of young and aged rats (Fig. 3). However, enrichment neither reversed the effects of age on the proportion of $BrdU^+$ cells that expressed neuronal phenotypes nor increased the proportion in young rats (Fig. 4). Other studies showing that exposure to enriched environments potentiates neuronal differentiation in young and aged have employed running wheels, larger social groups, and earlier more extended exposures to enriched environments, which could each potentiate different components of neurogenesis and probably each require more detailed investigation (Lazarov et al., 2010; Lugert et al., 2010). Overall, we show that just a few weeks of exposure to environmental enrichment can increase net neurogenesis (Fig. 5) in the hippocampus of aged rats by robustly enhancing new cell survival to the extent that it overcomes the effects of age on NPC proliferation (Fig. 3) and neuronal fate choice (Fig. 4).

We expanded upon work showing that exposure to environmental enrichment enhances the ability of aged rats to discriminate the spatial location of a platform hidden in water maze tasks that distribute training across days (Fernández et al., 2004; Frick and Fernandez, 2003; Lores-Arnaiz et al., 2006) by confirming that it also enhances their

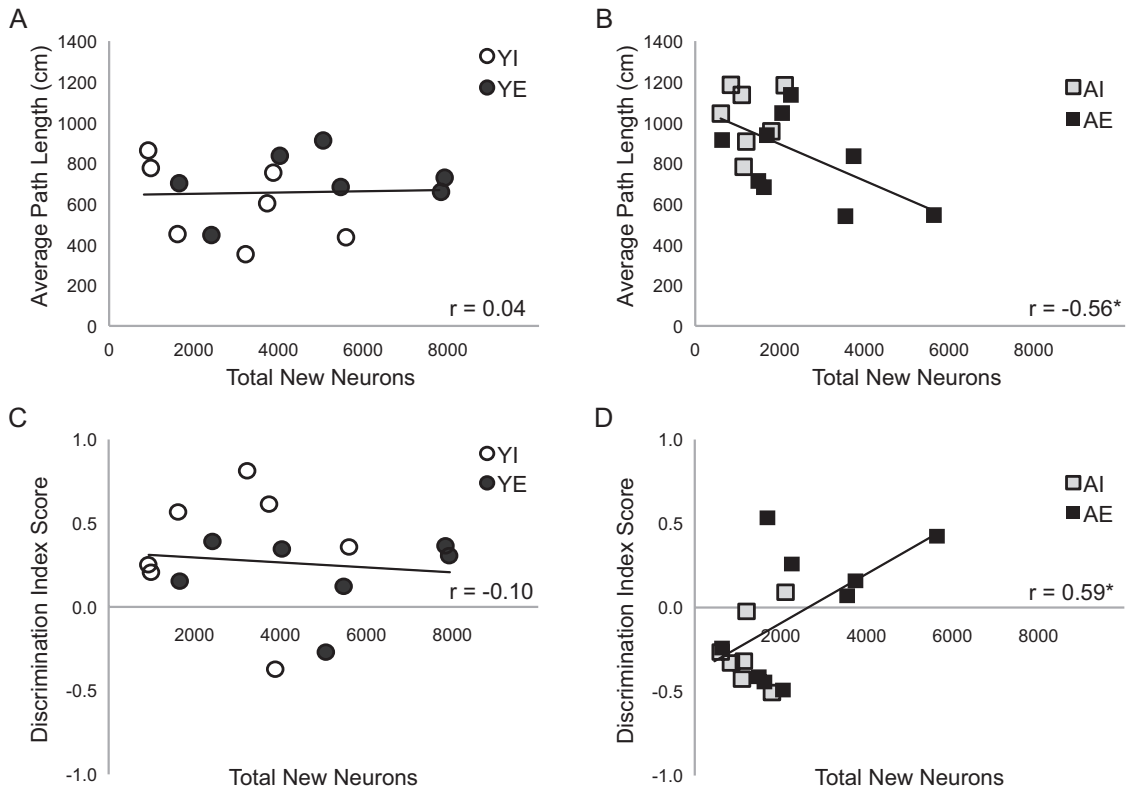

Fig. 6. New neuron number correlates with measures of spatial ability. Graphs depict total neuron number plotted against mean path lengths across hidden platform training blocks (A and C) or against probe trial discrimination index scores (B and D) for young rats housed individually (YI; white circles), young rats housed in an enriched environment (YE; dark gray circles), aged rats housed individually (AI; light gray squares), and aged rats housed in an enriched environment (AE; black squares). Mean path lengths correlated negatively with total new neuron number in aged rats ($r = -0.56$; A). Note that shorter path lengths indicate better performance. (D) The number of new neurons and discrimination index score are correlated positively in aged rats ($r = 0.59$). * $p \leq 0.05$.

