

Environmental enrichment: Effects on spatial memory and hippocampal CREB immunoreactivity

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Abstract

Environmental enrichment has been shown to improve performance in tests of spatial memory, induce neurogenesis in the hippocampus, enhance survival of newly formed granule cells, and inhibit spontaneous apoptosis. Although neuroplasticity of the mammalian brain declines with age, recent evidence suggests that the adult brain exhibits significant plasticity in response to environmental stimulation. The present study was designed to evaluate the effect of environmental enrichment on spatial memory and on immunoreactivity to cAMP response element binding protein (CREB) from the hippocampus. C57/BL/6 mice were trained in a Morris water maze after exposure to an enriched environment, either from 35 to 94 days or from 100 to 159 days of age. Hippocampal tissue from representative animals was later analyzed by Western blot for CREB immunoreactivity. Results indicate that environmental enrichment (particularly during the earlier period) improved performance on the Morris water maze and tended to increase immunoreactivity to CREB in the hippocampus. Social interaction by itself did not result in significant differences in navigational performance. Results with regard to social interaction and CREB immunoreactivity were mixed. Results are discussed in terms of evaluating the construct of enrichment, the correlation of CREB transcription and behavior change, and the importance of the developmental period for enrichment. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Environmental enrichment; Spatial memory; Water maze; CREB; Hippocampus

1. Introduction

Environmental enrichment has been shown to induce structural changes in the hippocampus. Such changes include increases in thickness [1], density of glial cells [1], and dendritic arborization [2]. In adult mice, neurogenesis occurs in the hippocampus, primarily in the subgranular zone of the dentate gyrus [3]. For spatial learning and memory in rodents [4], the neuronal progenitor cells residing in the subgranular zone are particularly important. These cells proliferate, migrate, and differentiate into granule cells. Their axonal processes become part of the mossy fiber pathway, which forms synaptic connections with targets in the CA3 region of hippocampus [5], a primary

site for long-term potentiation [6]. Thus, it is a primary site for the study of experience-dependent neuroplasticity.

An increased number of surviving, newly formed granule cells, as well as increased total neuron count, have been observed from the dentate gyrus of adult mice previously exposed to an enriched environment [3,7,8]. Thus, while neuronal loss may be permanent in much of the mammalian brain, neurogenesis within the granule cell layer of the hippocampus has been demonstrated throughout the adult life of rodents [7,8]. Although plasticity and proliferation of progenitor cells ultimately decline with age [9], enriched environmental conditions may serve as an effective buffer against rapid decline in plasticity related to age [4] or to cerebral injury [10]. The observed plasticity is both structural, functional, and behavioral in nature. Environmentally induced neurogenesis in the dentate gyrus is associated with improved performance in tests of spatial memory in adult rats [4].

In addition to its effects on neurogenesis, environmental enrichment has been shown to inhibit rates of spontaneous

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apoptosis in the rat hippocampus by 45% [11]. Enrichment also protected animals against experimentally induced seizures and excitotoxic injury. In senescent mice, improved cognitive performance has been found to be associated with enrichment-induced survival of newly formed granule cells in the hippocampal dentate gyrus [8]. Endogenous cAMP response element binding protein (CREB) activation has also been shown to promote neuronal survival in the dentate gyrus [12]. Thus, the neuroprotective properties [11] of enrichment may be mediated through activation of the transcription factor CREB.

Several reports implicate CREB activation in the formation of long-term memory in *Drosophila* [13,14], *Aplysia* [15–17], and mice [18]. Therefore, it is possible that the cognitive effects of environmental enrichment (i.e. improved spatial learning and memory) will also be mediated by CREB activation in the hippocampus. In this study, immunoreactivity to CREB was evaluated from the hippocampus of representative animals.

In humans, enrichment of the preoperative or posttraumatic environment has been utilized as an adjunct in the functional recovery from various types of brain injuries [10]. Moreover, Maguire et al. [19] have recently documented hippocampal reorganization in taxi drivers who are presumably confronted with greater navigational challenges than most other types of occupations. While evidence for neuroplasticity in experimental animals and humans is mounting, the environmental conditions that result in structural and functional alterations are less clear.

Environmental enrichment has been defined as a “combination of inanimate and social stimulation” [20]. This definition suggests that experience-dependent neuroplasticity may have a physical as well as a social component. As a result, recent reports documenting neurogenesis or neuroprotective properties of enriched environments have tended to assess the combined effects of both social and physical enrichment [4,11,21,22]. For example, the treatment group might be raised socially in an environment, which is also physically enriched with ‘toys’ and objects to manipulate. The control group is often housed individually in a physically impoverished environment. From these studies, it is impossible to assess the relative contribution of social vs. physical enrichment to experience-dependent neuroplasticity. In other studies, all animals (enriched and impoverished) are housed socially, with no individually housed (IH) controls [3,23]. In contrast, the design of the current experiment makes it possible to partition out the separate vs. combined effects of social vs. physical enrichment.

The developmental period during which environmental stimulation is administered can have enduring effects on adult behavior. For example, environmental enrichment from weaning to 100 days of age resulted in an improvement in shuttlebox avoidance learning, which remained significant 6 months after enrichment was discontinued [24]. In this study, environments were enriched during either an earlier (35–94 days of age) period or a later (100–159 days) period.

