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Environmental enrichment protects against the effects of chronic stress on cognitive and morphological measures of hippocampal integrity

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Abstract

Chronic stress has detrimental effects on hippocampal integrity, while environmental enrichment (EE) has beneficial effects when initiated early in development. In this study, we investigated whether EE initiated in adulthood would mitigate chronic stress effects on cognitive function and hippocampal neuronal architecture, when EE started one week before chronic stress began, or two weeks after chronic stress onset. Adult male Sprague Dawley rats were chronically restrained (6 h/d) or assigned as non-stressed controls and subdivided into EE or non-EE housing. After restraint ended, rats were tested on a radial arm water maze (RAWM) for 2-d to assess spatial learning and memory. The first study showed that when EE began prior to 3-weeks of chronic stress, EE attenuated chronic stress-induced impairments in acquisition, which corresponded with the prevention of chronic stress-induced reductions in CA3 apical dendritic length. A second study showed that when EE began 2-weeks after the onset of a 5-week stress regimen, EE blocked chronic stress-induced impairments in acquisition and retention at 1-h and 24-h delays. RAWM performance corresponded with CA3 apical dendritic complexity. Moreover, rats in EE housing (control or stress) exhibited similar corticosterone profiles across weeks, which differed from the muted corticosterone response to restraint by the chronically stressed pair-housed rats. These data support the interpretation that chronic stress and EE may act on similar mechanisms within the hippocampus, and that manipulation of these factors may yield new directions for optimizing brain integrity and resilience under chronic stress or stress related neuropsychological disorders in the adult.

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Keywords

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1. Introduction

Stressful life events are a critical determinant in depression, a disorder that is predicted to lead the disability and disease burden by 2020 (WHO, 2002). The stress response may be intertwined with depression because of overlapping brain regions, circuitries and mediators (Gold & Chrousos, 2002). Paradigms incorporating chronic stress in animal models reveal many core physiological and behavioral characteristics of depression that include anhedonia, hypothalamic-pituitary-adrenal (HPA) disruptions, motivational deficits and cognitive impairment (Willner, 2005). Additional works with chronically stressed rodents report that the hippocampus, a region important for the formation of spatial memories (Eichenbaum, 2000; O'Keefe and Nadel, 1978; Tolman, 1948), exhibits decrements in neurotrophic factors (Duman & Monteggia, 2006; Monteggia et al., 2004; Smith, Makino, Kvetnansky, & Post, 1995; Xu et al., 2006, 2007), decreased neurogenesis (Czeh et al., 2001; Mirescu & Gould, 2006; Oomen, Mayer, de Kloet, Joels, & Lucassen, 2007; Pham, Nacher, Hof, & McEwen, 2003; Xu et al., 2006, 2007) and marked alterations in neuronal dendritic complexity and spine density (Conrad, 2006; Magariños, McEwen, Flugge, & Fuchs, 1996; McLaughlin, Gomez, Baran, & Conrad, 2007; Sunanda, Rao, & Raju, 1995), with a range of antidepressants reversing these effects (Czeh et al., 2001; Duman & Monteggia, 2006; Magariños et al., 1996; Malberg, Eisch, Nestler, & Duman, 2000; Nestler et al., 2002). Reports that depressed individuals express similar alterations in the hippocampus that can also be reversed by antidepressants helped inspire a novel hypothesis that depression and chronic stress hinder neuroplasticity and function similarly, especially within the hippocampus (Pittenger & Duman, 2008). Therefore, understanding the neurobiology underlying these changes in hippocampal structure and function induced by stress in animal models is important to elucidate novel directions for the treatment of depression and other disorders with similar etiologies.

Environmental enrichment (EE) has a long record of promoting brain development, plasticity and cognitive performance, especially within the hippocampus (Fox, Merali, & Harrison, 2006; Hebb, 1947; Kramer, Bherer, Colcombe, Dong, & Greenough, 2004; Rosenzweig & Bennett, 1996; van Praag, Kempermann, & Gage, 2000). Investigations commonly implement EE paradigms in young rodents during development to compare the physiological and behavioral outcomes to standard or isolated housing conditions (Rosenzweig & Bennett, 1996). More recently, research has focused on either behavioral or neurobiological consequences of EE on the damaged or diseased brain (Dhanushkodi & Shetty, 2008; Fox et al., 2006; Mohammed et al., 2002; Rampon et al., 2000; Sifonios et al., 2009; van Praag et al., 2000; Yang et al., 2007) and after stress in adolescents (Cui et al., 2006; Yang et al., 2007). Importantly, the beneficial effects of the exposure to EE are evident not only when EE precedes, but also when EE follows a brain challenge. However, in spite of the bulk of studies on EE effects on brain functions, less is known about the effects of EE

exposure on chronic stress, and in particular whether the detrimental effects of chronic stress in adulthood on neuromorphology and function can be blocked. As such, this is the first study to investigate the impact of EE and chronic stress in adulthood on hippocampal plasticity and resilience, by examining morphological and behavioral measures within the same subjects. We hypothesized that adult-onset EE, begun before or after the onset of chronic stress, would prevent dendritic retraction and mitigate the detrimental effects of chronic stress on hippocampus-dependent spatial learning and memory.

2. Materials and methods

2.1 Subjects

Upon arrival, male Sprague-Dawley rats (275 gm, Charles River Laboratories, Wilmington, MA) were pair-housed in temperature (18–21 °C) and sound controlled chambers on a reversed light cycle (lights off 0600-h). Food and water were freely available unless otherwise noted. Rats acclimated for at least a week before procedures started. Body weights were taken weekly throughout the studies. Arizona State University Institutional Animal Care and Use Committee approved the procedures, which followed the Guide for Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Science, National Research Council, 1996).