ability to discriminate a platform spatially (Fig. 1A and B; Kumar et al., 2012) and visually (Fig. 1C) in a water maze task that masses training sessions into a single day. We did not observe the anticipated beneficial effect of enrichment on water performance in our young rats (Leggio et al., 2005; Schrijver et al., 2002). However, the effects of weeks rather than longer exposures to enrichment on spatial ability may be sex-dependent and only observable in water maze protocols that distribute training across days (for example Frick et al., 2003; Harburger et al., 2007). The rapidly asymptotic performances of YE and YI rats across hidden platform training blocks is consistent with the notion that the rapid water maze task may be sensitive to performance impairments but not enhancements in young rodents and precludes a meaningful evaluation of the relationship between their measures of neurogenesis and spatial ability.

Our data showing that AE and AI rats exhibited similar anxiety levels (percentage of time spent in the outer annulus), fitness (swim speeds and body mass), and perhaps visual acuity (similar performance was exhibited on early visible platform blocks), suggests that enrichment reverses age-related changes in systems mediating spatial and visual discrimination, independent of overt effects on sensorimotor ability. Exposure to enrichment improves cerebellar, in addition to hippocampal function (Camel et al., 1986; Greenough and Volkmar, 1973; Kumar et al., 2012), which could improve both spatial and visual discrimination. In addition, our unpublished data and previous research (Gerlai, 2001; Ormerod and Beninger, 2002) suggests that training on sequential tasks (including spatial vs. visual discrimination) may beneficially or detrimentally affect performance on the second task. Indeed, young rats appeared to readily employ procedural information they acquired on spatial discrimination trials about escaping the water maze on early visual discrimination blocks (Fig. 2A vs. C).

Exposure to an enriched environment produces many effects in the hippocampus that could relate to improved spatial discrimination in aged rats. For example, exposure to an enriched environment increases hippocampal and vascular volumes as well as morphological and electrophysiological measures of plasticity in aged rats (Hattiangady and Shetty, 2008; Kumar et al., 2012; Leventhal et al., 1999; Palmer et al., 2000). In support of other work employing water maze protocols with massed training schedules

(Drapeau et al., 2003; Driscoll et al., 2006), measures of neurogenesis and spatial ability correlated strongly (Fig. 6). This rapid task may be more sensitive to the relationship than distributed training water maze protocols (Bizon and Gallagher, 2003; Bizon et al., 2004; Merrill et al., 2003) because it taxes the hippocampus by requiring faster acquisition and more flexible use of spatial information (Foster, 2012). We also may have simply increased the variability within our measures enough to detect the relationship by exposing aged rats to differential experience.

We cannot conclude that neurogenesis mediates spatial ability from our correlational data. However, our data do suggest that neurogenesis may be a marker of spatial ability and hippocampal integrity in aged rats because aged rats with higher ongoing rates of neurogenesis exhibited better spatial ability than those with lower rates. Indeed, environmental enrichment increases the expression of factors associated with enhanced spatial ability and neurogenesis, such as brain-derived neurotrophic factor (Lee et al., 2002; Obiang et al., 2011) and stimulates the production of factors that are downregulated with age and are known to be neurogenic, such as fibroblast growth factor-2, vascular endothelial growth factor, and insulin growth factor-1 (Shetty et al., 2005). Our data do suggest that future work investigating the relationship between neurogenesis and hippocampal function across age may provide insight into the etiology and potential interventions for age-related cognitive decline.

In summary, we found that several weeks of daily exposure to an enriched environment partially reverses the effects of age on the rapid acquisition of a spatial search strategy in the water maze, potentially through its effects on neurogenesis because we found higher ongoing rates of neurogenesis in aged rats that exhibited better performance in the task. Our data suggest that engaging in mentally and physically stimulating activity could reverse some aspects of age-related cognitive decline perhaps by potentiating neurogenesis.

