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Different types of environmental enrichment have discrepant effects on spatial memory and synaptophysin levels in female mice

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Abstract

Environmental enrichment paradigms that incorporate cognitive stimulation, exercise, and motor learning benefit memory and synaptic plasticity across the rodent lifespan. However, the contribution each individual element of the enriched environment makes to enhancing memory and synaptic plasticity has yet to be delineated. Therefore, the current study tested the effects of three of these elements on memory and synaptic protein levels. Young female C57BL/6 mice were given 3 h of daily exposure to either rodent toys (cognitive stimulation) or running wheels (exercise), or daily acrobatic training for 6 weeks prior to and throughout behavioral testing. Controls were group housed, but did not receive enrichment. Spatial working and reference memory were tested in a water-escape motivated radial arm maze. Levels of the presynaptic protein synaptophysin were then measured in frontoparietal cortex, hippocampus, striatum, and cerebellum. Exercise, but not cognitive stimulation or acrobat training, improved spatial working memory relative to controls, despite the fact that both exercise and cognitive stimulation increased synaptophysin levels in the neocortex and hippocampus. These data suggest that exercise alone is sufficient to improve working memory, and that enrichment-induced increases in synaptophysin levels may not be sufficient to improve working memory in young females. Spatial reference memory was unaffected by enrichment. Acrobat training had no effect on memory or synaptophysin levels, suggesting a minimal contribution of motor learning to the mnemonic and neuronal benefits of enrichment. These results provide the first evidence that different elements of the enriched environment have markedly distinct effects on spatial memory and synaptic alterations.

Keywords: Radial arm maze; Working memory; Reference memory; Mouse; Exercise; Motor skill learning

1. Introduction

In the past few decades, much attention has been focused on the ways in which the mammalian brain can change in response to environmental experience. One of the most influential paradigms used to demonstrate this phenomenon is environmental enrichment. Environmental enrichment typically involves exposing rodents to a variety of stimuli such as toys, tunnels, running wheels, and social interactions with other cagemates. Enriched animals are generally compared with isolated controls (who are individually housed without any social interaction) or social controls (who are group housed but are not exposed to any other enriching stimuli). Of interest to the study of learning and memory is the fact that environmental enrichment can produce a wide range of morphological changes in regions of the brain critical for learning and memory, such as the hippocampus and neocortex. For example, enrichment increases dendritic branching and spine number, synaptic contacts and neurotransmission, and neuron size in the rat neocortex (Diamond, 1967; Diamond, Krech, & Rosenzweig, 1964; Globus, Rosenzweig, Bennett, & Diamond, 1973; Green & Greenough, 1986; Greenough & Volkmar, 1973; Greenough, Volkmar, & Juraska, 1973; Greenough, West, & DeVoogd, 1978; Rosenzweig & Bennett, 1996).

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Environmental enrichment in rats and mice also enhances many aspects of hippocampal physiology such as long-term potentiation (LTP) (Duffy, Craddock, & Nguyen, 2001), neurogenesis (Kempermann, Kuhn, & Gage, 1997; Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999), neurotrophin levels (Ickes et al., 2000; Pham et al., 1999; Pham, Winblad, Granholm, & Mohammed, 2002), dendritic spine growth and branching (Green, Greenough, & Schlumpf, 1983; Rampon et al., 2000), synaptophysin levels (Frick & Fernandez, 2003; Nithianantharajah, Levis, & Murphy, 2004), and NGF mRNA and CREB gene expression (Torasdotter, Metsis, Henriksson, Winblad, & Mohammed, 1996, 1998; Williams et al., 2001).

Consistent with effects of enrichment on the hippocampus and neocortex, enriched rodents also exhibit enhanced learning and memory abilities relative to rodents housed in social or isolated control conditions. Although early studies focused on mnemonic effects of enrichment early in development (Greenough, Wood, & Madden, 1972), more recent studies have demonstrated that enrichment initiated in adulthood can significantly improve performance on several tests of spatial and non-spatial memory. For example, spatial reference memory in the Morris water maze is improved in adult rats (Nilsson et al., 1999) and mice (Kempermann et al., 1997; Williams et al., 2001) exposed to 1–3 months of complex enrichment (including toys, running wheels, and cagemates) in the home cage relative to isolated or social controls. We recently reported that spatial working memory in a water-escape motivated version of the radial arm maze is significantly improved in adult C57BL/6 mice by 3 h/day of complex enrichment for several months (Gresack & Frick, 2004). Others have also shown that non-spatial memories, such as object recognition, social recognition, and contextual fear conditioning are enhanced by complex enrichment in mice (Duffy et al., 2001; Rampon et al., 2000; Tang, Wang, Feng, Kyin, & Tsien, 2001). In aging rodents, enrichment provides similar mnemonic benefits. For instance, complex enrichment in middle-aged and aged rats and mice reduces age-related deficits in numerous types of learning and memory including spatial reference memory tested in the Morris water maze (Frick & Fernandez, 2003; Frick, Stearns, Pan, & Berger-Sweeney, 2003; Kempermann, Kuhn, & Gage, 1998). In aged female mice, this spatial memory improvement was associated with significant increases in levels of the presynaptic protein synaptophysin in the hippocampus and neocortex (Frick & Fernandez, 2003).