2.2 Housing conditions

Upon arrival, rats were pair-housed (Pr) for at least a week before experimental procedures began. Rats that were randomly assigned to the environmental enrichment (EE) condition were pair-housed until EE commenced. Pr consisted of two rats/cage using Plexiglas housing (20 cm × 46 cm × 18 cm), while EE incorporated six rats/cage (74 cm × 91 cm × 36 cm) along with PVC pipes, nesting material, running wheels, and ceramic objects (Bernstein, 1973; Rosenzweig, Bennett, Hebert, & Morimoto, 1978; Wright & Conrad, 2008). The PVC pipes and ceramic objects were replaced or moved every three days to increase novelty.

2.3 Restraint stress procedure

Half of the Pr and EE housed rats were randomly divided into non-stressed control and stressed conditions, giving rise to four treatments ($n = 12/\text{group}$): non-stressed rats in standard housing (CON-Pr); chronically stressed rats in standard housing (STR-Pr); non-stressed rats housed in enriched housing (CON-EE) and chronically stressed rats in enriched housing (STR-EE). In experiment 1, rats in the STR group were restrained 6 h per day (0900–1500) for 3 weeks in wire mesh restrainers (16 cm circumference, 24 cm length) beginning one week after the onset of EE. In experiment 2, restraint began 2 weeks prior to the onset of EE and continued for a total of 5 weeks, until the end of the 3-week EE intervention. Restrained rats were never in the same housing chamber with non-stressed control rats to reduce the transfer of odor and sound between treatment groups. Pr rats were restrained in their home cages, while EE rats were moved from EE housing and placed in standard cages in chambers shared with the stressed, Pr rats during restraint. To reduce potential stress associated with the experimenter and behavioral testing, rats were handled

for 1 min/d for the 2 weeks preceding behavior testing. Separate investigators participated in handling duties and restraint procedures.

2.4 Radial arm water maze (RAWM)

The RAWM is sensitive to a variety of manipulations that disrupt hippocampal function (Alamed, Wilcock, Diamond, Gordon, & Morgan, 2006; Diamond, Park, Heman, & Rose, 1999; Morgan et al., 2000). The RAWM had symmetrical arms (27.9 cm long $\times$ 12.7 cm wide), radiating outward from a center annulus (diameter, 48 cm), constructed of black polypropylene, and filled with water (19 °C), rendered opaque with powder black tempera paint. An escape platform was located at the end of the one of the arms, submerged 2.5 cm below the surface of the water. The platform was in a constant location for all trials for a given rat, and the location was counterbalanced across rats to control for any potential location effects. Testing was conducted in a room containing numerous distal visual cues outside the maze that remained constant throughout testing.

Testing in the RAWM started one day after the chronic stress ended and took place over two consecutive days, between 0800 and 1600-h, to assess hippocampal spatial learning and memory (Diamond et al., 1999; Diamond et al., 2006). On the first day of testing, each rat received 12 massed trials, followed by a one-hour inter-trial interval (ITI) spent in their home cage, and concluded with 6 additional training trials. The next day, all rats were given a single retention trial (RT). A trial began by releasing a rat into an arm that did not contain the platform (the start arm). Rats had 3 min to locate the hidden platform. The start arm varied across trials and the goal arm held constant. If the rat failed to locate the platform within 3-min, the rat was guided to the platform and allowed to remain on it (15-s), and then returned to its home cage (15-s). The water was swept clean of debris after each trial.

An arm entry was manually recorded when the tip of the rat's nose reached 11-cm into an arm. A scorer, blind to the experimental manipulations, assessed acquisition and retention by measuring first-time and repeat incorrect entries into non-platformed arms. Because the platform location remained constant across trials, the rats had to learn and remember the position of the arm over the training trials and also retain the location of arms "already entered" within each trial. Consequently, first-time incorrect entries into non-platformed arms were scored as reference memory errors, and incorrect repeat entries into already entered arms within a given trial were scored as working memory errors.

2.5 Histological procedures

Immediately after the RAWM ended, rats were deeply anesthetized with isoflurane, decapitated, and then unperfused brains were rapidly removed. FD Rapid Golgistain™ kits (FD NeuroTechnologies, Baltimore, MD) were used for Golgi staining as previously described (Hoffman et al., 2011). For sectioning, brains were blocked, then frozen in 2-methylbutane and cut (100 μ m coronal sections) using a cryostat (25 °C). Slides were dehydrated (ethanol dilutions) and coverslipped with Permount (Fisher Scientific). Hippocampal CA3 neurons were identified and drawn as previously described (Hoffman et al., 2011; McLaughlin et al., 2007). Neurons were chosen on the basis of the following criteria: (i) the cell body and dendrites were fully impregnated and untruncated; (ii) the cell

was relatively isolated from surrounding neurons; and (iii) the cell was located in the CA3 region of the hippocampus. A camera lucida drawing tube attached to an Olympus BX51 microscope was used to trace all neurons. Dendritic length was quantified with a Scale Master II digital plan measuring system (Calculated Industries, Carson City, NV, USA) linked by a PC interface to a Dell PC. CA3 neurons were further labeled as short-shaft (SS) or long-shaft (LS), depending on their relative location in the stratum pyramidal and proximal apical shaft length. The apical dendrites of the SS neurons in the CA3 region are intrinsically more complex than the apical dendrites of the LS neurons, so the two values for the SS and LS neurons were averaged to obtain one value for each rat. For a rat to be included in the analysis, the brain must have contained at least three successfully stained neurons of each SS and LS category (six neurons per animal). Dendritic length and branch points (number of dendritic bifurcations) were quantified for apical and basal sections of the SS and LS neurons.