Several hypotheses were tested. Animals housed socially were expected to perform more efficiently in the Morris water maze than animals housed individually. Physical enrichment, in addition to social interaction, was expected to improve Morris maze performance relative to that elicited by social housing alone. Finally, earlier enrichment was expected to have a greater impact on performance than later enrichment of environment. Since CREB transcription in the hippocampus is believed to be a mediator of spatial learning and memory [18], levels of CREB immunoreactivity from hippocampal tissue were expected to correlate positively with Morris water maze performance.

2. Materials and methods

2.1. Subjects

Forty female C57/BL/6 mice, an inbred strain noted for capable performance on the Morris water maze [25], were selected after weaning (Harlan, Indianapolis, IN). Animals were maintained on a reverse 12:12 light/dark cycle with light onset at 20:00 h. Food and water were available ad libitum. Room temperature was maintained at $23 \pm 1^\circ\text{C}$. All procedures involving mice were conducted in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and the guidelines set forth by the Institutional Animal Care and Use Committee (IACUC) of the University of Southern Mississippi.

2.2. Housing

At the outset, all animals were given substantial daily handling for 1 week. Care was taken to ensure that different groups of animals received the same approximate amount of handling, since stress-related differences in cell loss, cognitive performance [26], and basal cyclic AMP accumulation in hippocampus and cerebral cortex [21] have been demonstrated between handled and nonhandled rodents. Animals were assigned to one of four groups. To control for the potentially confounding effect of individual vs. social housing, two control groups were used. In the first control group (IH), animals ($n=10$) were housed individually in plastic tubs ($11.5 \times 7.5 \times 5$ in., Ancare, Bellmore, NY). In the second control group (SH), animals ($n=11$) were housed collectively in a larger plastic tub ($19 \times 10.5 \times 6$ 1/8 in.). Enrichment was defined in terms of physical environment and not social housing condition. Therefore, the two experimental groups (E1 and E2) were also housed socially in same-sized tubs ($19 \times 10.5 \times 6$ 1/8 in.). The only difference between these two groups was the particular period of time during which animals received enrichment. E1 ($n=9$) received enrichment from 35 to 94 days of age. E2 ($n=10$) received enrichment from 100 to 159 days of age. Enrichment consisted of nesting materials, “toys,” containers suitable for hiding, ladders, platforms, and/or walkways.

New toys were placed in the cage at the beginning of the dark cycle and again 4 h later. Digging and burrowing are species-typical behavior in rodents [27]. Therefore, food was hidden in the bedding and nesting materials.

2.3. Apparatus

The water maze was modified from Morris [28]. Animals were trained in a pool 1.5 m in diameter and 0.6 m high, containing water held constant at $22 \pm 1^\circ\text{C}$. The pool was in the center of a room (16×16 ft.) containing various salient visual cues. Illumination was held constant. A 10×10 cm transparent platform was hidden in a constant place in the pool with its top surface submerged 1 cm below the water level. An HVS tracking system (SA-3 Tracker using CRT402 program with an IBM-compatible PC, San Diego Instruments) was used to record behavior. The camera (Burle TC355AC/TC352A, San Diego Instruments) was mounted directly over the center of the maze. A nontoxic white poster paint was added to the water to facilitate tracking the black mice and to obscure the platform.

2.4. Behavioral measures

The timeline (Fig. 1) illustrates the temporal sequence of events. All animals performed the Morris maze during three separate 5-day blocks (30–34, 95–99, and 160–164 days of age). During these periods, each day consisted of four training trials (except for Day 5 of each block, which consisted of three training trials and one probe trial). The training trials were given in two trial blocks, with an approximate 30 min intertrial interval (ITI) between the two trials. The time between the first and second blocks each day was approximately 2 h. The ITI was held approximately constant across animals, since CREB-mediated transcription affects the ITI required for the formation of long-term memories [29] and maximal CREB activation may require an ITI of at least 3–8 min [30]. During training trials, the location of the transparent Plexiglas platform remained at a fixed location for each animal during that 5-day block of trials. The location of the platform was then changed for each subsequent 5-day block of trials. Thus,

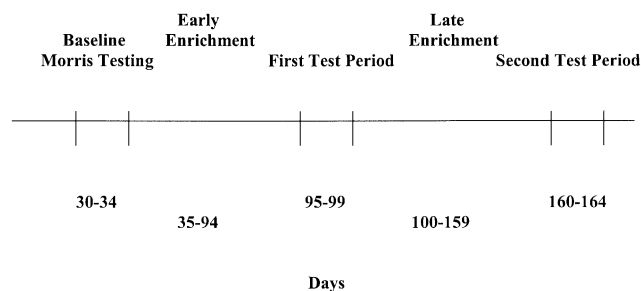


Fig. 1. Timeline for experimental protocol showing age of animals (days) during each enrichment period and age (days) of animals during each maze testing period.

each animal was given three separate training periods at three different ages. For each 5-day training block, the direction from which the animal started was randomly varied (i.e. North, South, East, and West) to prevent the association of the platform location with a single constellation of cues. If the animal located the platform during the 60-s trial, it was allowed to remain on the platform for an additional 10 s. Latency to reach the platform was recorded automatically by the tracking system. If the animal did not find the platform within the allotted 60 s, then the animal was guided to the platform, where it remained for 10 s before being removed from the pool. The three probe trials, which occurred on Days 34, 99, and 164, were the last trials for each 5-day block of trials. The probe trial consisted of a 60-s swim period during which time the platform was removed. On probe trials, the dependent variable was the percentage of time spent in the correct quadrant.

