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Striatal CREB Phosphorylation Following Cued/Response Learning in the Water Maze Differ in Two Inbred Strains of Mice

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ABSTRACT

Much evidence shows that the hippocampus and striatum play roles as important neural substrates for spatial/place and cued/response learning, respectively. This experiment was conducted to investigate the engagement of the striatum in cued/response learning. The engagement of the striatum was assessed after either place or cue training by determining levels of cAMP response element-binding protein (CREB) and phosphorylated CREB (pCREB) in these two mouse strains. Results revealed that striatal CREB levels in both strains of mice were not significantly increased after cued/response learning comparing to place training mediated by the hippocampus. However, striatal pCREB of DBA/2 mice was significantly higher after cued/response training in comparison to place learning, while striatal pCREB levels on C57BL/6 mice did not differ in cued learning versus place learning. These findings indicate that striatal pCREB, specifically associated with cued/response learning, is closely tied to differences in cued/responses strategy preference between C57BL/6 and DBA/2 mice.

Key words: cAMP response element-binding protein, pCREB, place learning, cued/response learning, striatum, water maze

INTRODUCTION

Much research with humans and rodents has shown that different neural systems are recruited to learn tasks that depend on information about relationships among stimuli (i.e., spatial/place) and associations between discrete cues and behavioral responses (i.e., cued/response) (McDonald and White, 1994; Packard and McGaugh, 1996; Hartley et al., 2003). The hippocampal system is used for acquiring spatial/place learning whereas the striatal system is used for cued/response learning. Subsequent evidence for multiple memory systems comes from studies that have linked specific molecular mechanisms in the hippocampus or striatum with the demands of a learning task. One such protein studied extensively is the cAMP response element-binding protein (CREB), which has been implicated in the formation of long-term memory (Abel and Kandel, 1998). For example, Colombo et al. (2003) trained rats with a cross maze task that could be solved by either a place strategy or a cued/response strategy. Then the strategy used was determined by a probe trial. The

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results of this study showed that levels of hippocampal phosphorylated CREB (pCREB) are higher in rats that choose a place strategy on a plus maze task in comparison to those that choose a cued/ response strategy, while the levels of striatal pCREB are higher in rats that choose a cued/ response strategy on a plus maze task in comparison to those that choose a place strategy (Colombo et al., 2003).

Studies of inbred strains of mice also support multiple memory systems. C57BL/6 and DBA/2 are background strains commonly used to construct genetically modified mouse models with the goal of identifying molecular and cellular mechanisms critical for learning and memory. These two strains perform differently on learning and memory tasks with different demands (Upchurch and Wehner, 1988; Paylor et al., 1993). It is reported that C57BL/6 mice strongly preferred a place strategy in a plus maze task and showed a strong c-Fos activation in the hippocampus, whereas no such place strategy preference and a strong c-Fos activation in the striatum was observed in DBA/2 mice (Passino et al., 2002).

To examine the striatal involvements in cued/ response learning with C57BL/6 and DBA/2 mice, this study compared levels of striatal CREB and pCREB across these strains following sessions of either place or cued training in the water maze. The phosphorylation of CREB through the activities of cAMP dependent protein kinase A (PKA) after a learning experience is one of the primary intracellular cascades implicated in learning and memory (Silva, 2003). Our data suggest that DBA/2 mice can accomplish cued/response learning through striatal activation via phosphorylation of CREB.

MATERIALS AND METHODS

Subjects

Twenty-four male C57BL/6 and twenty-four male DBA/2 mice (SPF) obtained from the Charles River Co. (Gapeung, South Korea) were 3 mo. at the beginning of the experiments. Mice were housed in groups of four to a cage, in a temperature and humidity-controlled room, with a 12 hr light/dark cycle (lights on, 07:00-19:00 h). Food and water were available *ad libitum*. All testing was conducted during the light cycle. Experiments were conducted in compliance with the Konkuk University's Council Directive for the use and care of laboratory animals. These animals had been used in a study of learning strategy selection and hippocampal pCREB (Sung et al., 2008).

Apparatus

The water maze consisted of a circular tank (1.50 m diameter and 0.46 m height) with an escape platform centered in one of the four maze quadrants. Water (27^{o}C) was made opaque with nontoxic white paint. The escape platform was located 0.5 cm beneath the surface on a hidden platform for place training and raised 2 cm above the water surface on visible platform for cued/ response learning. The maze was surrounded by white curtains to which were affixed black felt patterns for the purpose of providing distal visual (spatial) cues. Data were recorded with a HVS Image tracking system (Hampton, UK). During each trial, the distance of the mouse from the escape platform was sampled 10 times per sec, and these values were averaged in 1-sec bins. Cumulative search error was then calculated as the summed 1-sec averages of this proximity measure corrected for the particular start location on each trial (Gallagher et al., 1993).