Disclosure statement

The authors declare no potential conflicts of interest.

All rats used as subjects in this study were treated in accordance with the policies set forth by the University of Florida Institutional Animal Care and Use Committee and the National Institutes of Health and are in accordance with the guidelines established by the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Every effort was made to minimize the number of animals used and their suffering.

Acknowledgements

The authors thank Melissa Ferguson, Prasanna Durairaj, Jose Herrera, and Vijay Parekh for technical assistance and Dr. Gerry Shaw for his gift of chicken anti-GFAP. The study was supported by grants from the National Institutes of Health (AG014979, AG036800, and AG037984) to T.C.F., the McKnight Brain Research Foundation to T.C.F. and B.K.O., and the Broad Foundation for Biomedical Research to B.K.O. and a National Science Foundation Graduate Research Fellowship to R.B.S.

References

Aizawa, K., Ageyama, N., Yokoyama, C., Hisatsune, T., 2009. Age-dependent alteration in hippocampal neurogenesis correlates with learning performance of macaque monkeys. Exp. Anim. 58, 403–407.

Altman, J., Das, G.D., 1965. Post-natal origin of microneurones in the rat brain. Nature 207, 953–956.

Bizon, J.L., Gallagher, M., 2003. Production of new cells in the rat dentate gyrus over the lifespan: relation to cognitive decline. Eur. J. Neurosci. 18, 215–219.

Bizon, J.L., Lee, H.J., Gallagher, M., 2004. Neurogenesis in a rat model of age-related cognitive decline. Aging Cell 3, 227–234.

Camel, J.E., Withers, G.S., Greenough, W.T., 1986. Persistence of visual cortex dendritic alterations induced by postweaning exposure to a "superenriched" environment in rats. Behav. Neurosci. 100, 810–813.

Cameron, H.A., McKay, R.D., 1999. Restoring production of hippocampal neurons in old age. Nat. Neurosci. 2, 894–897.

Cameron, H.A., McKay, R.D., 2001. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J. Comp. Neurol. 435, 406–417.

Cameron, H.A., Woolley, C.S., McEwen, B.S., Gould, E., 1993. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neuroscience 56, 337–344.

Carter, C.S., Leeuwenburgh, C., Daniels, M., Foster, T.C., 2009. Influence of calorie restriction on measures of age-related cognitive decline: role of increased physical activity. J. Gerontol. A Biol. Sci. Med. Sci. 64, 850–859.

Coras, R., Siebzehnrubl, F.A., Pauli, E., Huttner, H.B., Njunting, M., Kobow, K., Villmann, C., Hahnen, E., Neuhuber, W., Weigel, D., Buchfelder, M., Stefan, H., Beck, H., Steindler, D.A., Blümcke, I., 2010. Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans. Brain 133, 3359–3372.

Correa, D.D., DeAngelis, L.M., Shi, W., Thaler, H., Glass, A., Abrey, L.E., 2004. Cognitive functions in survivors of primary central nervous system lymphoma. Neurology 62, 548–555.

Crossen, J.R., Garwood, D., Glatstein, E., Neuwelt, E.A., 1994. Neurobehavioral sequelae of cranial irradiation in adults: a review of radiation-induced encephalopathy. J. Clin. Oncol. 12, 627–642.

Dalla, C., Papachristos, E.B., Whetstone, A.S., Shors, T.J., 2009. Female rats learn trace memories better than male rats and consequently retain a greater proportion of new neurons in their hippocampi. Proc. Natl. Acad. Sci. U. S. A. 106, 2927–2932.

Deng, W., Aimone, J.B., Gage, F.H., 2010. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nat. Rev. Neurosci. 11, 339–350.

Drapeau, E., Mayo, W., Aurousseau, C., Le Moal, M., Piazza, P.V., Abrous, D.N., 2003. Spatial memory performances of aged rats in the water maze predict levels of hippocampal neurogenesis. Proc. Natl. Acad. Sci. U. S. A. 100, 14385–14390.

Drapeau, E., Montaron, M.F., Aguerre, S., Abrous, D.N., 2007. Learning-induced survival of new neurons depends on the cognitive status of aged rats. J. Neurosci. 27, 6037–6044.