2.5. Western blot

Following the completion of the behavioral experiments, randomly selected animals were sacrificed by cervical dislocation and brain tissue was removed immediately. Samples of hippocampal tissue and cerebral cortex were dissected and stored separately in aliquots at -70°C . The hippocampal tissue was homogenized in 500 μl of the lysis buffer containing 10 mM Tris-HCl, pH 7.5, 2 mM MgCl_2 , and 0.25 M sucrose. The samples were then sonicated for 20 s and microfuged for 10 min. The supernatants were used as tissue extracts. Total protein from each sample was assayed using a BCA kit from Pierce (Rockford, IL) and protein concentrations were equalized. An equal amount of SDS sample buffer was added to the lysate, which was then boiled for 4 min and loaded for SDS-PAGE (12.5% acrylamide gel). After transfer of samples from SDS-PAGE gel to Immobilon-P membrane (Bio-Rad, California), blots were blocked with 5% milk in PBS-T (0.05% Tween-20) for at least 30 min at room temperature. The primary antibody, anti-CREB (Santa Cruz, CA), was diluted in 5 ml of blocking solution (1:500) and was incubated for 1 h at room temperature. Blots were washed five times at room temperature with PBS-T followed by incubation with the secondary antibody, antirabbit IgG, which was diluted in 5 ml blocking solution (1:8000). Blots were washed and developed with a chemiluminescence kit (Amersham).

2.6. Statistical analyses

All analyses were conducted using SPSS software. The α level of significance used for all statistical tests was set at $P \leq .05$. Behavioral data consisted of two measures: (1) mean \pm S.E.M. latency in seconds to locate the hidden platform and (2) mean \pm S.E.M. time spent in the correct quadrant, expressed as a percentage of the total time in maze. Orthogonal contrasts were used to analyze the behavioral data. Because certain predictions were made a priori,

