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Exposure to Enriched Environment Improves Spatial Learning Performances and Enhances Cell Density but Not Choline Acetyltransferase Activity in the Hippocampus of Ventral Subicular–Lesioned Rats

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The authors demonstrated the efficacy of enriched housing conditions in promoting the behavioral recovery and neuronal survival following subicular lesion in rats. Chemical lesioning of the ventral subiculum impaired the spatial learning performances in rats. The lesion also induced a significant degree of neurodegeneration in the CA1 and CA3 areas of the hippocampus and entorhinal cortex. Exposure to enriched housing conditions improved the behavioral performance and partially attenuated the neurodegeneration in the hippocampus. The choline acetyl transferase (ChAT) activity in the hippocampus remained unchanged following ventral subicular lesion and also following exposure to an enriched environment. The study implicates the effectiveness of activity-dependent neuronal plasticity induced by environmental enrichment in adulthood following brain insult.

Keywords: subicular lesion, neurodegeneration, ChAT activity, enriched housing, behavioral recovery

The subiculum, positioned between the hippocampus proper and the entorhinal cortex, is considered to be the output station of the CA1 hippocampus. Compared with the in-depth analysis of the hippocampus proper and other associated areas for their role in spatial learning and memory functions, the subiculum has received less attention. Few studies have reported that lesioning of the subiculum impairs spatial learning (Devi, Diwakar, Raju, & Kutty, 2003; Govindaiah, Rao, Raju, & Meti, 1997; Laxmi, Bindu, Raju, & Meti, 1999; Morris et al., 1990; Oswald & Good, 2000). Laxmi et al. (1999), by means of discrete lesions of the subiculum, showed an impairment in the acquisition of a reward alternation test in a T maze without altering the retention capacity. Devi et al. (2003) observed an impairment in both the acquisition and retention of an eight-arm radial maze task. Bindu, Rekha, and Kutty (2005) reported an impairment in a water maze task following ventral subicular lesion. In addition, ventral subicular lesion produces neurodegeneration in the entorhinal cortex and the CA1 and CA3 regions of the hippocampus (Devi et al., 2003; Galani, Jarrard, Will, & Kelche, 1997; Govindaiah et al., 1997; Morris Schenk, Tweedie, & Jarrard, 1990). The anatomical connections between the subiculum and other structures of the hippocampal formation suggest the possibility of both retrograde and anterograde degeneration following subicular lesion. Neurodegeneration of the pyramidal neurons in the entorhinal cortex and hippocampus, coupled with cognitive dysfunction following ventral subicular lesion, is of particular concern because of the susceptibility of these brain regions to neurodegenerative diseases, such as Alzheimer's disease (Davies, Horwood, Isaacs, & Mann, 1992; Dickson, Crystal, et al., 1992, Dickson, Ksiezak-Reding, et al., 1992; Struble et al., 1991; Wang, Tanila, Puolivali, Kadish, & Van Groen, 2003). Developing strategies to ameliorate the hippocampus-dependent memory deficits and neurodegeneration following ventral subicular lesion may have clinical significance.

Exposure to an enriched environment provides better opportunities for physical and social stimulation (Leggio et al., 2005; Rosenzweig, Bennett, & Diamond, 1972; Rosenzweig, Krech, Bennett, & Diamond, 1962). The adult rat brain exhibits significant plasticity in response to environmental stimulation, leading to increased cortical thickness, neurogenesis, glial density, and dendritic arborization (Diamond, Ingham, Johnson, Bennett, & Rosenzweig, 1976; Diamond, Lindner, Johnson, Bennett, & Rosenzweig, 1975; Kempermann, Brandon, Gage, 1998; Kempermann & Gage, 1999; Kempermann, Kuhn, & Gage, 1997, 1998; Leggio et al., 2005; Mohammed et al., 1993; Mohammed, Winblad, Ebendal, & Larkfors, 1990; Rosenzweig, 2003; Rosenzweig & Bennett, 1996). Other studies have delineated the importance of appropriate enrichment in accordance with the functional demands of the task and in bringing therapeutic effectiveness (Will, Galani, Kelche, & Rosenzweig, 2004). Bennett, McRae, Levy, and Frick (2006) observed an overall enhancement of spatial memory functions in aged mice when exposed continuously to an enriched environment for a long period rather than when provided with enrichment on a

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daily basis. Enriched environments have been shown to alter the expression of several neurotrophic factors, such as nerve growth factor and basic fibroblast growth factor, thereby enhancing postlesion recovery (Dahlqvist et al., 1999; Mohammed et al., 1990, 1993). Gobbo and O'Mara (2004) demonstrated that ischemic rats housed in an enriched environment performed better in odor discrimination, object exploration, and the water maze tasks than did those housed under standard conditions. Will et al. (2004) summarized the various aspects of enriched housing conditions' ability to evoke neuronal plasticity in order to remodel neuronal circuitry and how such reorganization contributes to the recovery of behavior compromised by brain injury. Few studies have demonstrated the beneficial effect of an enriched housing condition on the behavioral performance of spatial tasks in subicular-lesioned rats (Bindu et al., 2005; Galani et al., 1997). In the present study we investigated the possible effect of an enriched environment in promoting the behavioral recovery and neuronal survival in the hippocampus of ventral subicular–lesioned rats.

Method

Subjects

Experiments were carried out with 2-month-old male Wistar rats. They were maintained at the Central Animal Research Facility at the National Institute of Mental Health and Neuro Sciences in Bangalore, India. Experiments were initiated after obtaining clearance from the Institutional Animal Ethics Committee. The rats were randomly divided into 5 groups: normal control rats (NC), sham control rats (SC), ventral subicular–lesioned rats exposed to a standard housing condition (VSL), ventral subicular–lesioned rats exposed to an enriched housing condition (VSL + EE), and normal control rats exposed to an enriched housing condition (EE).

Standard Housing Condition

The NC, SC, and VSL rats were housed in standard polypropylene cages with a dimension of 29 × 22 × 14 cm. Food and water were provided ad libitum. Two to 3 rats were kept together in individual cages, and 12-hr light–dark cycles were maintained. The room temperature was kept at 26 ± 2°C.

Socially Enriched Housing Conditions

The rats in the EE and VSL + EE groups were kept in specially designed cages that were 81.5 × 61 × 45 cm. Eight to 10 rats were housed together to provide social stimulation. Various exploratory materials such as plastic tunnels, platforms, sandboxes, balls, rattles, and sawdust, along with wooden objects of different textures and toys of different sizes and shapes, were provided. In addition, the cages also contained metal platforms kept at different heights connected by staircases for the animals to explore. The objects were changed daily for novelty stimulation.

Lesioning of the Ventral Subiculum

Rats were anesthetized with sodium pentabarbitone (40 mg/kg b.w. i.p.) and positioned in a stereotaxic instrument. The flat skull stereotaxic coordinates were adapted from Paxinos and Watson's (1982) rat brain atlas for ventral subiculum: AP = –7.0 to –7.2 mm, ML = 5.0–5.2 mm, and DV = 5.0–5.3 mm. Ibotenic acid (0.5 μg/0.5 μl/site) was injected bilaterally into the ventral subiculum at a speed of 0.5 μl/min with a micro injector. SC rats underwent the same surgical procedures, with the exception that they received 0.5 μl of phosphate-buffered saline instead of ibotenic acid. The NC rats were not exposed to any surgery and were reared in standard cages.