Behavioral training procedure

1. Spatial/place training: C57BL/6 (n=12) and DBA/2 (n=12) mice received 4 trials/day, starting each trial from one of four equidistantly located positions at the perimeter of the maze. The location of the platform remained constant across all training trials. The mice were placed into the water facing the wall and allowed 60 s to escape. The trial ended when the mice climbed onto the available platform or after the 60 s interval had elapsed. If a mouse did not locate the platform during a trial, it was placed on the platform by the experimenter. Mice were left on the platform for 20 s and then were moved to a holding cage for a 10 min intertrial interval.

2. Cued/response training: C57BL/6 (n=12) and DBA/2 (n=12) mice received 4 trials/day (10 min intertrial interval, maximum trial duration of 60 sec with 20 sec on the platform at the end of each trial)

in which the visible platform was moved to different locations in the pool between trials. Blank white curtains were drawn around the pool during cue training to occlude extramaze cues.

Thirty minutes after the last training trial on the 4th day in both protocols, all mice were sacrificed. The striatum were rapidly dissected and frozen at −80°C until further processing.

Western blot analysis

Proteins for the analysis of CREB and pCREB were extracted in the following manner. Individual tissue samples were weighed and then homogenized in 5 vol of ice-cold buffer containing 20 mM Tris, pH 7.5, 5% glycerol, 1.5 mM EDTA, 40 mM KCl, 0.5 mM dithiothreitol, and protease inhibitors (No. 539131, Calbiochem). Homogenates were centrifuged at 20,800×g for 30 min at 4°C. The supernatant was removed from each sample, and an aliquot was taken for determination of total protein concentration using Bradford Reagent. The proteins were then separated by SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Blots were blocked for 1 hr at room temperature with 5% skim milk in Tris-buffer saline [25 mM tris (pH 7.6) and 136 mM NaCl] solution containing 0.1% tween-20. The membrane was incubated with a primary antibody (Ab) against CREB (1 : 1,000, Cell Signaling) and pCREB, phosphorylated on serine-133 (1 : 1,000, Upstate). Following primary incubation, blots were incubated with the HRP-conjugated secondary Ab (1 : 2,500, Amersham Biosciences). Blots were visualized using an ECL system, and developed using Hyperfilm (Amersham). The relative expression levels of CREB and pCREB were determined by densitometry and normalization to β-actin (1 : 5,000, Sigma), an invariant cytoskeletal protein.

Data analysis

Search error during training was analyzed using repeated measures two factor analysis of variance (strain × trial block (day)) to evaluate acquisition in the place and cue learning tasks. T-test was conducted to discern differences of CREB and pCREB between place learning and cued learning in C57BL/6 and DBA/2. A p-value less than 0.05 was considered significant.

RESULTS

Spatial/place and cued/response training

We trained separate groups of mice from both strains on either place or cue learning protocols.

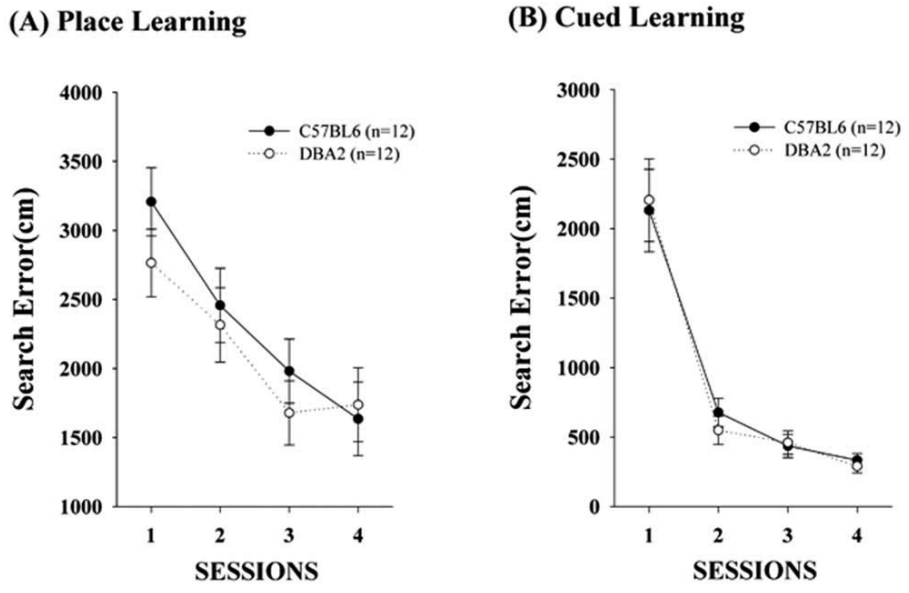

Fig. 1. Place (A) and cued/response (B) training performance of C57BL/6 and DBA/2 mice. No strain differences were observed on either place or cued/response performance. See text for statistical analysis.