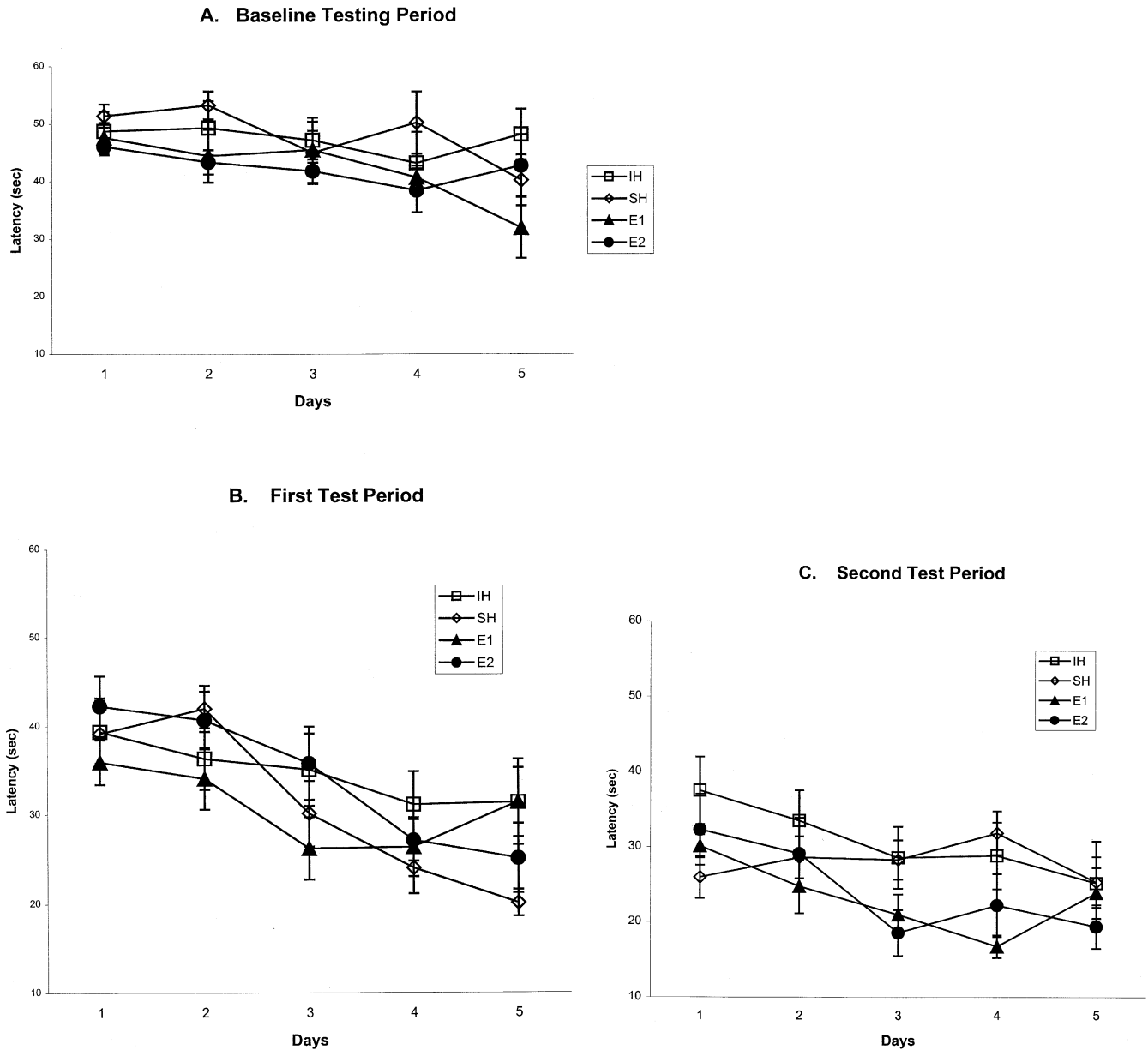


Fig. 2. Learning curve for Morris water maze performance: (A) baseline testing: Days 30–34; (B) first test period: Days 95–99; (C) second test period: Days 160–164. Mean time required to reach the submerged platform was recorded across test days for each of the four conditions. Mice serving in the control groups were housed either individually (IH: $n = 10$) or socially (SH: $n = 11$) but without a physically enriched environment. The two treatment groups were both housed socially. Group E1 ($n = 9$) received physical enrichment of environment during an early period (35–94 days). Group E2 ($n = 10$) received enrichment during a later period (100–159 days).

the information derived from different comparisons was assumed to be independent and nonoverlapping [31]. Orthogonal contrasts were performed for the following planned comparisons: (1) IH control vs. a composite of the three socially housed (SH) groups (SH, E1, and E2), (2) SH control group vs. a composite of the two enriched groups (E1 and E2), and (3) early (E1) vs. late (E2) enrichment group. One-way ANOVA was used to assess data collected during probe trials.

To assess the possibility that differences between groups in Morris maze performance might be due solely to differ-

ences in swim speed, a general linear model (GLM) repeated-measures ANOVA was conducted. Swimming speed (distance traveled per time) was calculated over all trials on the first and last days of the last training session. One-way ANOVA was used to assess whether there were differences in swim speed between the groups on Days 1 and 5 of Morris maze training.

For Western blot analysis, hippocampal tissue was evaluated from 12 animals; three animals randomly selected from each of the four groups. The immunoreactivity to CREB was quantitated by Alpha Imager 2000 with accom-

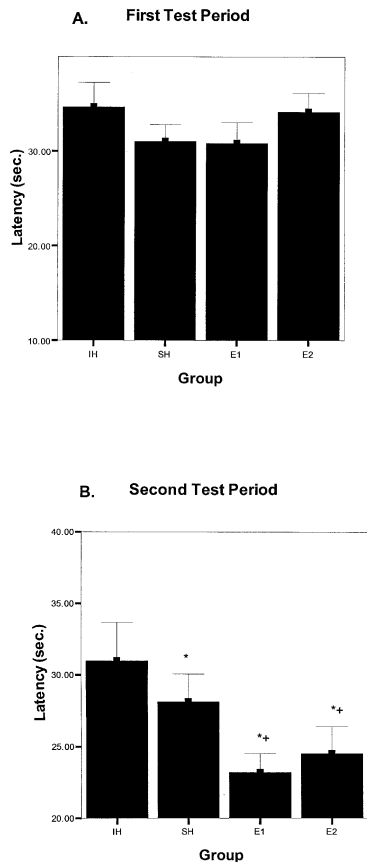


Fig. 3. Mean latencies across trials [4] in the Morris water maze task. (A) Mean \pm S.E.M. time required to reach the hidden platform across trials during the first test period (95–99 days). (B) Mean \pm S.E.M. time required to reach the hidden platform across trials during the final test period (160–164 days). * SH, E1, and E2 are significantly different from IH ($P \leq .05$). † E1 and E2 are significantly different from SH ($P \leq .05$).

panying software, which determined integrated density values (IDV) for each sample of tissue. A one-way ANOVA was performed on the IDV data using SPSS software.

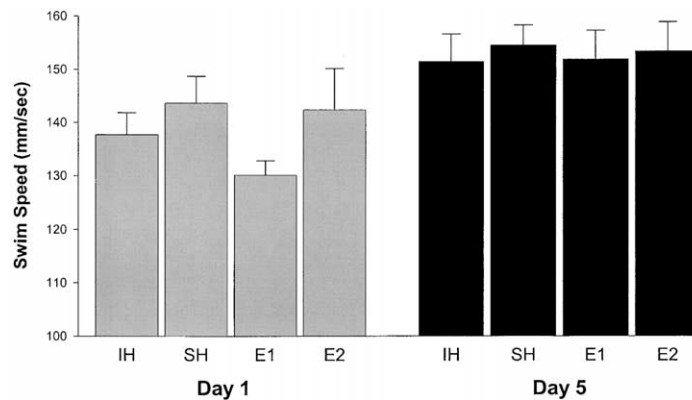


Fig. 4. Swim speed (mm/s) in the Morris water maze averaged across all trials on Days 1 and 5 during the second test period (160–164 days of age). The software (SA-3 Tracker using a CRT402 program), which accompanies the HVS tracking system provides the distance traveled, which was then divided by the latency to reach the hidden platform for each trial. Error bars represent S.E.M. Although animals swam faster on Day 5 than on Day 1 [$F(1,27)=32.67$, $P < .001$], there were no significant differences in swim speed between the four enrichment conditions.