Following ventral subicular lesion, rats were housed in either standard cages (VSL rats) or in enriched cages (VSL + EE rats) for 10 days continuously. Immediately after exposure to environmental enrichment, the rats were subjected to various experiments.

Experiment 1: Behavioral Assessment

Behavioral assessment of spatial learning was carried out with an eight-arm radial maze (Columbus Instruments, Columbus, OH). During the training period, the rats were kept in standard cages only and not in enriched cages. A total of 50 rats from different groups (NC, SC, VSL, VSL + EE, and EE; n = 10/group) were used for the behavioral study.

Acquisition of the Spatial Task

Training. The rats were semistarved for 48 hr to reduce their body weight to 85%. Then they were placed in the center of the radial maze for 15 min on the 1st day and allowed to freely explore and familiarize themselves with it. Each rat was given two trials daily, with an intertrial interval of 15 min. To avoid olfactory cues, we wiped the maze with 70% ethanol prior to each session.

Acquisition. During the acquisition phase, all eight arms of the maze were baited with food pellets, and the rats were trained to retrieve the pellets from each arm. Reentry into an already visited arm was considered as a working memory error. Entry into an arm was considered complete when all four paws had entered an arm of the maze. A trial was terminated when a rat had either taken the food reward from all eight arms or spent 10 min in the maze. The performance of a rat was scored by calculating the percentage of correct responses (a correct entry was when the rat had not previously entered the arm) divided by the total number of entries made by the rat. Acquisition was considered successful based on attaining 87.5%–100% correct choices, that is, when the rats entered seven out of eight arms correctly (Devi et al., 2003).

Retention Test

A retention test was carried out 10 days following the acquisition phase. In the retention test, performance in a single trial was assessed.

Experiment 2: Histology

A total of 30 rats from different groups (NC, SC, VSL, VSL + EE, and EE groups; n = 6/group) were used for histological verification of neurodegeneration. Ten days following exposure to an enriched environment, the rats from all the groups were anesthetized and perfused transcardially with 0.9% saline followed by 10% formalin. Their brains were removed and kept in 10% for-

malin for 48 hr. Coronal sections of 10 μm thickness were taken at the level of the dorsal hippocampus with vibratome (Vibratome, St. Louis, MO) and were stained with 0.1% Cresyl violet. The cell density in the CA1 and CA3 subsectors of the hippocampus and entorhinal cortex was quantified as per the method described by Govindaiah et al. (1997) and Devi et al. (2003). That is, the number of healthy, viable cells over 120 μm long in the CA1 and CA3 subsectors of the dorsal hippocampus and in the entorhinal cortex (at the level of the lateral hippocampus) over an area of 125 μm^2 were counted under a light microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of 500×. All the slides were coded to avoid any bias by the experimenter. A total of 36 brain sections from each group (6 sections/rat/group) were used to assess the cell density.

Experiment 3: Choline Acetyl Transferase (ChAT) Immunohistochemistry

For an immunohistochemistry assay, a total of 15 rats from different groups (NC, VC, VSL, VSL + EE, and EE groups; $n = 3$/group) were used. The rats from all the groups were anesthetized and perfused transcardially with a phosphate buffer saline (PBS; pH 7.4) followed by 4% paraformaldehyde. Their brains were removed, postfixed for 48 hr at 4ºC, and cryoprotected in a 30% sucrose solution for 2 days. A serial section of 30 μm thickness was taken with cryostat at the level of the dorsal hippocampus (Bregma AP is –3.8 mm) and stored at –20ºC. Mounted sections were washed three times in 0.1 M PBS (pH: 7.4) and incubated in 0.1% triton X–100/SDS for 20 min. Following several washes in PBS, the sections were incubated in 10% methanol for 20 min and subsequently in 2% bovine serum albumin for 1 hr. The sections were then washed in PBS three times and incubated in the primary antibody Goat Anti-ChAT at 1:100 dilution (AB144P; Chemicon International, Temecula, CA) for 24 hr at room temperature. After several washes in PBS, the sections were incubated with a secondary antibody (Anti-Goat FITC, 1:200 dilution; Sigma-Aldrich, St. Louis, MO) for 1 hr 15 min. Following several more washes in PBS, the sections were cover-slipped with 65% glycerol and observed under a fluorescence microscope (Leica, Germany) at 486 nm, and the images were captured with a CCD camera. Negative controls were processed in the same manner, except that the primary antibody incubation step was replaced by incubation with PBS. A double blind study was adapted to avoid any bias by the experimenter.

Experiment 4: SDS Polyacrylamide Gel Electrophoresis and Western Blotting

Five rats from each group (NC, VC, VSL, VSL + EE, and EE) were used for Western blotting. The rats were decapitated, and the hippocampus from both sides was dissected out and stored at –80°C. The hippocampus was homogenized in 1 ml of ice cold buffer (10% sucrose, 1 mM EDTA in 20 mM Tris–HCl; pH 7.4) and centrifuged at 12,000 rpm for 12 min at 4°C with a refrigerated centrifuge (Mikro22R, Hettich, Zentrifugen, Germany). The homogenate was used for protein concentration, and levels were normalized for gel loading with a loading buffer. Prior to gel loading, mercaptoethanol was added and samples were boiled for 10 min. The samples (150 μg) were then resolved onto 4%–10% SDS-polyacrylamide gels by electrophoresis at 70 V for 30 min followed by 150 V for an additional hour. Protein from the gels was transferred to polyvinylidene difluoride membranes (BioTraceTM PVDF, Muncie, IN) at 150 mA for 90 min. The membrane was washed with TBS–Tween (250 nM Tris–HCl, 0.9% NaCl, and 0.1% Tween 20) blocked with 5% nonfat milk in TBS–Tween for 2 hr.

The membrane was incubated with goat polyclonal antibody against ChAT (1:1000, Chemicon International) overnight at 4°C. Subsequently the membranes were washed in TBS–Tween and incubated with biotinylated antigoat secondary antibody (1:500, Vector Laboratories, Burlingame, CA) for 1 hr. The blots were further developed with avidin–biotin–alkaline phosphatase and Vector Black substrate kits (ABC–AP Vectastain Kits AK 5002, Kit–SK 5200; Vector Laboratories, Burlingame, CA); β tubulin (Abcam, Cambridge, United Kingdom) was used as loading control.

Quantification of Western Blotting

Quality 1 Gel documentation system was used to quantify the blots (Gel Doc 2000, Bio-Rad, Hercules, CA), and the peak optical intensity of bands was detected with Image analysis software.

Statistical Analysis

A two-way analysis of variance (ANOVA) with repeated measures followed by a least significant difference post hoc test was applied for acquisition of spatial learning performance. Retention of spatial learning performance and cell density was analyzed by a one-way ANOVA followed by a least significant difference post hoc test. The peak optical density of ChAT bands (Western blots) was analyzed with a one-way ANOVA.

Results

Acquisition of the Spatial Task

Figure 1A depicts the spatial learning performance of rats from different groups. The control groups (NC and SC) attained 100% acquisition of the spatial task by 8–9 days (i.e., by 16–18 trials). As the training advanced, the errors committed by the control rats decreased progressively. These rats followed an egocentric pattern to retrieve the food reward. The performance of the EE groups was comparable to that of the NC and SC groups ($p < .97$; see Figure 1A). However, the VSL rats (ventral subicular–lesioned rats maintained under standard housing conditions) committed more errors and made random entries irrespective of whether they had already visited the arms or not. The VSL + EE rats (those exposed to an enriched housing condition) performed better in the radial arm maze when compared with the VSL rats. They were able to attain the 100% criterion by the 9th day, and their behavioral performance was comparable to that of the control groups (NC and SC).