For the purpose of the neurobiological assessment that followed, the procedures were implemented across protocols such that training session duration was equated (see methods for procedural details). In the spatial learning protocol, mice received four training trials per day to a hidden platform for four successive days. Both C57BL/6 and DBA/2 mice improved over the course of training, as measured by reduced search errors ($F(3, 66)=14.64$, $p<0.001$; Fig. 1). There was no significant main effect of strain ($F(1, 22)=0.67$, ns) nor a significant strain by day interaction ($F(3, 66)=0.57$, ns; see Fig. 1). Similarly, in visible, cued/response training protocol, separate groups of two strain mice received four training trials per day to a visible platform for four successive days. Both C57BL/6 and DBA/2 mice improved over the course of training, as measured by reduced search errors ($F(3, 66)=56.84$, $p<0.001$; Fig. 1) and there was no significant main effect of strain ($F(1, 22)=0.02$, ns) nor a significant strain by day interaction ($F(3, 66)=0.02$, ns; Fig. 1).

CREB and pCREB levels after spatial/place and cued/response training

Mice in these groups were used to evaluate hippocampal CREB and pCREB following sacrifice thirty minutes after the completion of training. Fig. 2 shows representative immunoblots of hippocampal CREB and pCREB. In both strains, striatal CREB levels were significantly not changed after place training and cued training. In DBA/2 mouse strains, striatal pCREB levels were significantly increased after cued training in comparison to place training

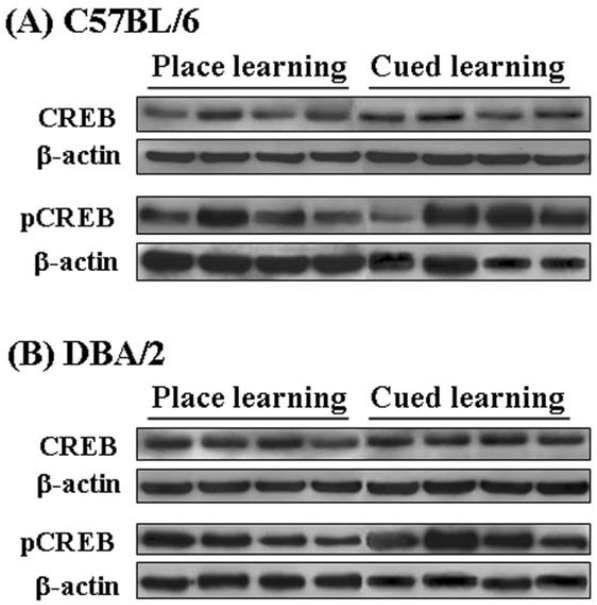

Fig. 2. Representative immunoblots of striatal CREB and pCREB from C57BL/6 (A) and DBA/2 (B) mice thirty minutes after place or cued/response training.

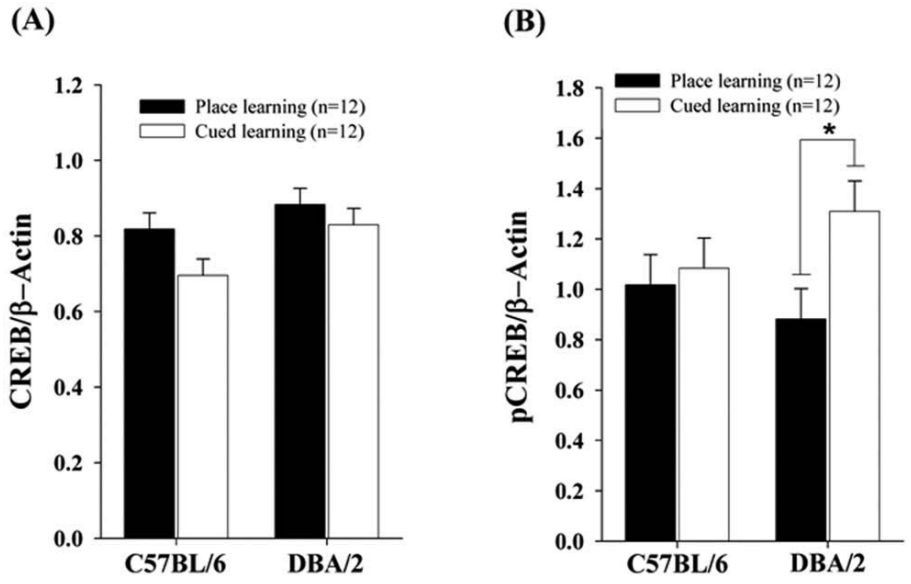

Fig. 3. Quantification of striatal CREB (A) and pCREB (B) levels (mean±S.E.M.) from C57BL/6 and DBA/2 mice thirty minutes after place or cued/response training. Data are expressed as the ratio of CREB/actin and pCREB/actin. (*) indicates significantly greater striatal pCREB following cued/response in comparison to place learning in DBA/2 mouse strains.

values, while, in C57BL/6 mouse strains, striatal pCREB levels were not different in comparisons with place and cued training values (Fig. 3).