3. Results

3.1. Water maze performance

When comparing performance on the water maze across all three testing periods, a practice effect was clearly evident. Fig. 2A depicts the learning curve for animals in all four conditions during the baseline condition (Days 30–34). Fig. 2B shows the learning curve during the first test period (Days 95–99). A substantial degree of “savings” is illustrated when Fig. 2A is compared to Fig. 2B. Specifically, during the baseline testing, the mean latencies on the first day of testing ranged from 46.05 to 51.41 s. Between the baseline and first test period, a period of approximately 2 months, the animals had no additional exposure to the maze. During the first test period (Days 95–99), the mean latencies (Fig. 2B) on the first day of testing ranged from 35.95 to 42.28 s, substantially lower than the baseline latencies. Further evidence for retention of spatial cues is evident when one compares Fig. 2B–C. Specifically, the mean latencies on Day 1 of the last test period (Days 160–164) have now been reduced to a range of 25.95–37.48 s. Although this incremental reduction might reflect retention of the constant spatial cues, it is also possible that the improvements in swim time to the hidden location are due to nonspecific practice effects.

The differences in mean latencies during each of the three testing periods were statistically evaluated. During the 5-day baseline condition (Days 30–34), there were no significant differences in latency to reach the hidden platform between the four experimental conditions. Indeed, the largest difference in mean latencies among the four conditions was less than 5 s during the 5-day baseline period. There were still no significant differences in latency between the four experimental conditions during the first test period (Fig. 3A). During these five trials, the maximum difference in mean latencies between the four conditions was less than 4 s. However, there were significant differ-