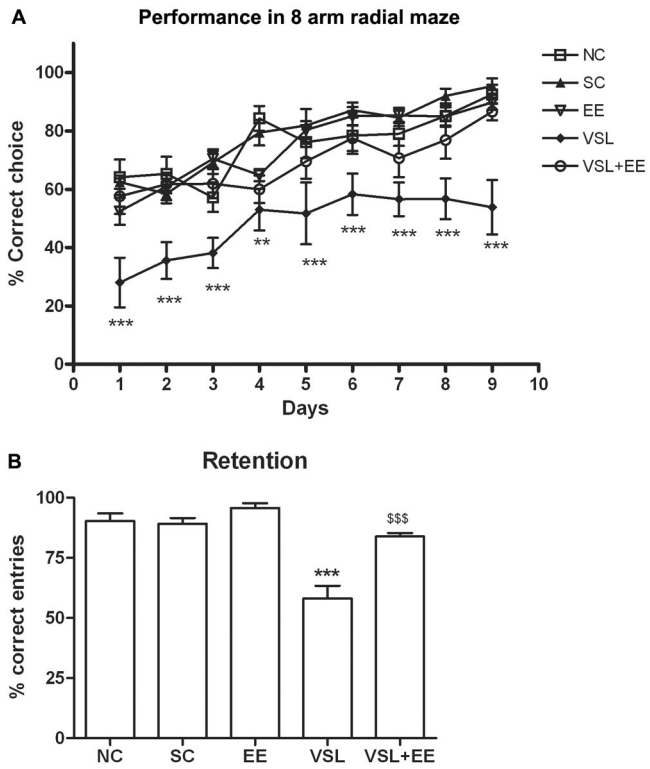

Figure 1. Performance of rats in the eight-arm radial maze. (A) Acquisition of spatial task: The graph plots the mean percentage of correct choices made by the rats as a function of time. Each rat received two trials per day. Note the severe behavioral impairment in the ventral subicular lesioned rats maintained in standard housing conditions (VSL) throughout the trial compared with normal control rats (NC), sham control rats (SC), and normal control rats exposed to an enriched environment (EE). The NC, SC, and EE groups attained the criterion of correct choice within 8–9 days of training, whereas the VSL rats could attain only up to 45%–55% of correct choices by the 9th day. Also note the behavioral recovery in the ventral subicular–lesioned rats reared in enriched housing conditions (VSL + EE). Each value represents the mean ± *SEM* ($n = 10$). $^{***}p < .0001$ and $^{**}p < .01$, comparison of the NC, SC, and EE groups with the VSL group by two-way ANOVA with repeated measures followed by least significant difference post hoc test. (B) Retention of spatial performance in the eight-arm radial maze task. The bar graph shows the five rat groups' degree of retention of the task 10 days after acquisition of it. The NC, SC, and EE rats demonstrated almost 100% retention, whereas the VSL group showed impaired performance. $^{***}p < .0001$ comparison of the NC, SC, and EE groups with the VSL group, and $^{\$\$\$}p < .0001$ comparison of the VSL group with the VSL + EE group, by one-way ANOVA followed by least significant difference post hoc test.

Retention

As seen in Figure 1B, the VSL rats showed impaired performance during the retention test when compared with other groups ($p < .0001$). The NC, SC, and EE rats showed almost a 100% correct performance; they were able to reach the set criterion of 7–8 choices without making many errors. The performance of VSL + EE rats was also comparable to that of the control groups.

In summary, exposure to an enriched environment helped to overcome the ventral subicular lesion–induced spatial learning impairment in rats.

Histological Verification of Lesion Site

Ibotenic acid produced complete lesioning of the ventral subiculum (see Figure 2B) with a lesion size of 1.5–2 mm^3. The lesion did not spread to neighboring areas, and no differences in the lesion size were observed between the VSL and VSL + EE groups. Figure 2C depicts the schematic representation of the extent of the subicular lesion following the ibotenic acid infusion.

Histological Assessment of Cell Density in the Hippocampus and Entorhinal Cortex

Bilateral ventral subicular lesioning has resulted in profound neurodegeneration in the CA1 and CA3 subfields of the dorsal hippocampus (see Figure 3A) and the entorhinal cortex (see Figure 3B). As shown in Figure 4, CA1 and CA3, the cell density decreased by a significant 83.76% (*M* ± *SEM*; 3.23 ± 0.62 viable cells/120 μm; $p < .0001$) in the CA1 subfield and by 71.69% (4.56 ± 1.28 viable cells/120 μm; $p < .0001$) in the CA3 area of the dorsal hippocampus. Similarly, the lesion produced a profound decrease in cell density in the entorhinal cortical layers (see Figure 4, EC Layers I–VI); cell density was reduced by 62.52% in Layer I (1.78 ± 0.3 viable cells/120 μm^2; $p < .0001$), by 55% in Layer II (6.93 ± 0.52 viable cells/120 μm^2; $p < .0001$), and by 30% in Layers III, V, and VI (13.02 ± 0.85; 13.05 ± 0.63; 15.50 ± 0.49 viable cells/120 μm^2; $p < .0001$, respectively). However, Layer IV pyramidal cells did not show any degeneration following ventral subicular lesion.

Exposure to an enriched environment helped to partially attenuate the ventral subicular lesion–induced neurodegeneration. There were higher numbers of healthy, viable cells in the hippocampus and the entorhinal cortex when compared with the VSL rats housed under standard housing conditions. The cell density in the CA1 and CA3 regions of VSL + EE rats was 57.5% (11.44 ± 0.94; $p < .0001$; see Figure 4, CA1) and 67.77% (10.91 ± 1.4; $p < .0001$; see Figure 4, CA3), respectively. Similarly, in the entorhinal cortex, the different layers showed different degrees of recovery. The cell density in Layers II, V, and VI was 59.3% (9.16 ± 0.85 viable cells/120 μm^2; $p < .005$), 75.53% (18.44 ± 0.67 viable cells/120 μm^2, $p < .0001$), and 94.57% (21.97 ± 0.66 viable cells/120 μm^2, $p < .0001$), respectively, whereas Layers I and III did not show any significant recovery (2.4 ± 0.56 viable cells/120 μm^2; $p < .133$ and 15.13 ± 0.85 viable cells/120 μm^2; $p < .358$, respectively) and were comparable to that of the VSL rats exposed to standard housing conditions.

Bilateral subicular lesion did not produce any neurodegeneration in Layer IV of the entorhinal cortex. However, following exposure to enriched housing conditions, there was a significant enhancement in cell density in Layer IV in the VSL + EE group ($p < .001$). Similarly, both EE and VSL + EE rats also showed a profound increase in cell density in the dentate gyrus (see Figure 3A).

