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CBP-dependent memory consolidation in the prefrontal cortex supports object-location learning

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Abstract

Recognition of an object's location in space is supported by hippocampus-dependent recollection. Converging evidence strongly suggests that the interplay between the prefrontal cortex and hippocampus is critical for spatial memory. Lesion, pharmacological and genetic studies have been successful in dissecting the role of plasticity in the hippocampal circuit in a variety of neural processes relevant to spatial memory, including memory for the location of objects. However, prefrontal mechanisms underlying spatial memory are less well understood. Here we show that an acute hypofunction of the cyclic-AMP regulatory element binding protein (CREB) Binding Protein (CBP) histone acetyltransferase (HAT) in the medial prefrontal cortex (mPFC) results in delay-dependent disruption of object-location memory. These data suggest that mechanisms involving CBP HAT-mediated lysine acetylation of nuclear proteins support selectively long-term encoding in the mPFC circuits. Evidence from the object-location task suggests that long-term memory encoding within the mPFC complements hippocampus-dependent spatial memory mechanisms and may be critical for broader network integration of information necessary for an assessment of subtle spatial differences to guide appropriate behavioral response during retrieval of spatial memories.

Keywords

mPFC; Spatial memory; Object-location; Histone acetyltransferase; Epigenetic regulation

Introduction

Considerable evidence from studies in humans and rodents indicates that the ability to learn and remember spatial locations is connected to the hippocampus (Burgess et al., 2002; Maguire et al., 1998; Maguire et al., 1999; Morris et al., 1982; Morris et al., 1990; O'Keefe and Dostrovsky, 1971; Rawlins and Olton, 1982). While the cortical organization of spatial memory is not understood, there are strong data implicating the prefrontal cortex and hippocampal circuits in spatial memories (Fletcher and Henson, 2001; Fuster, 1997; Wiltgen et al., 2004). A disconnection between the hippocampus and prefrontal cortex produces

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deficits in spatial learning (Floresco et al., 1997). The prefrontal circuit is also involved in the retrieval of remote contextual fear memory (Bontempi et al., 1999; Frankland et al., 2004; Maviel et al., 2004; Wiltgen et al., 2004). In addition, electrophysiological studies in rodents have shown that the prefrontal cortex is involved in spatial working memory (Horst and Laubach, 2009) and neurons in the medial prefrontal cortex are phase-locked to the hippocampal theta rhythm (Siapas et al., 2005).

Recent studies in the field of learning and memory suggest that epigenetic mechanisms may play a role in synaptic plasticity and memory consolidation (Peixoto and Abel, 2012; Sweatt, 2009). The most widely considered molecular mechanism for synaptic plasticity and memory postulates that N-methyl-D-aspartate receptor (NMDAR)-mediated mechanisms induce phosphorylation of the cyclic-AMP regulatory element binding protein (CREB) transcription factor, which in turn recruits CREB Binding Protein (CBP) (Chawla et al., 1998; Chrivia et al., 1993; Gonzalez et al., 1989; Hardingham et al., 1999). CBP comprises intrinsic histone acetyltransferase activity (HAT) (Bannister and Kouzarides, 1996; Korzus et al., 1998), which regulates the local chromatin structure required for NMDAR- and CREB-dependent gene expression (Lu et al., 2003), believed process thought to control long-term memory consolidation(Alarcon et al., 2004; Barrett et al., 2011; Korzus et al., 2004; Maddox et al., 2013; Valor et al., 2011; Wood et al., 2005). The cognitive effects of CBP hypofunction depend on the temporal and spatial neuronal patterns of the molecular dysfunction. When expressed in neurons during postnatal development, CBP mutations can produce additional cognitive deficits including the inability to form short-term memories (Chen et al., 2010). A number of independent manipulations to down-regulate CBP acetyltransferase activity target the adult living brain in order to avoid developmental confounds. These studies provide strong evidence for the selective role of CBP in the adult brain in LTP and long-term memory consolidation (Barrett et al., 2011; Korzus et al., 2004; Maddox et al., 2013; Valor et al., 2011). Some studies described specific effects, e.g., focal CBP knockout targeted to the dorsal CA1 area of the hippocampus has no effect on basal synaptic transmission, upstream signaling including CREB phosphorylation and short-term memory for object-location, whereas obvious deficits in LTP and long-term memory for object-location existed (Barrett et al., 2011). This observation is consistent with hippocampal lesion studies implicating the hippocampus in the object-location task (Barker and Warburton, 2011). In addition, these studies suggest that CBP-dependent plasticity in the hippocampus is part of the mechanism underlying the consolidation of memory for object-location and demonstrate that CBP hypofunction causes a delay-dependent memory deficit.