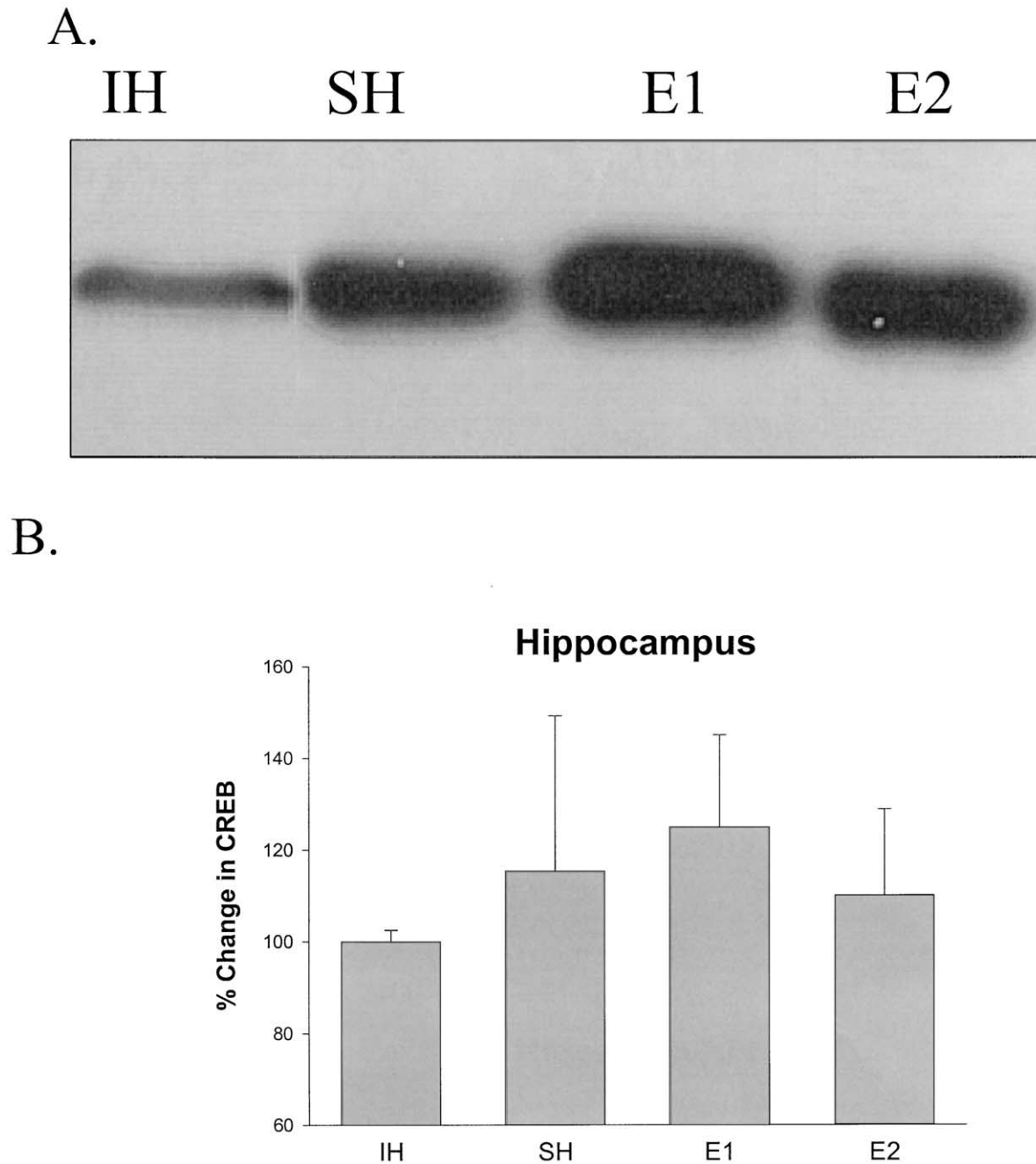


Fig. 5. (A) Representative Western blot for CREB protein expression in hippocampus. The hippocampi were retrieved from three mice randomly selected from each of the four conditions: IH controls, SH controls, and SH animals receiving physical enrichment during either the early (E1: 35–94 days of age) or the late (E2: 100–159 days of age) periods. Equal amounts of protein were loaded on each lane. CREB expression was detected by Western blot (see Materials and Methods) using specific antibody against CREB (Santa Cruz, CA). (B) Quantitative analysis of CREB immunoreactivity. The mean IDV was determined for each of the four conditions ($n=3$ animals per group) using Alpha Imager 2000 with accompanying software. The percentage (\pm S.E.M.) change in mean IDV was determined for Groups SH, E1, and E2 and plotted on the graph relative to the IDV for the baseline (IH) condition.

ences during the second test period (Fig. 3B). Animals in the control groups reached the hidden platform in 28.13 ± 1.99 and 30.99 ± 2.74 s for SH and IH groups, respectively. In contrast, the mean \pm S.E.M. latency for animals receiving early enrichment (Days 35–94) was 23.25 ± 1.33 s. Animals enriched during the later enrichment period (Days 100–159) reached the platform in 24.57 ± 1.88 s.

Based on a priori predictions, three orthogonal contrasts were conducted on mean latencies averaged over all 5 days of training during the last test period (Days 160–164). First, latency of the IH control group was compared to latency of the three SH groups (SH, E1, and E2). SH animals required significantly less time to reach the submerged platform (Fig. 3B) compared to the IH group [$P=.012$ (one-tailed)]. The

second orthogonal contrast tested the difference in latency between the composite of the two enriched groups (E1 and E2) vs. the SH control group. Latency for the enriched groups was significantly reduced in comparison to SH [$P=.05$ (one-tailed)]. Finally, the third planned comparison evaluated the difference in latency between the two enriched groups (E1 and E2). The difference in mean latency between E1 and E2 was not significantly different ($P=.335$).

To further clarify whether the specific time period for enrichment was important, *t* tests were conducted a posteriori. When environment was enriched during the earlier time period (35–94 days of age), latency to reach the hidden platform was significantly lower than latency for SH controls [$t(18)=1.95$, $P=.034$ (one-tailed)]. Enrichment administered during the later period (100–159 days) did not have a significant effect on latency compared to the SH group [$t(18)=1.18$].

Probe trials did not reveal any significant results. There were no differences between groups in the percentage of time spent in the correct quadrant during baseline testing [$F(3,36)=0.75$], the first test period [$F(3,36)=1.37$], or during the second test period in the water maze [$F(3,36)=2.70$].

Concerning the analysis of swim speed in the water maze, the GLM repeated-measures ANOVA revealed a significant main effect of days [$F(1,27)=32.67$, $P<.001$] during the last training period in the Morris maze (160–164 days of age). Animals swam significantly faster on Day 5 than on Day 1 during this period (Fig. 4). However, the interaction effect (Days \times Housing condition) was not significant [$F(3,27)=1.01$]. Furthermore, one-way ANOVAs revealed no significant differences in swim speed between the four treatment conditions either on Day 1 [$F(3,31)=1.22$] or on Day 5 [$F(3,38)=0.09$] of training in the Morris water maze. All animals, irrespective of how they were housed, swam faster on Day 5 than on Day 1, but there were no differences in swim speed between the four groups either on Day 1 or 5 of the later period of water maze testing. Hence, the shorter latencies to reach the hidden platform among enriched animals, particularly the E1 group, cannot be attributed to differences in swim speed (see Fig. 4).

3.2. Western blot

To determine the correlation of behavioral measures with molecular changes, hippocampal tissue from 12 animals (i.e., three randomly selected from each of the four groups) was evaluated by Western blot using specific antibody against CREB (Fig. 5). Results of the ANOVA indicated that there were no statistically significant differences in CREB immunoreactivity between the four conditions [$F(3,11)=2.23$]. Comparing IH animals to the SH animals, there was a tendency towards increased CREB immunoreactivity in the SH group. A trend ($P<.1$) toward increased immunoreactivity in the E1 group (Fig. 5) indicated a possibility that the effects of early physical enrichment and social housing may

be additive. On the other hand, animals receiving enrichment during the later period (E2) exhibited CREB immunoreactivity, which was nearly equivalent to the SH condition (Fig. 5). However, it must be emphasized that these visually inspected tendencies were not statistically significant and thus could represent only random variability between groups.