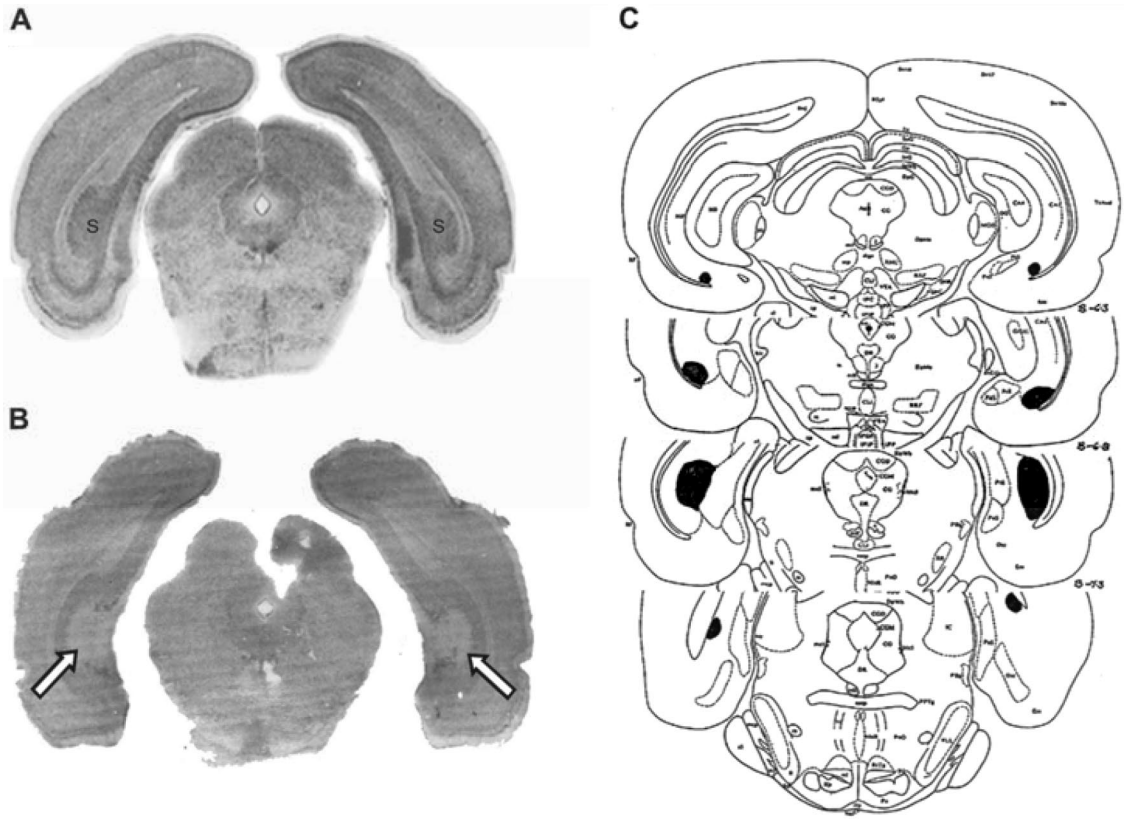

Figure 2. Photomicrographs of cresyl violet–stained coronal brain sections at the level of the ventral subiculum in the normal control rat (A) and the ventral subicular lesioned rat exposed to standard housing conditions (B; lesion sites are indicated by arrows). Bilateral injections of ibotenic acid into the ventral subiculum resulted in complete cell loss in the subiculum (see S in Section A). (C) Schematic representation of the extent of lesion of the ventral subiculum by ibotenic acid. Shaded areas indicate the cell damage by ibotenic acid in different planes, from bregma –6.3 to –7.3 mm. Adapted from *The Rat Brain in Stereotaxic Coordinates,* G. Paxinos and C. Watson, plates –5.8, –6.3, –6.8, and –7.3, copyright 1982, with permission from Elsevier.

Qualitative Assessment of ChAT Immunohistochemistry

The VSL rats showed reduced expression of ChAT immunopositive terminals in the dorsal hippocampus when compared to the control rats (see Figure 5). The expression of ChAT immunopositive terminals appears similar in the NC, VSL + EE, and EE groups.

Western Blot Analysis of ChAT Activity in the Hippocampus

Western blot analysis and the relative optical density of ChAT in the hippocampus showed a specific band at 72 kDa. No significant change in ChAT levels was observed between the five groups, $F(4, 20) = 2.255$, $p < .099$. The ChAT activity remained unchanged following lesion and also following enriched housing in the lesioned rats when the whole hippocampus was analyzed. However, an increase in ChAT expression was observed in the EE group, though not statistically significant (see Figures 6A and B).

Discussion

In the present study, we have demonstrated that postlesion exposure to an enriched environment ameliorates the subicular lesion–induced memory deficits and partially mitigates neurodegeneration in the hippocampus and entorhinal cortex. Will et al. (1986) have demonstrated the positive influence of enriched housing conditions in hippocampus lesioned rats but not in rats with entorhinal cortex lesions. They have reported that enriched housing conditions improved the maze learning, spontaneous alteration, and extinction test in dorsal hippocampus lesioned rats. Galani, Coutureau, and Kelche (1998) further elucidated the efficacy of an enriched environment on certain tasks, such as the Hebb–Williams maze task (Galani, Coutureau, & Kelche, 1998), but not on a novelty test or the Morris water maze task (Galani, Coutureau, & Kelche, 1998). These studies emphasized that an enriched environment would enable the recovery of certain behavioral tasks but not all and that recovery is lesion-locus dependent. Bindu et al. (2005) have also reported that ventral subicular–lesioned rats exposed to enriched housing experienced behavioral recovery in the eight-arm radial maze task but not in the water maze task. They have concluded that the recovery may be task-dependent and accordingly appropriate environmental stimulations may be required to observe behavioral recovery in water maze tasks. Although functional recovery was observed in ventral subicular–lesioned rats, normal rats housed in enriched cages (EE) did not

show such enhanced performance. Few studies have detected any behavioral enhancement after enrichment in normal animals (Biernaskie & Corbett, 2001; Farrell, Evans, & Corbett, 2001; Kolb & Gibb, 1991), although others have observed some beneficial effect (Kempermann et al., 1997; Moser & Andersen, 1994).

As mentioned in the above studies, various factors, including the duration of exposure to enriched environment, may be critical to inducing behavioral changes in adult rats. In the present study, we have observed behavioral recovery in ventral subicular–lesioned rats following continuous exposure to enriched housing for 10 days. The radial arm maze explicitly requires working memory, and the hippocampus is essential for mediating working memory. Following enriched housing the rats made fewer errors in the radial maze, and accordingly their performance also improved. Environmental enrichment may not compensate for the damaged function per se, but it may influence the unaffected abilities of the rat (Will, Rosenzweig, & Bennett, 1976). Additionally, various aspects, such as continuous exposure to an enriched environment (Bennett et al., 2006) and physical exercise on the voluntary running wheel (Pietropaolo, Feldon, Alleva, Cirulli, & Yee, 2006), exert multiple effects on the brain functions. For the present study, we adopted a short-term, continuous-enrichment approach without physical exercise that has resulted in enhanced behavioral recovery.