The enriched environment utilized in most rodent studies consists of a combination of complex social and sensorimotor stimuli such as bigger cages, numerous toys, tunnels, and running wheels. For this reason, the relevance of any single contributing factor, such as cognitive stimulation or exercise, cannot be easily ascertained, and it seems likely that an interaction between multiple factors is important to the mnemonic enhancement seen in enriched rodents. However, some evidence suggests that single factors, such as voluntary exercise, can affect the brain in a similar manner as complex enrichment, with changes including enhanced hippocampal LTP (Kim et al., 2004) and neurogenesis (van Praag, Christie, Sejnowski, & Gage, 1999a; van Praag, Kempermann, & Gage, 1999b), increased hippocampal and neocortical neurotrophin mRNA expression (Neeper, Gomez-Pinilla, Choi, & Cotman, 1995, 1996), and reduced age-related hippocampal synaptophysin decline (Chen, Chen, Lei, & Wang, 1998). Exercise also improves spatial memory and preserves cognitive function during aging in both rats (Anderson et al., 2000; Fordyce & Farrar, 1991) and mice (Fordyce & Wehner, 1993b; van Praag et al., 1999a). Furthermore, work in humans indicates that physical fitness can enhance cognitive and memory functions (Clarkson-Smith & Hartley, 1989; Dustman et al., 1990; Lupinacci, Rikli, Jones, & Ross, 1993), and that long-term aerobic training may prevent age-related decline in cognitive function (Hill, Storandt, & Malley, 1993; Rogers, Meyer, & Mortel, 1990; Yaffe, Barnes, Nevitt, Lui, & Covinsky, 2001).

Other studies have focused on motor learning associated with acrobatic training, as opposed to repeated physical exercise, as a single enriching variable. Acrobatic training consists of repeated trials through a course designed to encourage problem solving and coordination. For instance, animals may need to learn to traverse various bridges (made of such items as chains, ropes, and wires) and obstacles to reach a series of platforms. This type of motor learning (as opposed to the motor activity involved in voluntary or forced exercise) significantly increases synapse formation in the cerebellar cortex (Kleim et al., 1998; Kleim, Vij, Ballard, & Greenough, 1997), whereas repetitive physical exercise increases cerebellar blood vessel density (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990), suggesting that individual elements of the enriched environment may have markedly different effects on specific components of neural plasticity. Acrobatic training in rats also enhances synaptogenesis in the motor cortex and motor coordination following lesions of the sensorimotor cortex (Jones, Chu, Grande, & Gregory, 1999). If the motor skills acquired during the course of more conventional enrichment procedures contribute to memory improvement, then acrobatic training alone may benefit memory. However, the effects of motor skill learning on memory have yet to be examined.

Although a few attempts have been made to test the importance of single enriching factors such as socialization (Lu et al., 2003; Rosenzweig, Bennett, Hebert, & Morimoto, 1978) and general activity (Bernstein, 1973), no comparison has been made between different elements of the enriched environment in the same study. It is possible that separate elements of environmental

enrichment differentially enhance certain aspects of memory and neural function. Thus, the goal of the present study was to assess the influence of several individual aspects of enriched environments on spatial memory and synaptic protein levels. To this end, young female mice (all group housed) were exposed to either standard control conditions, cognitive stimulation only (CS), voluntary exercise only (EX), or acrobat training (AC) for 6 weeks. Spatial reference and working memory were then tested in a water-escape motivated version of the radial arm maze (Gresack & Frick, 2003). Previously, we have reported that a complex enrichment paradigm that combines the CS and EX conditions significantly improves the spatial working memory of young female C57BL/6 mice in this version of the radial arm maze (Gresack & Frick, 2004). Following behavioral testing, synaptic alterations were assessed by measuring levels of the presynaptic protein synaptophysin in the frontoparietal cortex, hippocampus, striatum, and cerebellum. Synaptophysin is a 38-kDa calcium-binding glycoprotein found in the membranes of neurotransmitter-containing presynaptic vesicles (Jahn, Schiebler, Ouimet, & Greengard, 1985; Wiedenmann & Franke, 1985). Among aging rodents, high synaptophysin levels are associated with better spatial reference memory (Calhoun et al., 1998; Chen, Masliah, Mallory, & Gage, 1995; Smith, Adams, Gallagher, Morrison, & Rapp, 2000), and enrichment-induced improvements in spatial reference memory are associated with increased synaptophysin levels in the hippocampus and frontoparietal cortex (Frick & Fernandez, 2003). Although an increase in synaptophysin is often interpreted to indicate an increase in presynaptic terminal density (e.g., Chen et al., 1995; Eastwood, Burnet, McDonald, Clinton, & Harrison, 1994; Terry et al., 1991), it is the number of vesicles per terminal, not terminal density, that seems to be increased in response to enrichment (Nakamura, Kobayashi, Ohashi, & Ando, 1999). Thus, enrichment-induced increases in synaptophysin levels may lead to increased neurotransmission and, consequently, improved spatial memory. Among young rodents, it is less clear how synaptophysin levels relate to spatial memory, or whether enrichment-induced improvements in spatial memory are associated with altered synaptophysin levels. The present study will be the first to examine this relationship.