DISCUSSION

In this behavioral comparison of C57BL/6 and DBA/2 mice, performance during place and cued/response training did not differ between these inbred strains. In the place training that is often used for assessing spatial reference memory, both C57BL/6 and DBA/2 mice performed with similar accuracy by decreased search errors (Fig. 1). There were also no differences of performance between C57BL/6 and DBA/2 mice in the cued/ response training. Differences in the place learning performance have been reported previously for these strains, albeit using somewhat different training protocols (Paylor et al., 1993; Owen et al., 1997; Nguyen et al., 2000; Brooks et al., 2005; Wahlsten et al., 2005). Interestingly, studies using a similar protocol to ours agree with the current findings in reporting no difference between the two strains (Owen et al., 1997; Brooks et al., 2005; Sung et al., 2008).

Against a background of similar performance in spatial learning, some studies have reported a notable behavioral difference when C57BL/6 and DBA/2 mice were given an option to select a strategy (Passino et al., 2002; Sung et al., 2008). The DBA/2 mice demonstrated a strong preference for selecting a cued/response strategy in tests when place and cued/response strategies were put in competition. On the other hand, the C57BL/6 mice demonstrated a strong preference for selecting a place strategy. These studies suggest that DBA/2 mice have the strong propensity to select the cued/response strategy, but lacked the propensity to select the place strategy, as exhibited by the C57BL/6 mice.

It has been shown that the hippocampus and dorsal striatum mediate place and cued/response strategies, respectively (McDonald and White, 1994; Packard and McGaugh, 1996; Packard, 1999; Packard and Knowlton, 2002). Although both neural systems have access to the same information from situation in which learning occurs, each system appears to be specialized to process a different kind of information (Malamut et al., 1984; Packard and Knowlton, 2002; White and McDonald, 2002). Consistent with this frame work, regionally specific pCREB and c-Fos expression in hippocampus and striatum were shown to correlate with place and cued/response strategy selection in individual rats (Passino et al., 2002; Colombo et al., 2003). In addition to these studies supporting multiple memory systems, the companion study of this experiment reported that C57BL/6 mice process spatial cues more actively than DBA/2 despite the similarities in spatial learning performance of the two stains of mice. That is, levels of hippocampal pCREB in C57BL/6 were significantly higher after place learning compared to cue learning, whereas hippocampual pCREB levels in DBA/2 did not differ across place and cue learning conditions (Sung et al., 2008).

On the other hand, the present study examined the involvement of the striatum in relation to place learning and cued/response learning. The striatal CREB and pCREB levels of C57BL/6 and DBA/2 were assessed thirty minutes after either place learning or cue learning in the water maze. Training duration was matched across these two protocols (training duration=40 minutes). The levels of striatal CREB were not significantly increased in either C57BL/6 or DBA/2 after cued/response training in comparison to place training, In contrast, levels of striatal pCREB in DBA/2 were significantly higher after cued/response learning compared to place learning, whereas striatal pCREB levels in C57BL/6 did not differ across place and cue learning conditions. These data indicate that the DBA/2 mice process response/cues information more actively than the C57BL/6 mice despite the similarities in cued/response learning performance of the two stains of mice. While C57BL/6 mice can accomplish cued/response learning, these mice appear to prefer a non-striatal learning strategy and did not exhibit a corresponding induction in striatal pCREB-dependent signaling.

Sung et al. (2008) and the present results are consistent with the theory that multiple parallel memory systems including the hippocampus and striatum access much of the same information, but each system has a unique information processing style. That is, the transcription factor CREB was

activated after spatial/place learning in the hippocampus of C57BL/6 mouse, a good place learner, whereas elevated pCREB was observed after cued/response learning in the striatum of DBA/2 mouse, a cued/response learner.

To elucidate the roles of genes and intracellular signaling pathways in learning and memory, genetically modified mice are widely used to discover key molecular and cellular mechanisms (Picciotto and Wickman, 1998; Wehner et al., 2001). Genetically-defined inbred strains of mice could also provide informative research models for such analysis, as indicated by the occurrence of a striatal phenotype or a hippocampal phenotype that differs between the comparison strains in this investigation and the companion study (Sung et al., 2008). Because DBA/2 mice present a behavioral profile together with a superior capability in a major plasticity pathway with the striatum, this strain may provide a suitable model for experimental analysis with the goal of enhancing striatum-dependent learning and memory.

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