Our study has shown an attenuation of cell death in the hippocampus and entorhinal cortex of ventral subicular–lesioned rats following short-term enriched housing. Exposure to an enriched environment has been shown to reduce spontaneous apoptotic cell death in the CA3 and dentate gyrus following seizures (Young, Lawlor, Leone, Dragunow, & During, 1999). Passineau, Green, and Dietrich (2001) have indicated that an enriched environment attenuates tissue destruction in order to preserve cell integrity. They observed an overall decrease in lesion size, microglial infiltration, and tissue vacuolization with behavioral recovery in the Morris water maze task. Bindu et al. (2007) have reported the efficacy of enriched housing on inducing morphological plasticity in terms of enhanced dendritic arborization and spine density of hippocampal pyramidal neurons in ventral subicular–lesioned rats. They have attributed behavioral recovery to the positive effect of enriched housing in inducing hippocampal neuronal plasticity. Perhaps improved radial arm performance of VSL + EE rats in the present study may be correlated with the positive effect of an enriched environment on cell survival in the hippocampal and entorhinal cortex.

ChAT activity in the hippocampus remained unchanged following lesion and after an enriched environment. Park, Pappas, Murtha, and Ally (1992), however, have reported enhanced ChAT activity in the hippocampus and cortex of rats exposed to environmental enrichment for a longer duration of 50 days and following spatial learning in the Morris water maze. No change in ChAT activity was observed in untrained rats exposed to either environmental enrichment or impoverished conditions. Park and

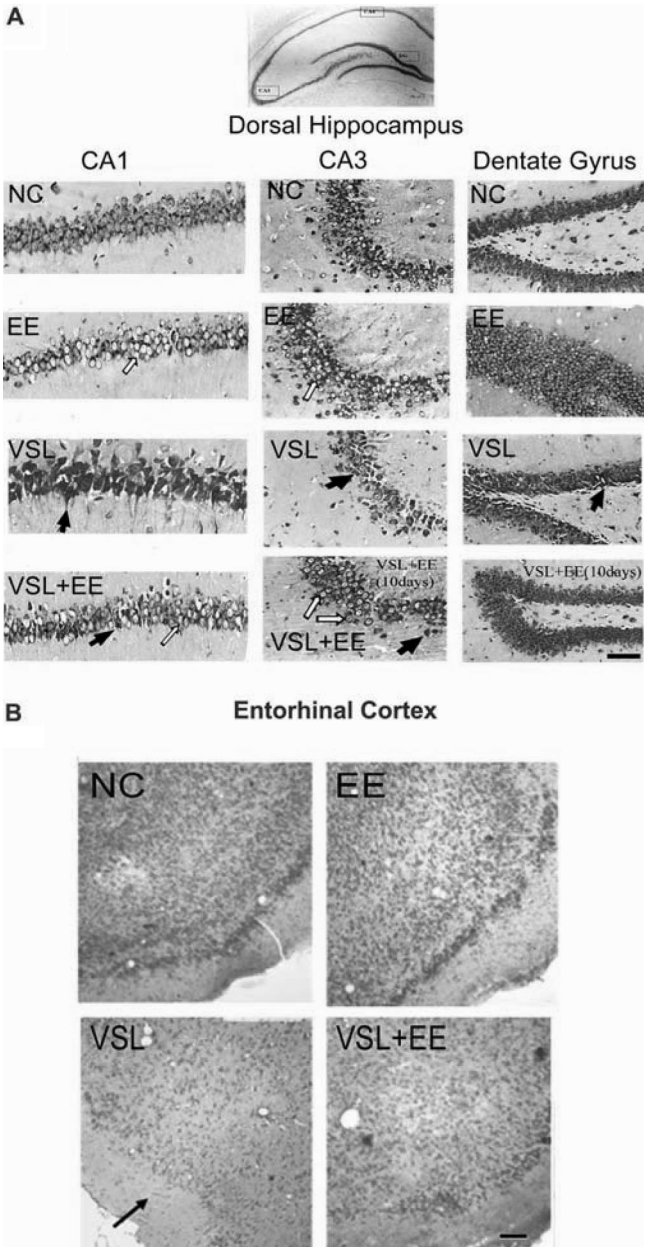

Figure 3. (A) Photomicrographs of the cresyl violet–stained coronal brain sections CA1, CA3, and dentate gyrus at the level of the dorsal hippocampus in normal control rats (NC), normal control rats exposed to enriched housing conditions (EE), and ventral subicular lesioned rats exposed to either standard housing conditions (VSL) or enriched housing conditions (VSL + EE). Note the prominent degeneration of cells in the CA1 and CA3 hippocampus of the VSL rats. And note the significant attenuation of neurodegeneration in the VSL + EE rats housed in socially enriched conditions for 10 days. Viable neurons could be easily distinguished from degenerating neurons by their large size, medium intensity staining, and dark nucleoli (see white arrows). In contrast, most of the degenerating neurons were shrunken and darkly stained (pyknotic cells; see black arrows). Note the increased cell density in the dentate gyrus of the EE and VSL + EE rats following exposure to an enriched environment. Scale bar = 120 μm. (B) Photomicrographs of the cresyl violet–stained coronal brain sections at the level of the entorhinal cortex of the NC, EE, VSL, and VSL + EE rats. Note a marked decrease in cell density in entorhinal cortical layers I, II, III, V, and VI of VSL rats (see arrow). Also note the minimal cell loss in the VSL + EE rats. Scale bar = 90 μm.