2. Materials and methods

2.1. Subjects

Subjects were 33 young female C57BL/6 mice obtained at 9 weeks of age from Charles River Laboratories (Wilmington, MA). Mice were handled for 5 min/day for 5 days after arrival in the laboratory vivarium. Environmental enrichment began at 11 weeks of age and behavioral testing was initiated at 14 weeks of age. All mice were housed four or five per shoebox cage (30 cm long × 18.5 cm wide × 13 cm high) in a room with a 12-h light/dark cycle (lights on at 06:00), and enrichment sessions and behavioral testing were performed during the light phase of the cycle. Food (LabDiet 5P00 ProLab RMH 3000) and water were provided ad libitum. All procedures conformed to the standards set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee of Yale University.

2.2. Environmental enrichment

Mice were divided into four groups: control ($n = 9$), cognitive stimulation (CS; $n = 8$), voluntary exercise (EX; $n = 8$), and acrobat training (AC; $n = 8$). Controls were group housed but were never exposed to the enrichment chamber or enriching stimuli. All other groups were enriched prior to (6 weeks, 7 days/week) and during testing (mice were enriched in the morning and tested in the afternoon).

2.2.1. Cognitive stimulation

Two large Rubbermaid containers (HiTop Storage Box, 56.5 cm long × 41.5 cm wide × 22 cm high) were used for enrichment sessions as described previously (Frick & Fernandez, 2003). The bottom and sides of the chambers were constructed of translucent plastic and the removable covers were made of white opaque plastic. During enrichment sessions, covers with 75–80 holes (6 mm in diameter) were placed on top of the chambers to allow for air circulation and prevent escape. The floors of the chambers were covered with bedding and mice were provided with both food (3–4 pieces of rodent diet) and water (in a medium sized weigh boat).

Two chambers were placed next to each other in a well-lit room with various visual cues including a table, shelves, and boxes. Several objects were present in the chambers during each enrichment session. Objects included rodent toys (e.g., a plastic toy in the shape of a boat, balls, and plastic mouse houses), PVC pipe fittings in different shapes, hollow metal cylinders, Legos in various configurations, and a toy rope suspended in the cage in various ways. No running wheels were ever present in this condition. For each daily session, 4 or 5 different objects were presented in different combinations and in different locations in the chamber. Four CS mice from the same home cage were placed in each chamber, allowing the mice to interact with both the objects and their cagemates. Enrichment sessions lasted 3 h, after which time the mice returned to their home cages. Enrichment chambers and objects were cleaned thoroughly with soap and water after each session.

2.2.2. Exercise

The same chambers were used as in the CS condition. Two chambers were positioned next to each other in the same room as the CS chambers and four EX mice were placed in each chamber. Three running wheels (11.5 cm in diameter) were placed in each chamber, in different arrangements, each day. No rodent toys or other stimuli were present in the chambers. Enrichment sessions lasted 3 h, after which time the mice returned to their home cages. Chambers and running wheels were cleaned with soap and water at the conclusion of each session.

2.2.3. Acrobat training

The AC group was run daily on an acrobatic course designed to encourage problem solving and coordination. The course was based on that used by Kleim et al. (1998) and was composed of a series of 10 bridges connecting six wooden platforms (10 × 10 cm, 34.5 cm high). The platforms were arranged in two rows of three, with the rows separated by 31 cm and the columns separated by 29 cm. The bridges were made of various materials, such as chains of different sizes, rubber bands of different tensions, metal rods with loose bolts, wires, and ropes of different thickness and lengths, and wooden obstacles. The composition and order of the bridges was changed every week. Mice were run one at a time through the 10-bridge course five times/session for a total of 50 bridge crossings in one session. One session lasting approximately 30 min was conducted per day. The experimenter gently held the mice by the tail to prevent them from falling. Platforms and bridges were cleaned with 70% ethanol after each mouse completed their session.

2.3. Water-escape motivated radial arm maze (WRAM)

This task was designed to assess spatial working and reference memory, as described previously (Gresack & Frick, 2003). Briefly, the maze consisted of an opaque central arena (diameter = 44 cm) with eight clear Plexiglas arms (38 × 12 cm) radiating equidistantly from the center. The maze was placed in a large pool of water (24 ± 2 °C) made opaque with white non-toxic tempera paint. Hidden escape platforms were submerged just below the water surface (approximately 9 cm below the top of the maze) and were placed at the ends of four arms. One arm was designated as a start arm and it never contained a platform. The sequence of arms with platforms was randomized between mice, but remained unchanged within a mouse for all sessions. Platforms were never located in more than two consecutive adjacent arms. Numerous extra-maze cues surrounded the maze.