Driscoll, I., Howard, S.R., Stone, J.C., Monfils, M.H., Tomanek, B., Brooks, W.M., Sutherland, R.J., 2006. The aging hippocampus: a multi-level analysis in the rat. Neuroscience 139, 1173–1185.

Epp, J.R., Galea, L.A., 2009. Hippocampus-dependent strategy choice predicts low levels of cell proliferation in the dentate gyrus. Neurobiol. Learn. Mem. 91, 437–446.

Epp, J.R., Haack, A.K., Galea, L.A., 2010. Task difficulty in the Morris water task influences the survival of new neurons in the dentate gyrus. Hippocampus 20, 866–876.

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nat. Med. 4, 1313–1317.

Fernández, C.I., Collazo, J., Bauza, Y., Castellanos, M.R., López, O., 2004. Environmental enrichment-behavior-oxidative stress interactions in the aged rat: issues for a therapeutic approach in human aging. Ann. N. Y. Acad. Sci. 1019, 53–57.

Foster, T.C., 1999. Involvement of hippocampal synaptic plasticity in age-related memory decline. Brain Res. Brain Res. Rev. 30, 236–249.

Foster, T.C., 2012. Dissecting the age-related decline on spatial learning and memory tasks in rodent models: N-methyl-D-aspartate receptors and voltage-dependent Ca^2 channels in senescent synaptic plasticity. Prog. Neurobiol. 96, 283–303.

Foster, T.C., Dumas, T.C., 2001. Mechanism for increased hippocampal synaptic strength following differential experience. J. Neurophysiol. 85, 1377–1383.

Foster, T.C., Kumar, A., 2007. Susceptibility to induction of long-term depression is associated with impaired memory in aged Fischer 344 rats. Neurobiol. Learn. Mem. 87, 522–535.

Foster, T.C., Sharrow, K.M., Kumar, A., Masse, J., 2003. Interaction of age and chronic estradiol replacement on memory and markers of brain aging. Neurobiol. Aging 24, 839–852.

Frick, K.M., Fernandez, S.M., 2003. Enrichment enhances spatial memory and increases synaptophysin levels in aged female mice. Neurobiol. Aging 24, 615–626.

Frick, K.M., Stearns, N.A., Pan, J.Y., Berger-Sweeney, J., 2003. Effects of environmental enrichment on spatial memory and neurochemistry in middle-aged mice. Learn. Mem. 10, 187–198.

Gage, F.H., Dunnett, S.B., Björklund, A., 1984. Spatial learning and motor deficits in aged rats. Neurobiol. Aging 5, 43–48.

Galea, L.A., Ormerod, B.K., Sampath, S., Kostaras, X., Wilkie, D.M., Phelps, M.T., 2000. Spatial working memory and hippocampal size across pregnancy in rats. Horm. Behav. 37, 86–95.

Gerlai, R., 2001. Behavioral tests of hippocampal function: simple paradigms complex problems. Behav. Brain Res. 125, 269–277.

Gould, E., Beylin, A., Tanapat, P., Reeves, A., Shors, T.J., 1999. Learning enhances adult neurogenesis in the hippocampal formation. Nat. Neurosci. 2, 260–265.

Greenough, W.T., Volkmar, F.R., 1973. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. Exp. Neurol. 40, 491–504.

Harburger, L.L., Lambert, T.J., Frick, K.M., 2007. Age-dependent effects of environmental enrichment on spatial reference memory in male mice. Behav. Brain Res. 185, 43–48.

Hattiangady, B., Shetty, A.K., 2008. Aging does not alter the number or phenotype of putative stem/progenitor cells in the neurogenic region of the hippocampus. Neurobiol. Aging 29, 129–147.

Kempermann, G., Gast, D., Gage, F.H., 2002. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol. 52, 135–143.

Kempermann, G., Kuhn, H.G., Gage, F.H., 1997. More hippocampal neurons in adult mice living in an enriched environment. Nature 386, 493–495.

Kempermann, G., Kuhn, H.G., Gage, F.H., 1998. Experience-induced neurogenesis in the senescent dentate gyrus. J. Neurosci. 18, 3206–3212.