4. Discussion

Plasticity, adaptive ability, and rate of neurogenesis decline as the adult nervous system ages [9,32]. Nonetheless, recent evidence suggests that the adult mammalian nervous system continues to exhibit significant neural and behavioral plasticity in response to a challenging, enriched environment [3,4]. Consistent with this view, we report that C57 mice housed in a physically enriched environment exhibit more efficient behavior, as evidenced by lower latencies to reach the hidden platform relative to a control group of similarly housed animals.

Performance averaged over all 5 days of the last testing period for the two combined enriched groups (E1 and E2) was significantly improved when compared to the SH control group (see Fig. 3B). Post hoc comparisons revealed that performance in the Morris maze was improved specifically when physical enrichment of the environment was administered during the early period (35–94 days of age). Enrichment during the later period (100–159 days) did not have a significant effect on latency. It is possible that the extensive training regimen employed in this experiment may have contributed to the lack of differential effects on behavior. Nonetheless, when data from both early and late (E1 and E2) enrichment periods were combined, maze performance was significantly improved compared to SH animals that had never received physical enrichment at any time period.

Results of the analyses of swim speed reveal that the differences between groups in latency to reach the hidden platform cannot be attributed to faster swimming (Fig. 4). Other investigators have observed faster swim speeds in animals that were housed under enriched conditions [33]. However, the shorter latencies of the enriched animals were found to be independent of the faster swim speeds, suggesting that the effect may be attributed to learning. In the current study, a uniform increase in swim speed from Days 1 to 5 was observed across all four groups. Thus, differences in latency to reach the hidden platform reflect an improvement in spatial learning and memory, particularly for animals that had their environment enriched during the earlier developmental period (35–94 days of age).

A trend toward increased CREB immunoreactivity was observed in both of the physically enriched groups (Fig. 5). Reactivity in the early enrichment group (E1) was the highest, while CREB immunoreactivity in the late (E2) enriched group tended to be lower and nearly identical to immunoreactivity to the SH condition. The differences were not statistically significant, perhaps because of the low number

of animals sampled for immunoblotting. Independent confirmation using a larger sample is necessary before definitive conclusions can be reached regarding the putative role of CREB in mediating enrichment-induced improvements in spatial memory. Nonetheless, the most efficient behavioral performance and the highest degree of CREB immunoreactivity were both observed in animals from the early enrichment (35–94 days of age) group. Thus, the data support the notion that behavioral change may be mediated, at least in part, by CREB-mediated transcription in the hippocampus. Additionally, less efficient performance and lower CREB immunoreactivity in the late enrichment group (E2), compared to the early enrichment group (E1), are observations consistent with the view that neural and behavioral plasticity declines with age.

The concept of environmental enrichment is complex, typically involving the opportunity for social interaction, as well as the opportunity to sense and manipulate a variety of physical objects. The relative contribution of these two components of enrichment to neural and behavioral plasticity has been evaluated previously. Behavioral recovery from hippocampal lesions was evaluated in rats reared under an enriched (i.e., both physically and socially) condition, a social condition, or an isolated condition [34]. While isolation tended to diminish variability in behavior on a number of tasks, enrichment tended to enhance response variability. Early enrichment (weaning to 60 days of age) alleviated lesion deficits in a spontaneous alternation task in a T-maze where salient, proximal cues were available. In the underwater radial-arm maze task, the animal must rely solely on distal cues. Early enrichment did not facilitate behavioral recovery from early hippocampal damage using this testing protocol. The modest effects of enrichment reported in the current study may reflect, in part, the lack of salient cues provided by the Morris maze task. The benefits of a physically enhanced environment may accrue from the enhanced experience that animals gained in attending to and manipulating objects that present contextual information about the environment.

While the behavioral effects observed in this experiment were not particularly prominent, they were persistent. We report here a significant reduction in latency to reach the hidden platform 65 days after the last day of enrichment. In terms of persistence, our findings are in agreement with those of Pacteau et al. [34] who observed significant effects approximately 60 days after the last day of enriched housing. Furthermore, a long-lasting behavioral effect has been demonstrated [24] using a critical enrichment period (weaning to 100 days of age) similar to the E1 enrichment period (35–94 days of age) shown to be effective in the current study. These investigators demonstrated improved shuttlebox avoidance learning in adult rats 6 months after cessation of enrichment [24]. The persistence of structural and neurochemical alterations in the brain was first reported more than 25 years ago. Increases in cortical brain weights (particularly occipital cortex) and alterations in cholinergic enzymatic activity were

both found to persist some 21–47 days after the last day of enrichment [35]. Collectively, these data suggest that the behavioral and structural effects of enrichment are persistent. However, the magnitude and sometimes even the direction [34] of the effect may be task-dependent. The lack of salient, proximal cues in Morris maze testing may limit sensitivity in detecting certain effects of enrichment.