Prior to the first test session, mice were acquainted with the escape platforms using a five trial shaping procedure in which only one arm contained a platform. This platform was made visible with red tape and by lowering the water level to approximately 0.3 cm below the platform surface. For the first four trials, the mouse was confined to the shaping arm. In the first trial, the mouse was placed directly on the platform for 15 s. With each successive trial, the mouse was placed at further distances from the platform such that for the fourth trial, the mouse was placed at the entrance to the shaping arm. During the last trial, the shaping arm was opened to allow access to the center of the maze (the other 7 arms remained blocked). The mouse was then placed in the center and allowed to climb on the platform in the shaping arm. If, on any trial, the platform was not found in 30 sec, the mouse was gently guided to it. No data were collected during shaping.

Testing began the day after shaping. Four trials/day (comprising one session) were conducted for 14 consecutive days as follows. At the start of Trial 1, the mouse was released from the start arm and given 120 s to locate and climb onto a submerged platform. If the mouse did not locate the platform, it was guided to the nearest one, upon which it remained 15 s. It was then removed from the platform, dried off with a towel, and placed in a holding cage for a 30-s inter-trial interval (ITI). During the ITI, the platform was removed, leaving three platforms in the maze. The mouse was then returned to the start arm for Trial 2. This procedure was repeated until all four platforms were located (1 platform/trial). At the end of the fourth trial, the mouse was removed from the maze, dried with a towel, and returned to its home cage.

An arm entry was recorded when the entire body (excluding the tail) crossed into the arm. Three types of errors were recorded during each trial (Gresack & Frick, 2003). Working memory errors were entries into arms from which a platform had been removed during a daily session. Initial reference memory errors were first entries in each trial into arms that never contained a platform. Repeated reference memory errors were repeated entries into arms that never contained a platform. Although an error of this type involves a lapse of both reference and working memory, in that the mouse fails to remember that an arm does not contain a platform (reference) and that it has previously entered this arm during the session (working), this error is referred to as a reference memory error because it involves entries into arms that never contain platforms. In addition to determining the total number of working memory errors committed in each session, the number of working memory errors committed in Trials 2–4 of each session was determined (it was impossible to make a working memory error in Trial 1). This allowed working memory errors to be assessed as the trials progressed and the working memory information to be remembered (i.e., working memory load) increased (Hyde, Hoplight, & Denenberg, 1998).

2.4. Tissue collection

Each mouse was sedated briefly with CO_2 and decapitated (Berger-Sweeney, Berger, Sharma, & Paul, 1994).

The brain was removed immediately and frontoparietal cortex, striatum, hippocampus, and cerebellum were dissected bilaterally on ice. Brain tissue samples were weighed and stored at −70 °C until the day of assay. Samples were resuspended in 0.02% Triton X-100 in 0.1 mM Tris, pH 7.4, sonicated with a probe sonicator, and centrifuged for 10 min at 10,000*g*. The supernatant was diluted 1:5 and designated as the crude extract. This crude extract was further diluted as described below. The protein content of the samples was measured using a Bradford protein assay (Bradford, 1976).

2.5. Synaptophysin assay

Synaptophysin is a 38-kDa calcium-binding glycoprotein found in the membranes of neurotransmitter-containing presynaptic vesicles (Jahn et al., 1985; Wiedenmann & Franke, 1985), and increases in synaptophysin immunoreactivity have most often been interpreted as reflecting an increase in presynaptic terminals (Chen et al., 1995; Eastwood et al., 1994; Terry et al., 1991). Several studies report significant correlations between synaptophysin levels and spatial memory in the Morris water maze, such that more synaptophysin is associated with better spatial reference memory (Calhoun et al., 1998; Chen et al., 1995; Smith et al., 2000).

Synaptophysin was measured using an enzyme-linked immunosorbent assay (ELISA) as described previously (Frick & Fernandez, 2003; Frick, Fernandez, & Bulinski, 2002; Frick et al., 2003). Because purified synaptophysin was not available for use as a standard, synaptophysin levels in the samples are expressed as "equivalents" relative to synaptophysin immunoreactivity from whole mouse brain homogenate, termed mouse brain standard (MBS). An antibody sandwich ELISA assay using two different anti-synaptophysin antibodies (monoclonal anti-synaptophysin Clone SY 38 and polyclonal rabbit anti-synaptophysin; DAKO, Carpinteria, CA, USA) was used to determine the relative amounts of synaptophysin in the samples. Samples were diluted to 1:32,000 from the crude extract and were assayed in triplicate. Optical density was measured at a wavelength of 405 nm using a Labsystems Multiskan Plus microplate reader. The average absorbance of three wells containing no MBS was subtracted from each reading.

To calculate the relative amount of synaptophysin in the samples, the absorbance of each of four MBS concentrations was plotted versus the log of the total protein concentration. The equation of the straight line that resulted and the absorbance of each sample was used to determine the concentration of MBS which would have the absorbance exhibited by the sample. This apparent MBS concentration of the sample was divided by the total protein concentration of the sample (obtained from the protein assay described above) to yield the relative amount of synaptophysin in the sample versus the amount of synaptophysin in the MBS homogenate (termed "MBS synaptophysin equivalent").