2.6 Blood sampling

One subset of rats (STR-Pr, STR-EE, CON-EE) was used solely for blood sampling during restraint and EE and was not used for behavioral testing. Blood was taken on days 1, 7, 14 and 21 of restraint, with sampling occurring outside the animal colony. On each day of sampling, 300 μ L of blood was taken at hour 0, 1, and 3 of restraint from the lateral saphenous vein by quick puncture using a 20 gauge needle and drawn into heparinized capillary tubes (Fischer Scientific). The initial blood sampling from the STR-Pr group (0-h) on Day 1, when blood was taken within 2–4 min of disturbance in the animal colony room, represents the baseline to which all other measures are compared because these rats had no restraint experience prior to this time point.

Blood was centrifuged for 30-min at 3000 RPM. Serum was removed and stored at -70°C . Samples were diluted 1:30 and processed in duplicate. Final values for each subject were averaged and represented as $\mu\text{g}/100\text{ mL}$. Corticosterone (CORT) levels were determined using an Enzyme Immunoassay kit (Immuno Diagnostic Systems Limited (IDS), Fountain Hills, AZ). Antibody cross-reactivity to 11-desoxycorticosterone was 18.5% and 11-dehydrocorticosterone was 2%. The remaining steroid cross-reactivity did not exceed 2.0%. Optical density values were measured at 450 nm using a microplate reader (LabSystems Multiskan RC, Fisher Scientific).

2.7 Statistical analyses

Statistical analyses were done using the Statistica software package under the windows XP operating system. Parametric data were analyzed by ANOVA and significant main effects and interactions were investigated further with Neuman-Keuls post hoc tests or planned comparisons. Statistical significance was accepted for $p < 0.05$. Results are represented as means $\pm$ SEM.

For the RAWM, errors were grouped into blocks of two trials and analyzed as a repeated measured analysis of variance (ANOVA), with Stress and EE as independent measures. Rats in both experiments were excluded if their errors measured at least two standard deviations from the group mean on any given trial and/or when they made less than three entry errors

on the first trial, since adequate exploration is critical to the acquisition of spatial cues ($n = 1-4/\text{experiment}$). An additional three rats from three different groups were excluded from RAWM analyses in experiment 2 for poor health.

3. Results

3.1 Experiment 1: Prevention: EE started before chronic stress

In experiment 1, EE was initiated one week prior to chronic stress and then continued until the end of the chronic stress regimen. Chronic stress and EE altered the prevalence of first-time entry errors, but not repeat entry errors in the 6-arm RAWM (for first-time entry errors, there was a significant stress $\times$ EE $\times$ block interaction, $F_{5,195} = 3.74$, $p < 0.005$, a significant stress $\times$ block interaction, $F_{5,195} = 2.51$, $p < 0.05$, a significant repeated effect of block, $F_{5,195} = 25.40$, $p < 0.0001$, and a significant main effect of stress, $F_{1,39} = 6.45$, $p < 0.05$; no other effects were significant, $n = 10-12/\text{group}$, Fig. 1A and B). To probe the significant 3-way interaction, separate repeated measures ANOVAs were performed to determine the effect of block for each group alone. These revealed decreasing first-time entry errors across blocks 1–6 for all treatment groups ($p < 0.05$) except for STR-Pr ($p = 0.4$). Further, EE decreased arm entry errors during blocks 1 and 2 (significant stress $\times$ EE interaction for first-time entry errors on blocks 1 and 2, $F_{1,39} = 6.07$, $p < 0.05$, $F_{1,39} = 4.71$, $p < 0.05$, respectively). For block one, post hoc tests showed no significant group differences and an ANOVA performed on the first training trial of block 1 was not significant. For block 2, CON-EE made fewer errors than CON-Pr ($p = 0.05$). No other effects were significant. Consequently, chronic stress and EE altered the prevalence of first-time entry errors in opposing directions with chronic stress slowing and EE facilitating the acquisition of performance based upon first-time entry errors. We interpreted differences among groups on day 1 to be reflective of learning because performance was similar in the first training trial to suggest comparable levels of motivation, motor ability and/or attention. After a 1-h and 24-h delay, all groups performed similarly (Fig. 1A and B; blocks 7–9 and the RT).

In addition to preventing chronic stress-induced deficits in RAWM performance, EE, in chronically stressed rats, allowed the maintenance of dendritic branching at levels similar to that seen in controls (Fig. 1C–E). Specifically, while chronic stress decreased hippocampal CA3 apical branch points (significant main effect of stress, $F_{1,21} = 5.31$, $p < 0.05$), EE increased them to the level of non-stressed controls (significant main effect of EE, $F_{1,21} = 5.39$, $p < 0.05$). Moreover, EE but not stress, altered apical length (significant main effect of EE, $F_{1,21} = 9.25$, $p < 0.01$). There were no significant effects on basal dendritic measures (Table 1).

Separate cohorts of rats were used to assess hippocampal CA3 dendritic complexity and serum corticosterone (CORT) levels without exposing the rats to the RAWM in order to discount potential effects of cognitive testing that may influence dendritic properties, as was previously reported for spines in the hippocampal CA1 region (Diamond et al., 2006; Leuner, Falduto, & Shors, 2003; Moser, Trommald, & Andersen, 1994). In this experiment, EE prevented chronic stress-induced changes in hippocampal CA3 dendritic architecture, with chronic stress decreasing CA3 apical branch points and length in untested rats (significant stress $\times$ EE interaction for apical branch points, $F_{1,17} = 14.02$, $p < 0.005$, and

significant main effect of stress for apical length $F_{1,17} = 13.49$, $p < 0.005$). Specifically, STR-Pr showed evidence of decreased apical branch points compared to the other groups ($p < 0.05$). In contrast, EE maintained apical branch points and length in chronically stressed rats comparable to the level of controls (significant main effect of EE for apical length $F_{1,17} = 5.81$, $p < 0.05$, Table 1).