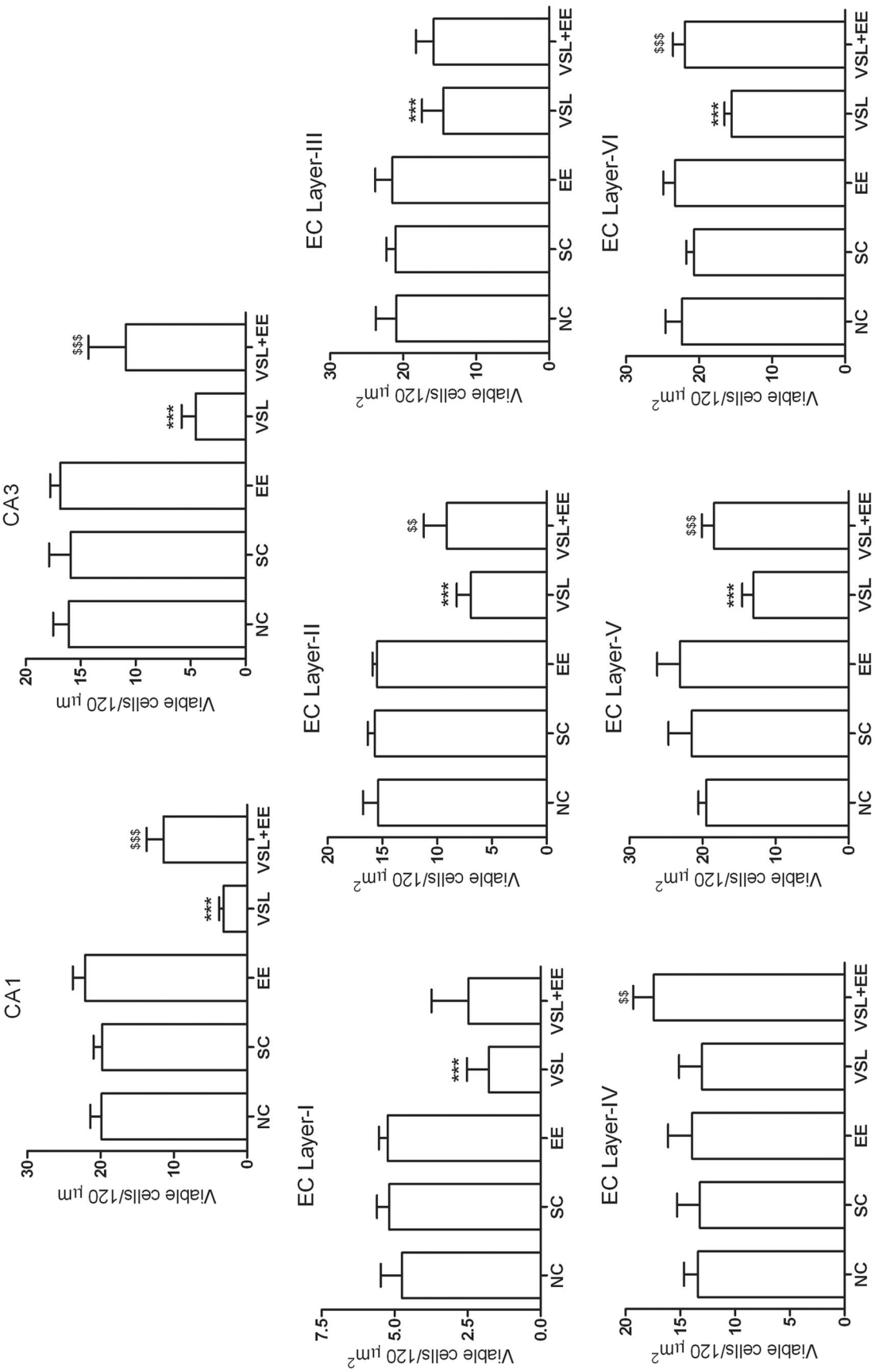

Figure 4. Cell density in the CA1 and CA3 areas of the dorsal hippocampus and entorhinal cortex (EC) in the five groups of rats. Data represent the mean ± *SEM* of healthy/viable cells over 120 μm in length in the dorsal hippocampus and 120 μm^2 in area in the EC. The cell density was significantly reduced in ventral subicular lesioned rats exposed to standard housing conditions (VSL) compared with all other groups of rats. Exposure to enriched housing has been shown to enhance cell survival, and accordingly, cell density in the ventral subicular lesioned rats exposed to enriched housing conditions (VSL + EE) was significantly higher than that of VSL rats. $^{***}p < .0001$ comparison of VSL group with control groups (NC = normal control rats; SC = sham control rats; EE = normal control rats housed in enriched environmental conditions). $^{\$\$}p < .01$ comparison of VSL group with VSL + EE group. $^{\$\$\$}p < .0001$ comparison of VSL group with VSL + EE group by one-way ANOVA followed by least significant difference post hoc test.

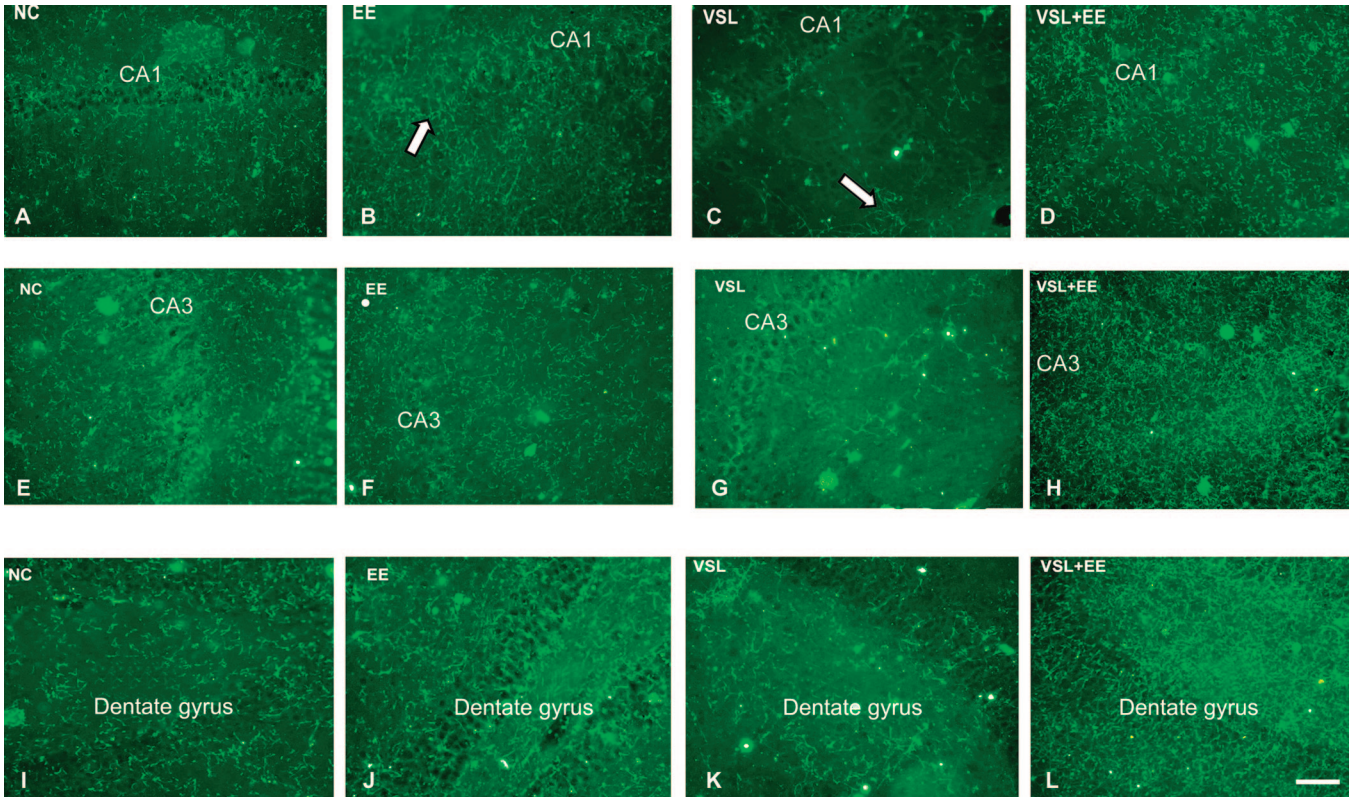

Figure 5. Photomicrograph showing the choline acetyl transferase (ChAT) expression in the CA1 (A–D), CA3 (E–H), and dentate gyrus (I–L) in various groups of rats. The ventral subicular–lesioned rats exposed to standard housing conditions (VSL) showed decreased expression of ChAT when compared with normal control rats (NC), sham control rats (SC), and normal control rats exposed to enriched environmental conditions (EE) and ventral subicular–lesioned rats exposed to enriched environment conditions (VSL + EE). The cholinergic terminals are indicated by white arrows. Scale bar = 90 μm.