2.6. Data analysis

Working memory errors, initial reference memory errors, and repeated reference memory errors were each analyzed separately using one between (Treatment) and one within (Sessions) subject repeated measures analyses of variance (ANOVA; SuperANOVA, Abacus Concepts, Berkeley, CA). Fisher's Protected least significant difference (PLSD) post hocs were performed to delineate between-group differences. Working memory errors made within each trial (working memory load) were analyzed using a one between (Treatment) and one within (Trials) repeated-measures ANOVA. One-way ANOVAs without repeated measures were used to analyze treatment-related differences in working memory errors within each trial. The first day of testing was considered a training day and thus, data from Session 1 were excluded from all analyses (e.g., Bimonte, Hyde, Hoplight, & Denenberg, 2000; Gresack & Frick, 2003). Because mice during Session 1 are introduced for the first time to the entire maze, the platform locations, hidden platforms, and the concept that platforms disappear once found, this session does not accurately measure any aspect of working or reference memory. Therefore, working and reference memory are measured starting in Session 2, at which point the mice have been fully exposed to the apparatus and rules of the task. In addition to the analyses described above, we conducted separate analyses on the first half (Sessions 2–7) and last half (Sessions 8–14) of testing to determine if the effects of the enrichment treatments differed during task acquisition from those later in testing (as in Gresack & Frick, 2003). However, there were no differential effects of any of the enrichment treatments on performance in either the first or last halves of testing (for both working and reference memory). Thus, these analyses are not reported below.

Synaptophysin data were analyzed for each brain region separately using one-way ANOVAs without repeated measures and Fisher's PLSD post hocs.

3. Results

3.1. Water radial arm maze

3.1.1. Working memory errors

The main effect of Treatment was significant for working memory errors ($F(3,29) = 3.9$, $p < .02$; Fig. 1A). Posthoc tests revealed that EX mice made significantly fewer errors than both controls ($p = .01$) and AC mice ($p < .005$). Although there was a trend for the EX group to make fewer errors than the CS group ($p = .08$),

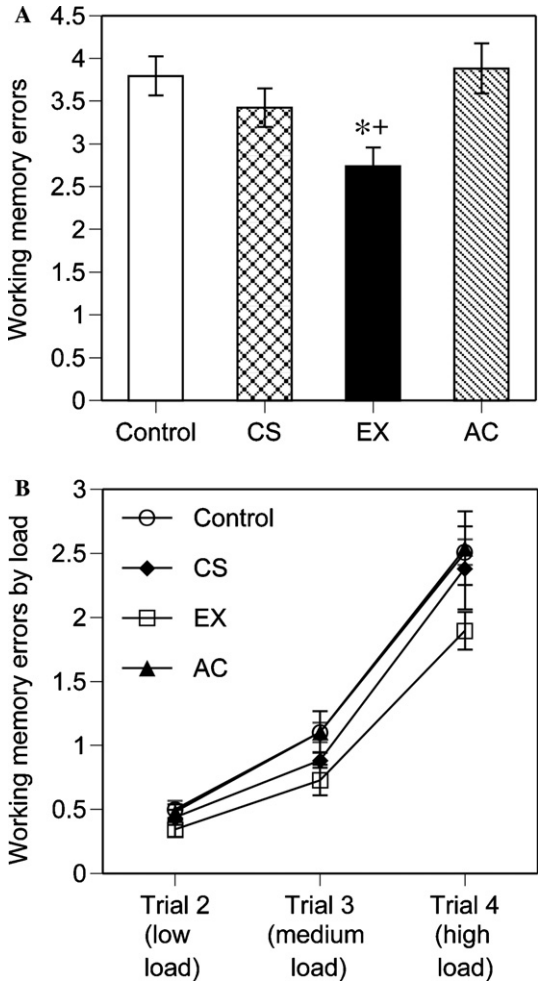

Fig. 1. (A) Mean (±SEM) working memory errors made during sessions 2–14 of testing. EX mice made significantly fewer errors than controls (*$p < .05$) or AC mice ($^{+}p < .05$). (B) Mean (±SEM) working memory errors made during each trial of sessions 2–14. EX mice made significantly fewer errors than controls or AC mice during Trial 3 ($p < .05$).

there were no other significant group differences. The main effect of Session was also significant ($F(12,348) = 4.0$, $p < .0001$), reflecting a decrease in the number of errors per session as training progressed. The Session × Treatment interaction was not significant.

The number of working memory errors made within a session increased with working memory load (main effect of Trial, $F(2,58) = 190.4$, $p < .0001$; Fig. 1B). The Treatment main effect was also significant ($F(3,29) = 3.4$, $p < .05$) and post hoc tests indicated that the EX group made fewer errors than the control and AC groups ($ps < .05$). In one-way ANOVAs performed on each trial, Treatment main effects were not significant for Trials 2 or 4, but were nearly significant for Trial 3 ($F(3,29) = 1.3$, $p = .06$). In this trial, EX mice made significantly fewer errors than the control and AC groups ($ps < .05$).