Additionally, total serum CORT levels, based upon biologically active and inactive CORT, were measured from an additional subset of untested rats at the start (baseline) of restraint and following the 1st and 3rd hour of restraint ($n = 6/\text{group}$). This process was repeated on the 7th, 14th and 21st day of restraint for a total of four different days of sampling. Control pair-housed rats were not used as a separate group because initial CORT levels from pair housed rats prior to stress (0-h) on Day 1 served as the control pair housed data. These values were used as the baseline to determine percentages of CORT alterations produced by chronic stress or EE. CORT levels were significantly modified by treatment, day and hour of sampling (significant treatment across days and hours, $F_{12,90} = 2.69$, $p < 0.005$). Subsequent analyses were conducted for each treatment condition separately. For the STR-Pr group, CORT levels exhibited a profile in the early sampling days (1,7) that differed by the last day (21) of restraint. Specifically, on days 1 and 7, CORT levels significantly increased within one hour of restraint ($p < 0.01$) and then significantly decreased three hours later ($p < 0.01$). By day 21, however, CORT levels flattened out and were statistically similar to the 1st and 3rd sampling hours of restraint (Fig. 2A). The CORT profiles for STR-EE and CON-EE differed from STR-Pr, but were similar to each other in that both had a robust CORT response within an hour of restraint ($p < 0.001$) and these CORT elevations were maintained at the 3-h sampling time throughout the four sampling days (significant effect of hour for STR-EE, $F_{1,15} = 102.58$, $p < 0.001$; CON-EE, $F_{1,15} = 32.05$, $p < 0.005$; without a significant main effect of day nor interaction, Fig. 2B and C).

3.2 Experiment 2: Intervention: EE started after chronic stress

In experiment 2, EE was initiated two weeks into a five week chronic stress procedure, because by two weeks, chronically restrained rats begin to show anhedonia, a characteristic of depressive behavior (Kleen, Sitomer, Killeen and Conrad, 2006; Nestler et al., 2002). Given the rapid acquisition of the CON-EE rats in the 6-arm RAWM task and the similar performance among groups at retention in experiment 1, task difficulty was increased by using an 8-arm RAWM to reduce potential floor effects.

EE initiated in adulthood two weeks into and continuing throughout restraint blocked stress-induced impairments in spatial learning and memory in the 8-arm RAWM ($n = 8\text{--}12/\text{group}$, Fig. 3A and B). While all groups learned the location of the hidden platform during acquisition (first-time and repeat entry errors decreased across blocks 1–6, $F_{5,190} = 40.47$, $p < 0.001$; $F_{5,190} = 23.05$, $p < 0.001$, respectively), chronic stress and EE altered entry errors in opposing directions (for first-time entry errors, there was a significant stress $\times$ EE $\times$ block interaction, $F_{5,190} = 4.04$, $p < 0.005$, a significant stress $\times$ EE interaction, $F_{1,38} = 12.67$, $p < 0.001$, and a significant main effect of EE, $F_{1,38} = 12.82$, $p < 0.001$; for repeat entry errors, there was a significant stress $\times$ EE $\times$ block interaction only, $F_{5,190} = 2.76$, $p < 0.05$). To probe the significant 3-way interaction, separate repeated measures ANOVAs were

performed to determine the effect of block for each group alone, which revealed decreasing first-time entry errors across blocks 1–6 for all treatment groups ($p < 0.05$). Subsequent analyses probed the significant three-way interactions for first-time entry errors and repeat entry errors by investigating stress and EE effects for each block separately. While chronic stress and EE did not alter first-time or repeat entry errors in the first block of the 8-arm RAWM, significant effects were found in the subsequent blocks. For first-time entry errors, chronic stress increased, whereas EE decreased first-time entry errors (significant stress $\times$ EE interaction for block two, $F_{1,38} = 19.12$, $p = 0.0001$, block three, $F_{1,38} = 6.87$, $p = 0.05$; block five, $F_{1,38} = 10.04$, $p = 0.005$; block six, $F_{1,38} = 4.19$, $p = 0.05$). Additional analyses revealed that STR-Pr rats made significantly more first-time entry errors than all other groups in blocks 2, 5 and 6 ($p \leq 0.05$), while STR-EE rats made first-time entry errors that were statistically similar to the non-stressed controls in blocks 2, 3, 5 and 6 ($p \geq 0.09$). Further, in block 2, STR-EE made fewer first-time entry errors than both CON-EE and STR-Pr ($p < 0.05$). For repeat entry errors, significant effects were found in block 3 only, with STR-EE making significantly fewer repeat arm entry errors than STR-Pr (significant stress $\times$ EE interaction, $F_{1,38} = 5.26$, $p < 0.05$, STR-EE compared to STR-Pr, $p = 0.05$). Interestingly, repeat entry errors reached zero by block 2 for STR-EE and by block 6 for CON-EE and these levels remained at zero for these groups until the retention trial.

After a 1-h delay, the pattern found during acquisition continued for first-time and repeat entry errors. For first-time entry errors, chronic stress increased arm entry errors, which was improved with EE (for first-time entry errors across blocks 7–9, there was a significant stress $\times$ EE interaction, $F_{1,38} = 9.13$, $p < 0.005$, a significant main effect of stress, $F_{1,38} = 4.21$, $p < 0.05$, and a significant main effect of EE, $F_{1,38} = 26.56$, $p < 0.001$). Post hoc analyses revealed that in blocks 7 and 8, STR-Pr made more first-time entry errors than all other groups ($p < 0.01$). For repeat entry errors, housing carried the predominate effect (significant EE $\times$ block interaction, $F_{2,76} = 4.77$, $p < 0.05$, significant main effect of EE, $F_{1,38} = 7.45$, $p < 0.01$, and significant effect for block, $F_{2,76} = 4.77$, $p < 0.05$). EE for both groups (STR-EE and CON-EE) reduced repeat arm entry errors by block 9 ($F_{1,38} = 7.02$, $p < 0.05$).