colleagues have attributed the increase in ChAT activity to learning-associated changes in the Morris water maze. In our study, ChAT activity was assessed following 10 days of continuous exposure to enriched housing following ventral subicular lesion. Neither ventral subicular lesion nor exposure to enriched housing affected ChAT activity in the hippocampus. Other studies have shown that an enriched environment enhances the expression of mRNAs that encode for trophic factors, such as neurotrophic factor-3 and nerve growth factors (NGFs), including brain- and glia-derived growth factors. We find it important that enhanced NGF protein expression and receptor density have been reported following enrichment (Mohammed et al., 1990, 1993; Pham, Soderstrom, Winblad, & Mohammed, 1999; Pham, Winblad, Granholm, & Mohammed, 2002; Torasdotter, Metsis, Henriksson, Winblad, & Mohammed, 1996, 1998). Because NGF and cholinergic neuronal survival are tightly coupled (Debeir, Marien, Ferrario, Rizk, Prigent, Colpaert, & Raisman-Vozari, 2004) and ChAT activity remained unchanged following subicular lesion, the enriched environment might have provided a suitable milieu for the behavioral recovery observed in our study. It has been suggested that a posttraumatic milieu can be an ideal environment for neuronal rewiring and functional reorganization, because enhanced trophic factor expression and neuronal plasticity have also been observed following brain injury (Cramer & Chopp, 2000; Jones & Schallert, 1994). Thus, exposing the brain-injured rats immediately to an enriched environment may have a synergetic effect that would lead to functional recovery or compensation for the lost function. Under normal circumstances, newly formed cells in the dentate gyrus following enriched exposure may not be essentially involved in behavioral performance (Kempermann et al., 1997). However, following lesion or brain injury, newborn cells could contribute to functional recovery as a compensatory effect. When a lesion or injury is present, an enriched environment could have a tremendous impact on functional recovery. Postlesion exposure to enriched housing has been shown to enhance cell density in the dentate gyrus (see Figure 3A). Although we have not studied the neurogenesis following environmental enrichment, the functional and morphological recovery in the VSL + EE rats could be attributed to the impact of enriched housing on functional recovery.

Conclusion

In summary, the present study demonstrated the positive influence of environmental enrichment on subicular lesion–induced neurodegeneration and spatial learning. Exposure to an enriched

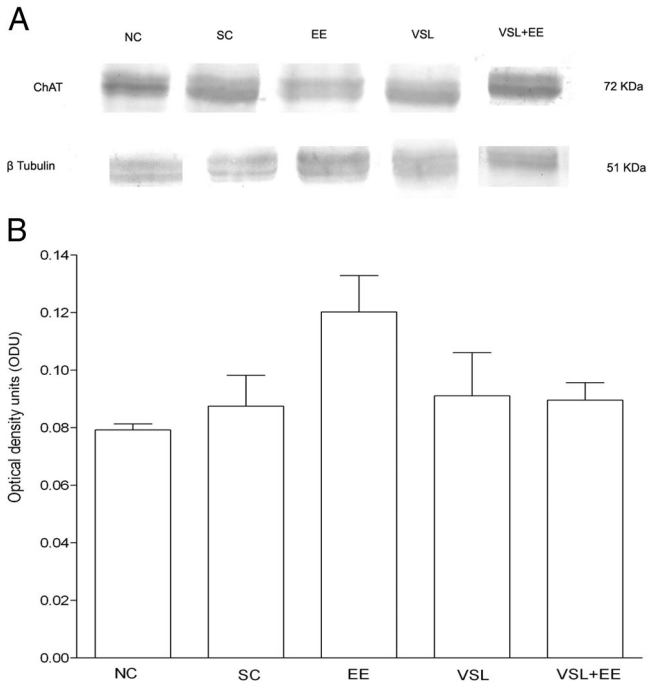

Figure 6. Representative immunoblots (A) and relative optical density units (B) for choline acetyl transferase (ChAT) in the hippocampus of the five groups of rats. NC = normal control rats; SC = sham control rats; EE = normal control rats exposed to enriched housing conditions; VSL = ventral subicular lesioned rats exposed to standard environmental conditions; VSL + EE = ventral subicular lesioned rats exposed to enhanced environmental conditions. Data are expressed as the mean ± *SEM*. One-way ANOVA showed no significant changes across groups.

environment ameliorated spatial working memory deficits and significantly enhanced neuronal survival in the hippocampus and entorhinal cortex. The functional recovery and enhanced neuronal survival observed in the present study would suggest that exposure to environmental enrichment offers protection from lesion–induced neuronal deficits.

References

Bennett, J. C., McRae, P. A., Levy, L. J., & Frick, K. M. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory, 85,* 139–152.

Biernaskie, J., & Corbett, D. (2001). Enriched rehabilitative training promotes improved forelimb motor function and enhanced dendritic growth after focal ischemic injury. *Journal of Neuroscience, 21,* 5272–5280.

Bindu, B., Alladi, P. A., Mansooralikhan, B. M., Srikumar, B. N., Raju, T. R., & Kutty, B. M. (2007). Short-term exposure to an enriched environment enhances dendritic branching but not brain-derived neurotrophic factor expression in the hippocampus of rats with ventral subicular lesions. *Neuroscience, 144,* 412–423.

Bindu, B., Rekha, J., & Kutty, B. M. (2005). Post insult enriched housing improves the 8-arm radial maze performance but not the Morris water maze task in ventral subicular lesioned rats. *Brain Research, 1063,* 121–131.

Cramer, S. C., & Chopp, M. (2000). Recovery recapitulates ontogeny. *Trends in Neurosciences, 23,* 265–271.

Dahlqvist, P., Zhao, L., Johansson, I. M., Mattsson, B., Johansson, B. B., Seckl, J. R., & Olsson, T. (1999). Environmental enrichment alters nerve growth factor-induced gene A and glucocorticoid receptor messenger RNA expression after middle cerebral artery occlusion in rats. *Neuroscience, 93,* 527–535.

Davies, D. C., Horwood, N., Isaacs, S. L., & Mann, D. M. (1992). The effect of age and Alzheimer's disease on pyramidal neuron density in the individual fields of the hippocampal formation. *Acta Neuropathologica (Berlin), 83,* 510–517.

Debeir, T., Marien, M., Ferrario, J., Rizk, P., Prigent, A., Colpaert, F., Raisman-Vozari, R. (2004). In vivo upregulation of endogenous NGF in the rat brain by the alpha2-adrenoreceptor antagonist dexefaroxan: Potential role in the protection of the basalocortical cholinergic system during neurodegeneration. *Experimental Neurology, 190,* 384–395.

Devi, L., Diwakar, L., Raju, T. R., & Kutty, B. M. (2003). Selective neurodegeneration of hippocampus and entorhinal cortex correlates with spatial learning impairments in rats with bilateral ibotenate lesions of ventral subiculum. *Brain Research, 960,* 9–15.

Diamond, M. C., Ingham, C. A., Johnson, R. E., Bennett, E. L., & Rosenzweig, M. R. (1976). Effects of environment on morphology of rat cerebral cortex and hippocampus. *Journal of Neurobiology, 7,* 75–85.

Diamond, M. C., Lindner, B., Johnson, R., Bennett, E. L., & Rosenzweig, M. R. (1975). Differences in occipital cortical synapses from environmentally enriched, impoverished, and standard colony rats. *Journal of Neuroscience Research, 1,* 109–119.

Dickson, D. W., Crystal, H. A., Mattiace, L. A., Masur, D. M., Blau, A. D., Davies, P., et al. (1992). Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiology of Aging, 13,* 179–189.

Dickson, D. W., Ksiezak-Reding, H., Liu, W. K., Davies, P., Crowe, A., & Yen, S. H. (1992). Immunocytochemistry of neurofibrillary tangles with antibodies to subregions of tau protein: Identification of hidden and cleaved tau epitopes and a new phosphorylation site. *Acta Neuropathologica (Berlin), 84,* 596–605.