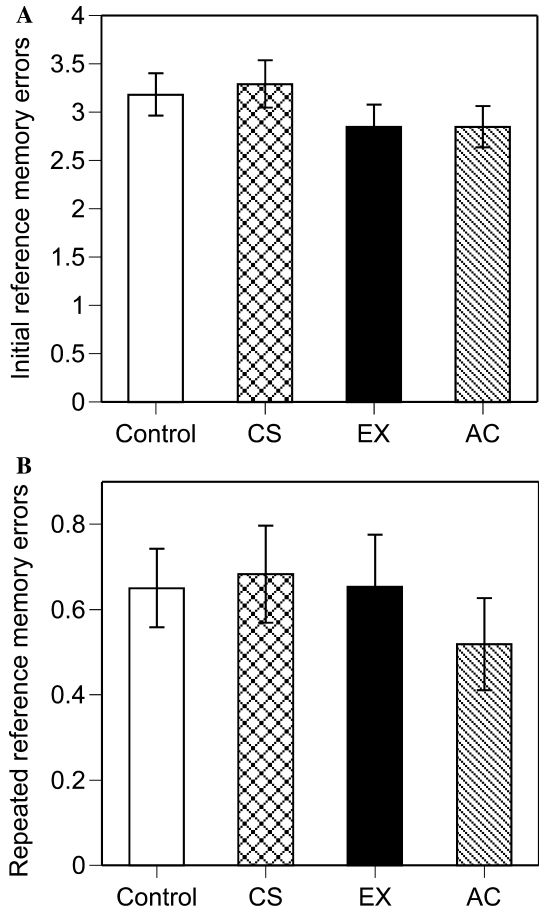

Fig. 2. Mean (±SEM) initial reference memory errors (A) and repeated reference memory errors (B) made during sessions 2–14 of testing. Enrichment had no significant effect on either error type.

3.1.2. Reference memory errors

There was no significant main effect of Treatment or Session × Treatment interaction for either initial (Fig. 2A) or repeated reference memory errors (Fig. 2B). The main effects of Session, however, were significant for both error types (Initial: $F(12,348) = 7.2$, $p < .0001$; Repeated: $F(12,348) = 3.1$, $p < .001$), reflecting a reduction in reference memory errors made during the course of testing.

3.2. Synaptophysin

Fig. 3 illustrates group differences in synaptophysin levels. Enrichment significantly affected synaptophysin levels in the neocortex ($F(3,29) = 3.0$, $p < .05$), hippocampus ($F(3,29) = 7.9$, $p < .0005$), striatum ($F(3,29) = 3.4$, $p < .05$), and cerebellum ($F(3,29) = 2.9$, $p = .05$). The CS group exhibited significantly higher synaptophysin levels than both the control and AC groups in the neocortex (Fig. 3A), hippocampus (Fig. 3B), and cerebellum (Fig. 3C; $ps < .05$). The EX group had significantly higher synaptophysin levels than controls in the neocortex and hippocampus, and than the AC group in the neocortex

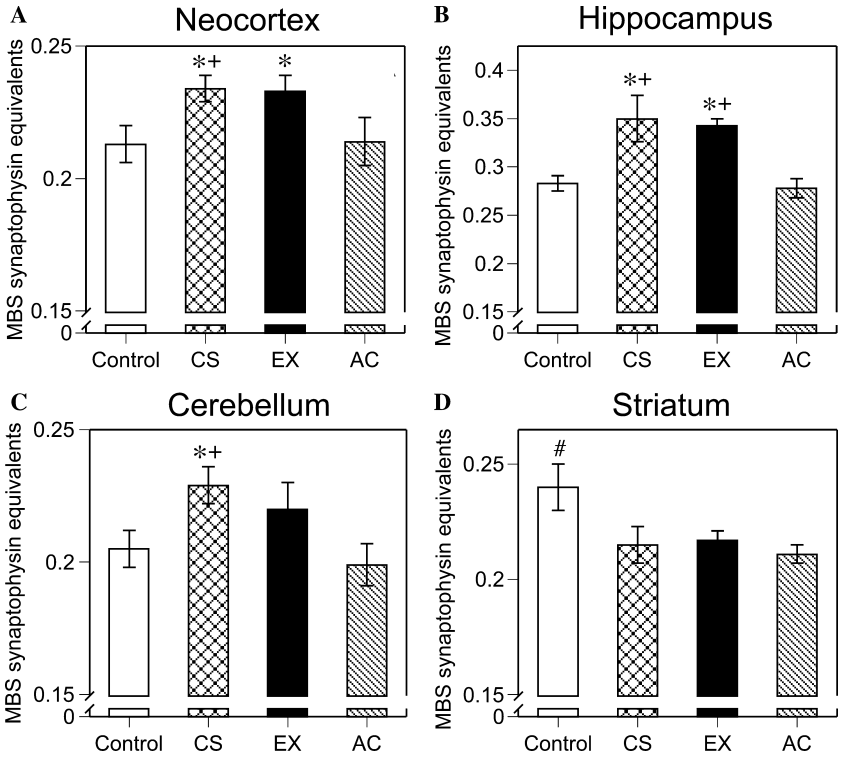

Fig. 3. Synaptophysin levels in the neocortex (A), hippocampus (B), cerebellum (C), and striatum (D). Synaptophysin values represent mean (±SEM) 'MBS synaptophysin equivalents' expressed as sample immunoreactivity relative to that of an equal amount of MBS. *$p < .05$ relative to controls, $^{+}p < .05$ relative to the AC group, $^{\#}p < .05$ relative to all other groups.