Kennard, J.A., Woodruff-Pak, D.S., 2011. Age sensitivity of behavioral tests and brain substrates of normal aging in mice. Front. Aging Neurosci. 3, 9.

Kolb, B., Pedersen, B., Ballermann, M., Gibb, R., Whishaw, I.Q., 1999. Embryonic and postnatal injections of bromodeoxyuridine produce age-dependent morphological and behavioral abnormalities. J. Neurosci. 19, 2337–2346.

Kuhn, H.G., Dickinson-Anson, H., Gage, F.H., 1996. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J. Neurosci. 16, 2027–2033.

Kumar, A., Rani, A., Tchigranova, O., Lee, W., Foster, T.C., 2012a. Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. Neurobiol. Aging 33, 828.e1–17.

Kumar, A., Thinschmidt, J.S., Foster, T.C., King, M.A., 2007. Aging effects on the limits and stability of long-term synaptic potentiation and depression in rat hippocampal area CA1. J. Neurophysiol. 98, 594–601.

Lazarov, O., Mattson, M.P., Peterson, D.A., Pimplikar, S.W., van Praag, H., 2010. When neurogenesis encounters aging and disease. Trends Neurosci. 33, 569–579.

Leal-Galicia, P., Castañeda-Bueno, M., Quiroz-Baez, R., Arias, C., 2008. Long-term exposure to environmental enrichment since youth prevents recognition memory decline and increases synaptic plasticity markers in aging. Neurobiol. Learn. Mem. 90, 511–518.

Lee, J., Duan, W., Mattson, M.P., 2002. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. J. Neurochem. 82, 1367–1375.

Leggio, M.G., Mandolesi, L., Federico, F., Spirito, F., Ricci, B., Gelfo, F., Petrosini, L., 2005. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav. Brain Res. 163, 78–90.

Lemaire, V., Koehl, M., Le Moal, M., Abrous, D.N., 2000. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 97, 11032–11037.

Leventhal, C., Rafii, S., Rafii, D., Shahar, A., Goldman, S.A., 1999. Endothelial trophic support of neuronal production and recruitment from the adult mammalian subependyma. Mol. Cell. Neurosci. 13, 450–464.

Lichtenwalner, R.J., Forbes, M.E., Bennett, S.A., Lynch, C.D., Sonntag, W.E., Riddle, D.R., 2001. Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. Neuroscience 107, 603–613.

Lores-Arnaiz, S., Bustamante, J., Arismendi, M., Vilas, S., Paglia, N., Basso, N., Capani, F., Coirini, H., Costa, J.J., Arnaiz, M.R., 2006. Extensive enriched environments protect old rats from the aging dependent impairment of spatial cognition, synaptic plasticity and nitric oxide production. Behav. Brain Res. 169, 294–302.

Lugert, S., Basak, O., Knuckles, P., Haussler, U., Fabel, K., Götz, M., Haas, C.A., Kempermann, G., Taylor, V., Giachino, C., 2010. Quiescent and active hippocampal neural stem cells with distinct morphologies respond selectively to physiological and pathological stimuli and aging. Cell Stem Cell 6, 445–456.

Madsen, T.M., Kristjansen, P.E., Bolwig, T.G., Wörtwein, G., 2003. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. Neuroscience 119, 635–642.

Merrill, D.A., Karim, R., Darraq, M., Chiba, A.A., Tuszynski, M.H., 2003. Hippocampal cell genesis does not correlate with spatial learning ability in aged rats. J. Comp. Neurol. 459, 201–207.

Monje, M.L., Vogel, H., Masek, M., Ligon, K.L., Fisher, P.G., Palmer, T.D., 2007. Impaired human hippocampal neurogenesis after treatment for central nervous system malignancies. Ann. Neurol. 62, 515–520.

Nacher, J., Alonso-Llosa, G., Rosell, D.R., McEwen, B.S., 2003. NMDA receptor antagonist treatment increases the production of new neurons in the aged rat hippocampus. Neurobiol. Aging 24, 273–284.

Obiang, P., Maubert, E., Bardou, I., Nicole, O., Launay, S., Bezin, L., Vivien, D., Agin, V., 2011. Enriched housing reverses age-associated

impairment of cognitive functions and tPA-dependent maturation of BDNF. Neurobiol. Learn. Mem. 96, 121–129.