After a 24-h delay, chronic stress and EE influenced entry errors in opposite directions (significant stress $\times$ EE interaction for first-time and repeat entry errors, $F_{1,38} = 11.84$, $p = 0.001$; $F_{1,38} = 7.00$, $p = 0.01$, respectively). Specifically, STR-Pr made more first-time and repeat entry errors in the single retention trial compared to the other groups ($p < 0.001$ for both first-time and repeat entry errors, Fig. 3A, see RT). Moreover, the STR-Pr group performed at chance levels at the 24-h test in first-time entry errors. Further, the number of first-time and repeat errors of the chronically stressed rats housed in EE were statistically similar to non-stressed controls ($p = 0.27$; $p = 0.64$, respectively).

In parallel with RAWM performance, EE blocked chronic-stress induced decreases in hippocampal CA3 dendritic branching and length when EE started two weeks after chronic stress began (Fig. 3C–E). While chronic stress decreased hippocampal CA3 apical branch points and length (significant stress $\times$ EE interaction for apical branch points and length, $F_{1,24} = 4.31$, $p = 0.05$; $F_{1,24} = 5.2$, $p < 0.05$, respectively), EE increased them to the level of controls (STR-Pr compared to STR-EE for apical branch points, $p < 0.05$; CON-Pr compared to STR-EE, $p = NS$). EE, but not stress, altered basal dendritic branch points and

length (significant main effect of EE for basal branch points and length, $F_{1,24} = 9.75$, $p < 0.01$; $F_{1,24} = 7.69$, $p = 0.01$, respectively; Table 1).

3.3 Body weight

In all experiments, chronic stress reduced body weight gain, demonstrating restraint effectiveness consistent with previous reports (Hoffman et al., 2011; McLaughlin et al., 2007; Tamashiro et al., 2007; Willner, 2005; Wright & Conrad, 2008). Further, EE rats gained significantly less weight than pair housed non-stressed rats ($p < 0.05$, data not shown).

4 Discussion

The current studies support the hypothesis that in adult rats, EE attenuates the detrimental effects of chronic stress on hippocampal morphology and function whether initiated prior to chronic stress or 2 weeks after the onset of chronic stress. Past reports provide clear evidence that chronic stress alters hippocampal structure by changing the hippocampal morphology in rats and tree shrews, including reversible remodeling of dendrites in the CA3 region (Conrad, Magariños, LeDoux, & McEwen, 1999; Kleen, Sitomer, Killeen, & Conrad, 2006; Magariños et al., 1996) and modification of dendritic spine number and shape (Diamond et al., 2006; McLaughlin, Baran, Wright, & Conrad, 2005; McLaughlin et al., 2010; Sunanda et al., 1995). This structural pruning in the hippocampus following chronic stress is thought to contribute to impaired hippocampal function, such as spatial learning and memory (Bellani, Luecken, & Conrad, 2006; Conrad, 2010; Conrad, Grote, Hobbs, & Ferayorni, 2003; Kitraki, Kremmyda, Youlatos, Alexis, & Kittas, 2004; Luine, Villegas, Martinez, & McEwen, 1994; Park, Campbell, & Diamond, 2001; Wright & Conrad, 2005). Here we show that EE can block these effects, even when initiated after chronic stress had commenced two weeks prior to the onset of EE. Thus, our data support the interpretation that chronic stress and EE may act on similar mechanisms within the hippocampus to influence hippocampal integrity, as measured by hippocampal function and dendritic architecture.

Very recently, a study investigated whether EE would prevent the detrimental effects of chronic stress on a land version of the radial arm maze (RAM) in adult rats (Veena et al., 2009). Similar to our paradigm, adult male rats were chronically stressed by restraint for 6 h/day for 21 days and then EE was started after chronic stress was completed. An important difference between the studies is the timing between the start and end of EE and chronic stress. Veena and colleagues (2009) started EE after chronic stress was completed, whereas we started EE two weeks after chronic stress began in experiment 2, and then both EE and chronic stress continued until RAWM assessment. This latter procedure was implemented to avoid the natural recovery process in hippocampal morphology and spatial ability when chronic stress ends (Conrad et al., 1999; Hoffman et al., 2011; Sousa, Lukoyanov, Madeira, Almeida, & Paula-Barbosa, 2000; Vyas, Pillai, & Chattarji, 2004). Importantly, our study reveals that EE can prevent the detrimental effects of chronic stress on spatial memory, even when chronic stress is ongoing. The present data and the findings of Veena and colleagues

(2009) complement each other, revealing that EE initiated in adulthood either before, during or after chronic stress can attenuate chronic stress-induced spatial learning deficits.

An additional novel behavioral finding in our study is that chronic stress influences spatial reference and working memory domains differently in the RAWM. Data were analyzed based on first and repeat entry errors as we have done previously (Hoffman et al., 2011), to reflect the domains of reference and working memory, respectively. During acquisition, three weeks of chronic stress in pair-housed conditions moderately slowed spatial learning as measured by first-time entry errors without significantly altering repeat entry errors. These findings are consistent with chronic stress impairing learning in spatial mazes (Conrad, 2010), and are especially consistent with spatial learning being mildly impaired in reference memory versions of water maze paradigms (Wright & Conrad, 2008). After a 1-h delay, performance under the domains of short-term reference and working memory were statistically similar among groups and nearly error free, which is consistent with previous studies showing intact spatial reference memory following a similar delay (45-min) (Wright & Conrad, 2008). After five weeks, however, chronically stressed rats showed additional metrics of poor performance that were not observed following three weeks of chronic stress, when compared to rats in EE who had undergone five weeks of stress, or when compared to non-stressed controls, including increased repeat entry errors during acquisition, poor short term (1-h) and long term (24-h) performance in the domains of reference and working memory. Consequently, the two memory domains reveal different time courses of detriment: the spatial reference memory domain decayed sooner than the spatial working memory domain in response to chronic stress, which is corroborated by another study (Veena et al., 2009), and reflects a developmental timeline for cognition that is consistent with accelerated aging (Conrad & Bimonte-Nelson, 2010).