($ps < .05$). Levels in the control and AC groups did not differ in the neocortex, hippocampus, or cerebellum. In contrast, controls exhibited higher synaptophysin levels than all other groups in the striatum ($ps < .05$; Fig. 3D).

4. Discussion

In the present study, exercise, but not cognitive stimulation or acrobat training, significantly reduced working memory errors in the radial arm maze. This finding is interesting in light of the fact that both the EX and CS groups exhibited significantly higher neocortical and hippocampal synaptophysin levels than controls, and calls into question the relationship between synaptophysin and memory in young females. In contrast to working memory, no enrichment treatment affected reference memory errors in the WRAM. Notably, acrobatic training had no effect on memory or synaptophysin levels, suggesting that motor learning may play little, if any, role in the mnemonic and neuronal benefits of enrichment.

Mice in the EX group made significantly fewer working memory errors in the WRAM than the control and AC groups. The EX data are consistent with a previous study of isolate-housed female rats in which 7 weeks of voluntary wheel running improved spatial working memory in a dry-land RAM relative to controls (Anderson et al., 2000). In contrast to working memory, no enrichment treatment, including EX, had any effect on spatial reference memory in the present study. Previous studies examining effects of 4–7 weeks of voluntary wheel running or forced treadmill exercise on another spatial reference memory task, the Morris water maze, have found a beneficial effect of exercise on this task in male (Fordyce & Wehner, 1993b) and female (van Praag et al., 1999a) mice. Thus, it is somewhat surprising that reference memory in the present study was not affected by exercise. However, the amount of reference memory information to be remembered differs significantly between these two tasks (4 platforms to be remembered in the WRAM versus 1 in the Morris water maze), and thus, it may take more extensive exercise treatment to influence reference memory in the WRAM. Alternatively, the number of reference memory errors, particularly repeated reference memory errors, was quite a bit lower than the number of working memory errors, and thus there may not have been much room for improvement in the two reference memory measures. Lastly, spatial working memory in the RAM is highly dependent on the hippocampus, whereas spatial reference memory in this task tends to be more dependent on the neocortex (Olton & Papas, 1979; Porter & Mair, 1997). The fact that the increase in synaptophysin levels in EX mice relative to controls was greater in the hippocampus (21%) than in the neocortex (9%) may have led to more of an

exercise-induced effect on working memory than on reference memory.

The EX-induced increase in hippocampal and neocortical synaptophysin levels is consistent with previous reports in female C57BL/6 mice of increased dentate gyrus LTP and neurogenesis after 4 weeks of social housing and voluntary wheel running (van Praag et al., 1999a, 1999b). They are also consistent with reports in rats and mice of increased hippocampal and neocortical neurotrophin mRNA expression (Neeper et al., 1995, Neeper, Gomez-Pinilla, Choi, & Cotman, 1996), hippocampal protein kinase C (PKC) activity (Fordyce & Wehner, 1993a), and hippocampal cholinergic activity (Fordyce & Farrar, 1991) after voluntary wheel running or treadmill exercise. In total, these data suggest a beneficial effect of exercise on hippocampal and neocortical synaptic plasticity. In two previous studies, increases in hippocampal PKC activity (Fordyce & Wehner, 1993a), LTP, and neurogenesis (van Praag et al., 1999a) were associated with improvements in spatial reference memory in the Morris water maze. Although our study used a different task, the data also suggest an association between exercise-induced increases in synaptophysin levels and spatial memory.

CS did not significantly improve memory in the WRAM, but increased synaptophysin levels relative to controls and AC in the neocortex, hippocampus, and cerebellum. It is difficult to compare these data to those from previous studies because no prior study has examined effects of cognitive stimulation in the absence of exercise-inducing stimuli on memory or the brain. A comparison to studies testing the effects of more complex enrichment protocols on Morris water maze performance (Duffy et al., 2001; Foster & Dumas, 2001; Kempermann et al., 1997; Nilsson et al., 1999; Rampon et al., 2000) suggests that the lack of effect of CS on spatial memory is somewhat unusual. One possible reason for the discrepancy is that, due to the increased difficulty of the WRAM over the standard reference memory version of the Morris water maze, CS alone may not be sufficient to improve performance. On the other hand, perhaps exercise is the critical component that allows complex enrichment protocols to affect spatial memory.