Animal lesion studies clearly link the mPFC to learning of spatial-object associations (Barker and Warburton, 2011). However, it is unclear what type of information is encoded into prefrontal circuitry during learning of spatial-object associations. For example, bilateral lesions or pharmacological inhibition of the mPFC have consistently shown a deficit in object-in-place test, e.g. (Barker et al., 2007; Barker and Warburton, 2011). However, inconsistent effects were observed on other versions of spatial memory tasks such as the object-location task (Barker et al., 2007; Ennaceur et al., 1997; Nelson et al., 2011). This may indicate that the mPFC is integrating information about task-specific rule learning and/or an object's spatial associations, and the differences between previous reports may

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arise from differences in task demands. In this study we examine the role of CBP-dependent mechanisms in the mPFC in spatial memory using mutant mice expressing a repressor of CBP acetyltransferase activity in the mPFC. Evidence from the object-location task, indicated that mPFC circuitry is critical for spatial memory recall in a delay-dependent manner.

Materials and Methods

Subjects

C57BL/6 mice from Jackson Laboratory were used for all experiments. Prior to any procedure, the mice are weaned at postnatal day 21, housed 4 animals to a cage with same-sex littermates, maintained on a 12 hr light/dark cycle, and had *ad libitum* access to food and water. Autoclaved bedding was changed every week. All procedures were approved by the UC Riverside Institutional Animal Care and Use Committee in accordance with the NIH guidelines for the care and use of laboratory mice. All of the physiological and behavioral tests were performed during adulthood (P70-P150) and counter-balanced for sex.

Surgery

The injection protocol has been previously described by Cetin et al. (Cetin et al., 2006). In this study, 2-4-month-old mice were individually housed and weighed to determine the appropriate drug ratios to use. Atropine was injected to help with breathing [.02 mg/kg body weight]. The mice were then placed into an isoflurane chamber to induce anesthesia, mounted in a heated stereotaxic apparatus and supplied with a constant flow of isoflurane/oxygen mix. The scalp was shaved and sanitized with 70% ethanol. The ear bars, bite bar and nose clamp were adjusted to firmly hold the head in place. A midline incision was made on the scalp and surgical hooks were placed to keep the skull exposed. Sterile PBS was added as needed to prevent the skull from drying. The head was leveled by comparing bregma and lambda coordinates until they were equivalent. Injection sites were calculated based on bregma coordinates, and a dental drill was used to thin the skull over the injection site. A 27G needle was then used to remove the thinned bone. A 5-μl calibrated glass micropipette [8 mm taper, 8 μm internal tip diameter] was fitted with a plastic tube connected to a 10-ml syringe and lowered onto a square of Parafilm containing a 4-μl drop of virus. The syringe was aspirated to fill the micropipette with 1 μl of solution before moving it to the injection site. The micropipette was slowly lowered to the proper stereotaxic coordinates and pressure was applied to the syringe to inject 0.6 μl of solution at a rate of 50 nl/min. After the total volume was injected, the micropipette was withdrawn slowly to avoid backflow, and the injection site was cleaned with sterile cotton swabs. The skin was sutured and antibiotic was applied to the scalp. Lidocaine was subcutaneously injected near the site followed by an intraperitoneal injection of sterile PBS [30 ml/kg body weight] to prevent dehydration. The mouse was kept warm by placing its cage on a heated plate and injected with buprenorphine [.05 mg/kg] for pain relief. On post-surgical days 1 and 2, the mouse received subcutaneous injections of meloxicam [1 mg/kg] to relieve pain. Animals were monitored for any signs of distress or inflammation for 5 days after surgery. Behavioral experiments were performed 5-20 days post-surgery.

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The infralimbic and prelimbic cortices were targeted at the following stereotaxic coordinates: Bregma; AP 2.0, ML±0.4, DV 1.4. The viruses were prepared by Dr. Rachael Neve (MIT, Viral Core Facility). HSV viruses are effectively expressed in neurons in the mPFC. We observed stable expression of the virus in the mPFC during the first 4 weeks post-injection.