Whereas physical enrichment of the environment enhanced spatial maze performance, social grouping by itself did not improve performance compared to the performance of IH animals. The effect of social housing may also be task-dependent. For example, social housing was just as effective as the enriched condition in improving efficiency of performance in an underwater radial-arm maze [34]. One limitation associated with the Morris maze used here is that it does not assess correct vs. incorrect arm choices. The Morris maze does, however, require continual spatial information processing from the beginning to the end of each trial. In the radial arm maze, once a decision is made to visit an arm, the ensuing motor response of swimming or running to the arm does not require significant processing of spatial information. An evaluation of what is being measured by each protocol is of paramount importance in understanding the limits to which one can generalize results across studies.

Although social interaction (SH) did not significantly improve navigational performance in this study, a tendency toward greater hippocampal CREB immunoreactivity was noted relative to the baseline (IH) condition (see Fig. 5). However, this observation was not consistent across all tissue samples from SH animals. Thus, the extent to which social interaction induces CREB-mediated transcription in the hippocampus remains equivocal. It is possible that other nonhippocampal loci, such as the hypothalamus or other limbic system structures, will also be involved in mediating the effects of social interaction. Moreover, the behavioral, cellular, and molecular consequences of social interaction may be task-dependent. Although social grouping did not alter navigational performance in this study, extent of social experience has been shown to alter performance on other tasks, such as memory of socially transmitted food preferences [29]. Typically, mice required only one 5-min social interaction to form lasting and stable memories for preferred foods [29]. In contrast, CREB-deficient mutant mice did not exhibit a socially transmitted food preference when tested 24 h later, even when they were given two opportunities for social interaction [29].

Several reports now suggest that CREB transcription plays a critical role in the formation or consolidation of new reference (i.e. long-term) memories [29,36–38] across a number of different memory modalities (e.g., simple classical conditioning, spatial memory, and socially transmitted food preferences) and across the phylogenetic scale from mollusks to mammals. The cAMP-responsive transcription factors are likely candidates for such a role since modulation of gene expression would result in continual protein synthesis [34] and could thus evoke enduring alterations in the

pattern of neuronal activity in the hippocampus. New protein synthesis is considered by most to be a necessary property for consolidation of a reference memory.

As stated earlier, both enrichment [8] and endogenous CREB activation [12] have been shown to promote survival of newly formed hippocampal granule cells of the dentate gyrus. These neuroprotective properties may underlie the positive effects on navigational performance observed in the present study. In addition to CREB, other factors are likely to play a role in mediating the effects of environmental enrichment on cognitive performance. Increases in protein kinase C from hippocampus has been observed following brief exposure (12 days) to an enriched environment, a condition that was sufficient for improving Morris maze performance in young (27-day-old) rats [23]. Enrichment of the environment increases nerve growth factor in the hippocampus [22,39], buffers stress-related hippocampal damage by inducing expression of the gene encoding for glucocorticoid receptors [39], and induces expression of both glial-derived neurotrophic factor and brain-derived neurotrophic factor [11]. Enrichment has been shown to induce expression of the neural cell adhesion molecule [11], which is, in turn, associated with neurogenesis in dentate granule cells [3]. Moreover, evidence suggests that the phosphorylation state of CREB in the hippocampus also plays an important role in learning and memory processes, which parallels CREB expression [40–42]. Thus, the transcription factor CREB, as well as phosphorylated CREB, are likely to be among the many central nervous and endocrine agents, which mediate the cognitive effects and neuroprotective properties associated with environmental enrichment.

Enrichment-induced plasticity has been observed in the human nervous system. For example, level of educational attainment, a form of environmental enrichment, is inversely related to the risk for Alzheimer's [43] and Parkinson's disease-related dementia [44]. Using structural MRI brain scan technology, Maguire et al. [19] have recently documented regional differences in the distribution of gray matter within the right hippocampus of experienced taxi drivers, whose occupation places a high demand on navigational skill. In taxi drivers, posterior regions of the hippocampus were expanded while anterior regions diminished in volume. Moreover, the magnitude of the volumetric difference was correlated with the years of experience the subjects had as taxi drivers, suggesting an overall reorganization of hippocampal circuitry in response to the need to store an increasingly detailed spatial representation. Thus, while some measures of plasticity may decline with age, significant reorganization of the hippocampus in response to environmental stimulation does occur in the adult human nervous system.

In conclusion, the results of the present study confirm that enrichment of the living conditions of 35–94-day-old mice improved performance in the Morris water maze when mice were tested at various times during adulthood. Immunoreactivity to the CREB protein was induced differentially in the hippocampus either by enrichment during the early or late

time periods, although the degree of CREB immunoreactivity was highest in early enriched animals. These results are consistent with recent demonstrations that the mature nervous system can be altered structurally, functionally, and behaviorally in response to environmental stimulation in adult animals [7,8] as well as healthy adult human subjects [19]. In the present study, both behavioral and neural plasticities were evident in young adult mice. Although plasticity may occur throughout the life span, our results are consistent with the view that plasticity related to environmental stimulation declines with age. A better understanding of the mechanisms, which underlie “nurture-induced” plasticity, holds promise as a paradigm for methods to buffer the aging nervous system from the decline in memory function associated with neurological disorders involving the hippocampus.

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