The behavioral findings in these experiments need to be considered in the context of the methodological differences between experiments 1 and 2. In experiment 1, a 6-arm maze was used to assess spatial learning and memory, while an 8-arm maze was used in experiment 2. The 6-arm maze did not appear to have the sensitivity to reveal the effects of both EE and chronic stress in all blocks that showed stress-induced deficits. Chronic stress produced the expected behavioral deficit, but the performance of the chronically stressed rats was not poor enough to allow EE to produce robust prevention of performance deficits. Given these findings from experiment 1, the task was modified in experiment 2 so that EE could potentially produce the expected recovery of function. As predicted, EE-induced functional recovery in chronically stressed rats was observed in experiment 2 with the 8-arm maze. The experimental differences between experiment 1 and 2 prevent strong conclusions regarding the effects of EE timing on chronic stress-induced learning deficits. However, the functional performance of STR-EE rats in experiment 1 and the previously reported finding that EE prevents chronic stress-induced learning deficits in the Morris water maze (Wright & Conrad, 2008) converge to suggest that EE can prevent chronic stress-induced spatial learning deficits. The findings from experiment 2 were more robust and support the previously untested hypothesis that EE can prevent chronic stress-induced learning deficits even when initiated after the onset of chronic stress. One other potential issue is that STR-Pr rats were housed in the same environment as restraint, which might result in a more sustained stress compared to STR-EE. While possible, we believe it is more likely that the

discrete cues associated with restraint play a larger role than does the home cage environment, as suggested by studies showing greater aversive learning with discrete tone or cued conditioning as compared to non-discrete contextual conditioning (Phillips & LeDoux, 1992,1994). Moreover, such a pattern of sustained HPA axis activity was not observed in rats restrained repeatedly in their home colony compared to rats that were transported to a different context for restraint (Grissom, Iyer, Vining, & Bhatnagar, 2007). Therefore, these experiments along with the literature support the overall conclusion that EE attenuates the detrimental effects of chronic stress on hippocampal function, when initiated before or after the onset of chronic stress.

In the present experiments, reductions in hippocampal CA3 dendritic complexity generally manifested in parallel with the development of the RAWM performance deficits. Specifically, three weeks of chronic stress produce retraction of apical dendrites in the CA3 region of the hippocampus that extended to CA3 basal dendritic retraction after five weeks and is concurrent with the timeframe of a breakdown in both reference and working memory domains in RAWM performance (see Conrad & Bimonte-Nelson, 2010). Importantly, EE attenuates stress-induced CA3 dendritic retraction and RAWM performance deficits even when chronic stress lasts for 5 weeks.

The present findings on the neuroprotective effects of EE in the hippocampus complement the recent findings by Veena and colleagues (2009) showing that EE restores hippocampal neurogenesis and hippocampal volume following chronic stress. While the stress and EE paradigms differ significantly between the present study and that reported by Veena et al. (2009), both studies reveal the protective effects of EE. Clearly, stress exerts a profound effect on the hippocampus that leads to functional impairment of hippocampal-dependent learning. The present findings and those of Veena et al. (2009) make the first steps at establishing this effect and determining the potential mechanisms by which EE exerts its protective effects in adults.

The HPA axis may be an important contributor to the plasticity and resilience of the adult brain following deleterious experiences. Indeed, overactivity of the HPA axis and the hypersecretion of glucocorticoids can lead to impaired spatial ability (Arbel, Kadar, Silbermann, & Levy, 1994; Bardgett, Taylor, Csernansky, Newcomer, & Nock, 1994; Bodnoff et al., 1995; Dachir, Kadar, Robinson, & Levy, 1993; Luine, Spencer, & McEwen, 1993; Sandi & Pinelo-Nava, 2007) and CA3 dendritic retraction (Conrad et al., 2007; Magariños & McEwen, 1995; Watanabe, Gould, Cameron, Daniels, & McEwen, 1992) and in turn, the breakdown of hippocampal dendritic architecture following chronic stress can lead to inefficient regulation of the HPA axis (Conrad, 2006). Consequently, a vicious cycle of elevated glucocorticoids and poor hippocampal functioning can result, making the hippocampus vulnerable to potential injury (Conrad, 2008). However, elevated glucocorticoids alone do not necessarily cause spatial learning and memory detriment, as chronic glucocorticoid exposure may still permit intact function (Coburn-Litvak et al., 2004; Conrad et al., 2007), and glucocorticoid levels can even be elevated under pleasurable conditions or exercise without impairing spatial function (O'Callaghan, Ohle and Kelly, 2007; Woodson, Macintosh, Fleshner, & Diamond, 2003). Similarly, functional spatial memory can be achieved despite the presence of hippocampal CA3 dendritic retraction

(Wright, Lightner, Harman, Meijer, & Conrad, 2006) or even CA3 lesion (Roozendaal et al., 2001). As such, it is possible for spatial memory to remain intact following an inefficient regulation of the HPA axis or CA3 disruption (i.e., dendritic retraction or lesion), but not when both occur together.