Ormerod, B.K., Beninger, R.J., 2002. Water maze versus radial maze: differential performance of rats in a spatial delayed match-to-position task and response to scopolamine. Behav. Brain Res. 128, 139–152.

Ormerod, B.K., Lee, T.T., Galea, L.A., 2004. Estradiol enhances neurogenesis in the dentate gyri of adult male meadow voles by increasing the survival of young granule neurons. Neuroscience 128, 645–654.

Palmer, T.D., Willhoite, A.R., Gage, F.H., 2000. Vascular niche for adult hippocampal neurogenesis. J. Comp. Neurol. 425, 479–494.

Raber, J., Rola, R., LeFevour, A., Morhardt, D., Curley, J., Mizumatsu, S., VandenBerg, S.R., Fike, J.R., 2004. Radiation-induced cognitive impairments are associated with changes in indicators of hippocampal neurogenesis. Radiat. Res. 162, 39–47.

Rapp, P.R., Rosenberg, R.A., Gallagher, M., 1987. An evaluation of spatial information processing in aged rats. Behav. Neurosci. 101, 3–12.

Roman, D.D., Sperduto, P.W., 1995. Neuropsychological effects of cranial radiation: current knowledge and future directions. Int. J. Radiat. Oncol. Biol. Phys. 31, 983–998.

Saxe, M.D., Battaglia, F., Wang, J.W., Malleret, G., David, D.J., Monckton, J.E., Garcia, A.D., Sofroniew, M.V., Kandel, E.R., Santarelli, L., Hen, R., Drew, M.R., 2006. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proc. Natl. Acad. Sci. U. S. A. 103, 17501–17506.

Schrijver, N.C., Bahr, N.I., Weiss, I.C., Würbel, H., 2002. Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol. Biochem. Behav. 73, 209–224.

Segovia, G., Yagüe, A.G., García-Verdugo, J.M., Mora, F., 2006. Environmental enrichment promotes neurogenesis and changes the extracellular concentrations of glutamate and GABA in the hippocampus of aged rats. Brain Res. Bull. 70, 8–14.

Seifert, A.W., Zheng, Z., Ormerod, B.K., Cohn, M.J., 2010. Sonic hedgehog controls growth of external genitalia by regulating cell cycle kinetics. Nat. Commun. 1, 23.

Shetty, A.K., Hattiangady, B., Shetty, G.A., 2005. Stem/progenitor cell proliferation factors FGF-2, IGF-1, and VEGF exhibit early decline during the course of aging in the hippocampus: role of astrocytes. Glia 51, 173–186.

Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y., Gould, E., 2002. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus 12, 578–584.

Siffert, J., Allen, J.C., 2000. Late effects of therapy of thalamic and hypothalamic tumors in childhood: vascular, neurobehavioral and neoplastic. Pediatr. Neurosurg. 33, 105–111.

Siwak-Tapp, C.T., Head, E., Muggenburg, B.A., Milgram, N.W., Cotman, C.W., 2007. Neurogenesis decreases with age in the canine hippocampus and correlates with cognitive function. Neurobiol. Learn. Mem. 88, 249–259.

Snyder, J.S., Hong, N.S., McDonald, R.J., Wojtowicz, J.M., 2005. A role for adult neurogenesis in spatial long-term memory. Neuroscience 130, 843–852.

Uylings, H.B., van Eden, C.G., Hofman, M.A., 1986. Morphometry of size/volume variables and comparison of their bivariate relations in the nervous system under different conditions. J. Neurosci. Methods 18, 19–37.

van Praag, H., Schinder, A.F., Christie, B.R., Toni, N., Palmer, T.D., Gage, F.H., 2002. Functional neurogenesis in the adult hippocampus. Nature 415, 1030–1034.

van Praag, H., Shubert, T., Zhao, C., Gage, F.H., 2005. Exercise enhances learning and hippocampal neurogenesis in aged mice. J. Neurosci. 25, 8680–8685.

West, M.J., Slomianka, L., Gundersen, H.J., 1991. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. Anat. Rec. 231, 482–497.

Winocur, G., Wojtowicz, J.M., Sekeres, M., Snyder, J.S., Wang, S., 2006. Inhibition of neurogenesis interferes with hippocampus-dependent memory function. Hippocampus 16, 296–304.