Behavioral Testing

Object-location task—The mice were habituated in a testing apparatus 3 times for 15 min each 2 days prior to training. The testing apparatus used in the study was an open field arena (17″ × 17″ × 12″) with clear Plexiglas walls, a white acrylic floor and visual cues (located on north and east sides), which were visible in the arena. A lamp and behavior recording camera was installed above the testing arena. The mice performed four training trials for 5 min each trial with an ITI of 3 min. During the first trial, the mice were re-habituated to the arena without objects. In trials 2-4, two identical objects were placed in the NW and NE corner of the arena, and the mice were allowed to explore the objects for 5 min for each trial. Memory was then assessed during a single, 5 min test trial after a 5 min delay (for short-term memory) or 24 hr delay (for long-term memory). During the test trial, replicas of the training objects were placed in a familiar [NW] corner and a novel [SE] corner. The position of the moved object was counterbalanced between mice. Wild type mice showed an exploratory preference for the novel location [SE]. Data collected for the 5min-delay test (Figure 2B) and the 24h-delay test (Figure 2C) were generated from two separate cohorts of mice.

Histology

Mice were anesthetized using CO_2 and transcardially perfused first with PBS and then 4% PFA. The extracted brain was soaked in 4% PFA overnight and then transferred to PBS until histological sectioning. For c-fos and p-CREB staining, neural activity was induced by a pentylenetetrazole (PTZ, Sigma) seizure protocol (Ferraro et al., 1999). Briefly, mice were injected with 50mg/kg of PTZ and sacrificed one hour after the first episode of a partial clonus seizure. In this study, 50-μm-thick sections of the mPFC were obtained using a Compresstome VF-300 (Precisionary Instr., Greenville, NC) and placed in a 24-well plate for free-floating immunohistochemistry (IHC) according to a previously described protocol (Vieira et al., 2014). The sections are washed 3 times for 10 min in a wash buffer (PBS, 0.3% Triton x-100, 0.02% NaN_2) followed by a 1-hr incubation in blocking buffer (5% normal goat serum in washing buffer), followed by a 10-min incubation in the wash buffer. The sections were incubated overnight at 4C° with primary antibodies: anti-c-Fos Rabbit IgG (Calbiochem, Cat No: PC38, 1:1000 dil.) or anti-pCREB (Santa Cruz, Cat No: SC7978-R, 1:500 dil.). After three washes with the wash buffer, the sections were incubated with secondary antibodies (Alexa568-goat anti-rabbit IgG (Molecular Probes, 1:2000 dil.) in blocking buffer for 4 hr at room temperature. TO-PRO-3 stain (Molecular Probes, 1:1000 dil.) was used as a nuclear marker. The sections were washed again three times with the wash buffer before mounting for viewing. Negative control slices were performed for each row of the well plate, undergoing the same IHC procedure but missing primary antibodies. After immunostaining, the tissue was mounted directly onto glass slides, covered, and sealed with nail polish before imaging.

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Imaging

Immunostained tissue was analyzed using a semi-automatic laser scanning confocal microscope (Olympus FV1000) controlled by the FluoView software. GFP, Alexa568, and TO-PRO3 were imaged using 473-nm, 559-nm, and 647-nm lasers, respectively. Brain sections were imaged using identical microscope settings. 50-micrometer z-stacks (representative images) or a single optical section (fluorescent intensity measurements) were obtained from the PL region in the mPFC. The fluorescence intensity was measured using 20x objective (XLUMPLFL20XW, NA 0.95) and quantification was performed using a single optical section. Gain and offset of each channel were balanced manually using Fluoview saturation tools for maximal contrast. All settings were tested on multiple slices before data collection and brain slices were imaged using identical microscope settings once established. The background fluorescence for each channel using established image acquisition settings was measured in negative control experiments for each group (secondary without primary) and subtracted from final image calculations. The fluorescence intensity quantification was performed on original images by the use of Olympus Fluoview software without any non-linear image adjustments. The region of interest (ROI) was a 5 μm circle placed on cells expressing GFP within the nucleus indicated by TO-PRO3 and fluorescence corresponding to P-CREB or c-Fos was measured from randomly selected 75-85 cells per animal. Fluorescence intensities were normalized to the mean of the control group before further statistical analysis.