Interestingly, the lack of a CS effect on the WRAM occurred despite robust increases in synaptophysin levels in the neocortex, hippocampus, and cerebellum. The observed CS-induced increases in neocortical and hippocampal synaptophysin levels are consistent with data from previous studies using complex enrichment protocols in female mice (Frick & Fernandez, 2003; Nithianantharajah et al., 2004). Nevertheless, the RAM is a task in which both the neocortex and hippocampus play a critical role (Olton & Papas, 1979; Porter & Mair, 1997), and therefore, it is surprising that increased synaptophysin levels in these regions did not lead to significantly improved memory. Although parallel enhancements in hippocampal synaptophysin levels and performance in the Morris water maze have been reported in aged mice (Calhoun et al., 1998; Frick & Fernandez, 2003), a similar relationship between increased spatial memory and synaptophysin levels may not be present in the young brain. Numerous neurobiological mechanisms likely account for intact spatial memory in young mice, and an increase in one mechanism may have little effect on an optimally functioning brain. In contrast, an increase in any one mechanism in the aged brain may make the difference between intact and impaired performance. Alternatively, it is possible that in young mice, synaptophysin levels have little to do with performance on the WRAM. A comparison of the CS and EX data suggests this may be the case. Although both treatments increase synaptophysin levels, the primary benefits of enrichment to WRAM performance may be produced by changes in other factors, such as cerebral blood flow (Black et al., 1990), neurotrophin levels (Neeper et al., 1995, 1996), or neurogenesis (Kempermann et al., 1997; van Praag et al., 1999a, 1999b). Furthermore, the exercise treatment may have rendered the EX group more physically fit than the CS group, allowing the EX group to better handle the physical demands of the WRAM and more easily focus on the mnemonic aspects of the task. The divergent behavioral effects of the CS and EX treatments provide an interesting model in which to test hypotheses about the critical neurobiological mechanisms underlying the mnemonic benefits of environmental enrichment.

Acrobatic training did not affect memory or synaptophysin levels. The fact that no changes in synaptophysin levels were observed in the cerebellum or neocortex (our dissection of which contains the motor cortex) is discrepant with previous studies showing that acrobatic training increased synaptic density in the cerebellum of female rats (Black et al., 1990; Kleim et al., 1998, 1997) and the motor cortex of male rats (Jones et al., 1999). Although species differences may account for this discrepancy, it is also possible that our ELISAs, which measured synaptophysin protein levels throughout an entire structure, were less able to detect presynaptic changes than the morphological procedures used in previous studies. Indeed, the more restricted focus on the paramedian lobules of the cerebellum (Black et al., 1990; Kleim et al., 1997, 1998) and the motor cortex (Jones et al., 1999) in previous studies compared with our dissections of the entire cerebellum and frontoparietal cortex may have obscured AC-induced changes in synaptophysin levels. However, even if alterations in synaptophysin levels in the current study were present, but undetectable, these changes were inadequate to significantly affect memory in the WRAM. When comparing the AC group to the other enrichment groups in this study, the fact that CS also did not affect memory in the WRAM supports the idea that exercise is the critical memory-

enhancing element of complex enrichment. However, the fact that CS and EX, but not AC, increased synaptophysin levels in several brain regions suggests that AC is qualitatively different from these treatments. Methodological differences between the acrobat treatment and the other enrichment conditions (e.g., total time spent enriching per day, amount of experimenter contact) may also have contributed to differences among these treatments.

The only way in which the control group differed from all other groups was in striatal synaptophysin levels. In contrast to all other brain regions, controls exhibited higher synaptophysin levels than all other groups. The striatum is not generally thought to be critical for the performance of spatial memory tasks such as the WRAM, but rather is usually associated with response learning and motor activity (Poldrack & Packard, 2003). Nevertheless, we might have expected to find an increase in striatal synaptophysin activity in the AC or EX groups due to the more physical nature of their treatments. Previous work has shown that enrichment procedures capable of increasing hippocampal neurotrophin expression produce no changes in the striatum (Faherty, Kerley, & Smeyne, 2003; Neeper et al., 1996). However, other data indicate that complex enrichment, but not exercise, enhances hippocampal dendritic branching, whereas neither treatment affects striatal dendritic morphology (Faherty et al., 2003). In the current study, controls differed from all other groups in that they received no physical activity prior to WRAM testing. Because the WRAM requires a tremendous amount of physical activity, testing may have produced the increased synaptophysin seen in controls. Testing may not have produced a similar increase in the other groups if they had already grown accustomed to increased physical activity as a result of treatment and thus, synaptophysin levels had returned to baseline by the time of testing.

In conclusion, the results of the present study suggest that each element of the enriched environment may contribute differently to the mnemonic and neurobiological alterations observed in enriched animals. In particular, exercise alone appears to be sufficient to improve working memory and increase synaptophysin levels. Moreover, enrichment-induced increases in synaptophysin levels do not, by themselves, appear to be sufficient to improve working memory in young mice. Further work will be needed to determine the ways in which various environmental components influence memory and the brain, and identify the enrichment-induced neurobiological changes that are more closely associated with improvements in memory in young animals. Nevertheless, this work provides a first step towards the goal of revealing the neurobiological mechanisms by which various environmental manipulations can improve memory.

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