Farrell, R., Evans, S., & Corbett, D. (2001). Environmental enrichment enhances recovery of function but exacerbates ischemic cell death. *Neuroscience, 107,* 585–592.

Galani, R., Coutureau, E., & Kelche, C. (1998). Effects of enriched postoperative housing conditions on spatial memory deficits in rats with selective lesions of either the hippocampus, subiculum or entorhinal cortex. *Restorative Neurology and Neuroscience, 13,* 173–184.

Galani, R., Jarrard, L. E., Will, B. E., & Kelche, C. (1997). Effects of postoperative housing conditions on functional recovery in rats with lesions of the hippocampus, subiculum, or entorhinal cortex. *Neurobiology of Learning and Memory, 67,* 43–56.

Gobbo, O. L., & O'Mara, S. M. (2004). Impact of enriched-environment housing on brain-derived neurotrophic factor and on cognitive performance after a transient global ischemia. *Behavioural Brain Research, 152,* 231–241.

Govindaiah, Rao, B. S., Raju, T. R., & Meti, B. L. (1997). Loss of hippocampal CA1 neurons and learning impairment in subicular lesioned rats. *Brain Research, 745,* 121–126.

Jones, T. A., & Schallert, T. (1994). Use-dependent growth of pyramidal neurons after neocortical damage. *Journal of Neuroscience, 14,* 2140–2152.

Kempermann, G., Brandon E. P., & Gage F. H. (1998). Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus. *Current Biology, 8,* 939–942.

Kempermann, G., & Gage, F. H. (1999). Experience-dependent regulation of adult hippocampal neurogenesis: Effects of long-term stimulation and stimulus withdrawal. *Hippocampus, 9,* 321–332.

Kempermann, G., Kuhn, H. G., & Gage, F. H. (1997, April 3). 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. *Journal of Neuroscience, 18,* 3206–3212.

Kolb, B., & Gibb, R. (1991). Environmental enrichment and cortical injury: Behavioral and anatomical consequences of frontal cortex lesions. *Cerebral Cortex, 1,* 189–198.

Laxmi, T. R., Bindu, P. N., Raju, T. R., & Meti, B. L. (1999). Spatial memory impairment in ventral subicular lesioned rats. *Brain Research, 816,* 245–248.

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. *Behavioural Brain Research, 163,* 78–90.

Mohammed, A. H., Henriksson, B. G., Soderstrom, S., Ebendal, T., Olsson, T., & Seckl, J. R. (1993). Environmental influences on the central nervous system and their implications for the aging rat. *Behavioural Brain Research, 57,* 183–191.

Mohammed, A. K., Winblad, B., Ebendal, T., & Larkfors, L. (1990). Environmental influence on behaviour and nerve growth factor in the brain. *Brain Research, 528,* 62–72.

Morris, R. G., Schenk, F., Tweedie, F., & Jarrard, L. E. (1990). Ibotenate lesions of hippocampus and/or subiculum: Dissociating components of allocentric spatial learning. *European Journal of Neuroscience, 2,* 1016–1028.

Moser, E. I., & Andersen, P. (1994). Conserved spatial learning in cooled rats in spite of slowing of dentate field potentials. *Journal of Neuroscience, 14,* 4458–4466.

Oswald, C. J., & Good, M. (2000). The effects of combined lesions of the subicular complex and the entorhinal cortex on two forms of spatial navigation in the water maze. *Behavioral Neuroscience, 114,* 211–217.

Park, G. A., Pappas, B. A., Murtha, S. M., & Ally, A. (1992). Enriched environment primes forebrain choline acetyltransferase activity to respond to learning experience. *Neuroscience Letters, 143,* 259–262.

Passineau, M. J., Green, E. J., & Dietrich, W. D. (2001). Therapeutic effects of environmental enrichment on cognitive function and tissue integrity following severe traumatic brain injury in rats. *Experimental Neurology, 168,* 373–384.

Paxinos, G., & Watson, C. (1982). *The rat brain in stereotaxic coordinates.* London: Academic Press.

Pham, T. M., Soderstrom, S., Winblad, B., & Mohammed, A. H. (1999). Effects of environmental enrichment on cognitive function and hippocampal NGF in the non-handled rats. *Behavioural Brain Research, 103,* 63–70.

Pham, T. M., Winblad, B., Granholm, A. C., & Mohammed, A. H. (2002). Environmental influences on brain neurotrophins in rats. *Pharmacology Biochemistry and Behavior, 73,* 167–175.

Pietropaolo, S., Feldon, J., Alleva, E., Cirulli, F., & Yee, B. K. (2006). The role of voluntary exercise in enriched rearing: A behavioral analysis. *Behavioral Neuroscience, 120,* 787–803.

Rosenzweig, M. R. (2003). Effects of differential experience on the brain and behavior. *Developmental Neuropsychology, 24,* 523–540.

Rosenzweig, M. R., & Bennett, E. L. (1996). Psychobiology of plasticity: Effects of training and experience on brain and behavior. *Behavioural Brain Research, 78,* 57–65.

Rosenzweig, M. R., Bennett, E. L., & Diamond, M. C. (1972). Cerebral effects of differential experience in hypophysectomized rats. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 79,* 56–66.

Rosenzweig, M. R., Krech, D., Bennett, E. L., & Diamond, M. C. (1962). Effects of environmental complexity and training on brain chemistry and anatomy: A replication and extension. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology, 55,* 429–437.

Struble, R. G., Polinsky, R. J., Hedreen, J. C., Nee, L. E., Frommelt, P., Feldman, R. G., & Price, D. L. (1991). Hippocampal lesions in dominantly inherited Alzheimer's disease. *Journal of Neuropathology and Experimental Neurology, 50,* 82–94.

Torasdotter, M., Metsis, M., Henriksson, B. G., Winblad, B., & Mohammed, A. H. (1996). Expression of neurotrophin-3 mRNA in the rat visual cortex and hippocampus is influenced by environmental conditions. *Neuroscience Letters, 218,* 107–110.

Torasdotter, M., Metsis, M., Henriksson, B. G., Winblad, B., & Mohammed, A. H. (1998). Environmental enrichment results in higher levels of nerve growth factor mRNA in the rat visual cortex and hippocampus. *Behavioural Brain Research, 93,* 83–90.

Wang, J., Tanila, H., Puolivali, J., Kadish, I., & Van Groen, T. (2003). Gender differences in the amount and deposition of amyloidbeta in APPswe and PS1 double transgenic mice. *Neurobiology of Disease, 14,* 318–327.

Will, B., Galani, R., Kelche, C., & Rosenzweig, M. R. (2004). Recovery from brain injury in animals: Relative efficacy of environmental enrichment, physical exercise or formal training (1990–2002). *Progress in Neurobiology, 72,* 167–182.

Will, B. E., Rosenzweig, M. R., & Bennett, E. L. (1976). Effects of differential environments on recovery from neonatal brain lesions, measured by problem-solving scores and brain dimensions. *Physiology & Behavior, 16,* 603–611.

Will, B., Toniolo, G., Kelche, C., Pallage, V., Deluzarche, F., & Misslin, R. (1986). The effects of postoperative physical environment on novelty seeking behaviour and maze learning in rats with hippocampal lesions. *Behavioural Brain Research, 19,* 233–240.

Young, D., Lawlor, P. A., Leone, P., Dragunow, M., & During, M. J. (1999). Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Medicine, 5,* 448–453.

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