Another critical aspect of the HPA axis is that it must be tightly regulated so that it can become engaged when needed and then terminated when the stressor has passed. In the current study, the CORT levels of the STR-Pr group were elevated at the onset of restraint, but with repeated restraint, CORT levels became muted, blurring the distinction between the onset and ending of restraint. In contrast, CORT profiles of the EE groups (CON-EE and STR-EE) appeared lower than the baseline measurement from the pair-housed rats, which is consistent with previous research (Belz, Ken-nell, Czambel, Rubin, & Rhodes, 2003; Campeau et al., 2010). Moreover, the EE groups demonstrated a robust elevation in response to the onset of restraint and these CORT elevations were maintained for at least three hours as restraint continued. While past findings show that maintaining elevated CORT after a stressor has terminated can be detrimental, these results show that muting CORT levels when the stressor is still ongoing can be unfavorable as well. Importantly, the elevated glucocorticoids of the EE rats more closely approximated the stressor situation than did the glucocorticoid levels from the pair-housed rats. Perhaps having an HPA axis response that approximates the stressor situation may be more important in promoting resiliency than terminating the stress response prematurely or having the HPA axis response mismatch the stressor, which is in agreement with previous evidence suggesting that EE renders the HPA axis response more adaptive (Larsson, Winblad, & Mohammed, 2002; Mohammed et al., 1993).

While the mechanisms by which EE may engender stress resiliency are poorly understood, EE has the opposite effect of stress on biochemical and morphological measures such as dendritic spine density, neuronal morphology, hippocampal neurogenesis, and neurotrophin expression (van Praag et al., 2000), so it seems that further research is necessary to determine how various mechanisms may interact to impart their potential therapeutic advantage. Mechanisms acting on synaptic plasticity may be involved and remain to be investigated in our experimental model. For instance, neurotrophins are a good candidate because EE increases their expression and they are involved in synaptic plasticity and neuronal signaling (Pham et al., 1999; Zhu et al., 2006). Further, increased glucocorticoid levels following stress may act in concert with altered neurochemical and molecular signaling pathways. Each could produce unique effects on dendritic morphology and behavior; their combination may produce a net retraction of dendrites. As such, it is plausible to hypothesize that when EE acts on individual or a combination of effects, different patterns of stress effects will result.

The present findings indicate that even when initiated in the adult, EE may benefit cognitive function and hippocampal neuronal architecture following chronic stress. This is consistent with recent studies which reveal that EE started either after weaning or in adulthood can prevent disruptions in spatial recognition memory (Chen et al., 2010), fear conditioning (Mitra & Sapolsky, 2009) and learned helplessness (Sifonios et al., 2009), perhaps through neuromorphological mechanisms (i.e., cytoskeletal reorganization or neurogenesis) (Mora,

Segovia, & del Arco, 2007; Schloesser, Lehmann, Martinowich, Manji, & Herkenham, 2010; Sifonios et al., 2009). These findings are not surprising given that exposure to a combination of learning opportunities, social networks and exercise appears to be essential in promoting and maintaining brain integrity in adult animals, as well as in humans. For instance, formal education in childhood has protective effects on adult human cognition and brain structure (Colsher & Wallace, 1991) and can even be beneficial when the education occurs in adulthood (Ball et al., 2002; Draganski et al., 2004). A rich social network also lowers the risk of cognitive impairment and dementia (Barnes, Mendes de Leon, & Wilson, 2004; Fratiglioni, Paillard-Borg, & Winblad, 2004). Further, studies conducted in adult humans point to beneficial effects of aerobic activity on brain structure and function (Aberg et al., 2009; Andel et al., 2008), for review (Hillman, Erickson, & Kramer, 2008).

In order to understand mechanism(s) of action, it may be necessary to dissociate EE subcomponents. Early studies attribute EE-induced morphological changes in the brain to inanimate object interactions (Rosenzweig et al., 1978). Subsequent findings suggest that both object and social interactions contribute to the beneficial effects of EE (van Praag et al., 2000). More recent studies suggest that exercise alone can improve working memory performance and hippocampal neurogenesis (Kobilo et al., 2011; Lambert, Fernandez, & Frick, 2005). However, some studies show that exercise increases hippocampal cell proliferation in isolated, but not socially housed mice (Kannangara, Webber, Gil-Mohapel, & Christie, 2009) and that duration of wheel running is critical in mitigating uncontrollable stress (Greenwood, Foley, Burhans, Maier, & Fleshner, 2005). As such, future studies are required to tease apart the respective contribution of each subcomponent of EE on the outcomes reported here.

Despite the need for further investigation, the preponderance of research indicates that separate or synergistic effects of single factors within EE, such as social interaction and exercise, exert considerable health benefits, and render the individual more resilient to effects of subsequent stressors. Evidence even demonstrates the protective nature of exercise (Babyak et al., 2000) and social networks (Dalgard, Bjork, & Tambs, 1995) for stress and depression (Barton, Griffin, & Pretty, 2011). Within this context, manipulation of environmental and experiential factors may yield new directions for optimizing brain integrity and resilience, even when one is experiencing ongoing stress or depression. The increasing prevalence of depressive disorder necessitate greater understanding of the basic mechanisms by which environment can both create (stress) and aid in the recovery (enrichment) of psychiatric illness. Enhanced understanding of these processes may enable the identification and development of alternative interventions.

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Fig. 1.

Prevention: EE initiated in adulthood prior to and throughout chronic stress ($n = 10\text{--}12/\text{group}$). (A) STR-Pr showed no statistically significant decrease in first-time entry errors across blocks 1 through 6 while first-time entry errors decreased across blocks for all other groups (CON-Pr, CON-EE and STR-EE). All groups were statistically similar during blocks 7–8 and at the 24-h retention trial (RT). A diagram of the RAWM illustrates swim areas within the 6 arms (in white) and the filled circle represents the hidden platform. (B) During acquisition (Blocks 1–6), repeat entry errors for all groups decreased. All groups were statistically similar at RT. (C) Camera Lucida-assisted reconstructions of representative Golgi-stained dorsal hippocampal CA3 neurons with quantitative group measurements illustrated in D. (D) Chronic stress decreased CA3 dendritic branch points, which was increased by EE. (E) EE increased CA3 apical dendritic length. Data are represented as means $\pm$ SEM and are slightly offset on the x -axis to assist with viewing in A and B. $§p < 0.05$ for significant main effect of stress, $‡p < 0.05$ for significant main effect of EE, $*p < 0.05$ for significant effect of blocks 1–6 for within each group alone. *Abbreviations:* CON,

control; EE, environmental enrichment; Pr, pair housed; RAWM, radial arm water maze; STR, stress.

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Fig. 2.

Prevention: RAWM inexperienced: EE initiated in adulthood prior to and throughout chronic stress modified CORT profiles ($n = 6/\text{group}$). (A). For the STR-Pr group, CORT levels exhibited a profile in the early sampling days (1,7) that differed by the last day (21) of restraint, such that CORT levels were muted. (B and C). The CORT profiles for STR-EE and CON-EE differed from STR-Pr, but were similar to each other in that both produced a CORT response within one hour of restraint and these CORT elevations were maintained at the 3-h sampling time throughout the four sampling days. The data are presented as mean percent of baseline CORT ($\pm \text{SEM}$). Please note, error bars for some means are smaller than is represented by the size of the symbol. ¥ $p < 0.05$ compared to sampling point "0" (i.e., baseline) on the x -axis. ¶ $p < 0.05$ compared to sampling point "1 h" on the x -axis.

Abbreviations: CON, control; CORT, corticosterone; EE, environmental enrichment; Pr, pair housed; STR, stress.

Fig. 3.

Intervention: EE initiated in adulthood two weeks after the start of stress ($n = 8-12/\text{group}$).

(A) For first-time entry errors during acquisition in the 8-arm RAWM, errors decreased for all groups (Blocks 1–6), indicating that the rats were learning. However, STR-Pr demonstrated reduced acquisition compared to other groups, which was attenuated by EE.

At the single 24-h retention trial, STR-Pr made more first-time entry errors than all other groups, which was prevented by EE. A diagram of the RAWM illustrates swim areas within the 8 arms (in white) and the filled circle represents the hidden platform. (B) For repeated entry errors during acquisition (Blocks 1–6), errors decreased for all groups, indicating that the rats were learning. At the 24-h retention trial, STR-Pr made more repeat entry errors than all other groups, which was prevented by EE. (C) Camera Lucida-assisted reconstructions of representative Golgi-stained dorsal hippocampal CA3 neurons with quantitative group measurements illustrated in D. (D) Chronic stress decreased CA3 dendritic branch points, which EE prevented. (E) Chronic stress decreased CA3 dendritic branch length, which EE prevented. Data are represented as means $\pm$ SEM and are slightly offset on the x-axis in panels A and B to assist with viewing. $\ddagger p < 0.05$ for significant main effect of EE, $+p < 0.05$

for STR-Pr compared to STR-EE, $\sqrt{p} < 0.05$ for STR-EE compared to Con-EE, $*p < 0.05$ for STR-Pr compared to STR-EE, CON-Pr, CON-EE. *Abbreviations:* CON, control; EE, environmental enrichment; Pr, pair housed; RAWM, radial arm water maze; STR, stress.

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Quantitative analysis of hippocampal CA3 dendritic morphology. Data are represented as mean $\pm$ SEM.

Table 1

	CON Pr	STR Pr	CON EE	STR EE
<u>Prevention: EE started before chronic stress: RAWM experienced (n = 10–12/group).</u>				
<u>Basal Dendritic Measures</u>				
Dendritic Branch Points	14.0 $\pm$ 0.6	15.0 $\pm$ 0.8	14.0 $\pm$ 0.8	14.6 $\pm$ 1.4
Total Dendritic Length (μ m)	1367.5 $\pm$ 51	1606.2 $\pm$ 93	1411.1 $\pm$ 62	1411.2 $\pm$ 92
<u>Prevention: EE started before chronic stress: RAWM inexperienced (n = 9/group).</u>				
<u>Basal Dendritic Measures</u>				
Dendritic Branch Points	15.7 $\pm$ 1.0	14.1 $\pm$ 0.6	14.1 $\pm$ 1.4	14.1 $\pm$ 1.2
Total Dendritic Length (μ m)	1435 $\pm$ 126	1405 $\pm$ 66	1453 $\pm$ 78	1289 $\pm$ 98
<u>Apical Dendritic Measures</u>				
Dendritic Branch Points	17.1 $\pm$ 0.3 ^a	12.7 $\pm$ 0.9 ^b	15.8 $\pm$ 0.7 ^a	15.8 $\pm$ 0.5 ^a
Total Dendritic Length (μ m)	1767 $\pm$ 92 ^a	1367 $\pm$ 80 ^b	1827 $\pm$ 40 ^a	1672 $\pm$ 67 ^a
<u>Intervention: EE started after chronic stress: RAWM experienced (n = 11 to 14/group).</u>				
<u>Basal Dendritic Measures</u>				
Dendritic Branch Points	15.1 $\pm$ 1.0 ^a	15.9 $\pm$ 1.5 ^a	19.9 $\pm$ 1.4 ^b	19.0 $\pm$ 1.1 ^b
Total Dendritic Length (μ m)	1543 $\pm$ 54 ^a	1511 $\pm$ 122 ^a	1785 $\pm$ 65 ^b	1722 $\pm$ 63 ^b

Abbreviations: CON, control; EE, environmental enrichment; Pr, pair housed; RAWM, radial arm water maze; STR, stress. Means with different letters indicate that groups are statistically significant and means with identical letters indicates means are statistically similar.