Data analysis

The discrimination Index (DI) was expressed as the absolute difference in the time spent exploring the moved and unmoved objects divided by the total time exploring both objects and calculated according to the formula $DI = ((Time_{Moved\ Object} - Time_{Unmoved\ Object}) / (Time_{Moved\ Object} + Time_{Unmoved\ Object}))$.

The experimenters were blind to the group conditions. Data are expressed as the means ± SEM. N indicates number of animals. Statistical analysis was performed using Prism 6 software (GraphPad Software, Inc.) The Student's *t*-test was used for statistical comparisons. A $p<0.05$ was considered statistically significant. The asterisks indicate statistical significance: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

Results

To gain insight into the prefrontal mechanisms controlling spatial memory we used mutant mice expressing CBP HAT in the mPFC (referred to as CBPHAT$^{PFC\text{-}hypo}$ mice) that are described previously (Vieira et al., 2014). The CBP HAT mutant carries a substitution mutation of two conserved residues (Tyr^{1540}/Phe^{1541} to Ala^{1540}/Ala^{1541}) in the acetyl CoA binding domain and is a potent repressor of CBP-dependent histone acetylation (Korzus et al., 1998). Previous data demonstrated that CBP HAT expressed in neurons blocks selectively long-term memory without affecting information acquisition or short-term memory (Korzus et al., 2004). CBPHAT$^{PFC\text{-}hypo}$ mice express CBP HAT and the fluorescent marker enhanced green fluorescent protein (eGFP) in the mPFC (Figure 1A-B). For control mice, we injected a virus expressing eGFP only in the mPFC. Analysis of

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CBPHAT$^{PFC\text{-}hypo}$ and control animals revealed that expression of mutant proteins were targeted to neurons in the mPFC (Vieira et al., 2014). Vieira et al. (2014) also reported that CBP HAT is an effective inhibitor of histone acetylation in prefrontal neurons in CBPHAT$^{PFC\text{-}hypo}$ mice (Vieira et al., 2014). Conditioned CBPHAT$^{PFC\text{-}hypo}$ mice displayed decreased level of acetylated histone H3 ($t(10) = 2.38$, $p = 0.038$) and decreased level of acetylated histone H4 ($t(10) = 2.97$, $p = 0.014$) in cells expressing GFP when compared to conditioned control animals (Vieira et al., 2014). These data are consistent with previous studies reporting downregulation of acetylated histones in CBP deficient mutant mice (Alarcon et al., 2004; Barrett et al., 2011; Chen et al., 2010; Korzus et al., 2004; Peixoto and Abel, 2012; Valor et al., 2011; Wood et al., 2005).

Environmentally driven neuronal activity is known to induce the expression of immediate-early genes, including *c-Fos* (Hunt et al., 1987; Morgan et al., 1987; Tischmeyer and Grimm, 1999) and *c-Fos* has been used for more than two decades to map neuronal activity. In addition, the *c-Fos* promoter has been useful for the detection of neuronal activity in optogentic studies (Schoenenberger et al., 2009) and in a design of genetic activity reporters (Reijmers et al., 2007). It has been demonstrated that CBP is recruited to the *c-Fos* promoter after induction of the NMDA receptor (Impey et al., 2002). NMDA receptor-dependent synaptic reinforcement has been recognized as a crucial process for memory consolidation (Tsien et al., 1996). Consistent with previous studies (Korzus et al., 2004), we demonstrated that the histone acetyltransferase activity of CBP was required for normal *c-Fos* gene expression in the mPFC. The expression of CBP HAT in mPFC neurons abolished neuronal activity-induced elevated levels of *c-Fos*. (Figure 1C. t-test for levels of c-Fos expression: $t(14) = 4.31$, $p = 0.0007$; Ctrl: 1.00 ± 0.04, $n = 8$; CBPHAT$^{PFC\text{-}hypo}$, 0.50 ± 0.11, $n = 8$;).

CREB is a transcription factor required for memory consolidation (Bourtchuladze et al., 1994; Josselyn et al., 2001; Kida et al., 2002; Pittenger et al., 2002), and CREB phosphorylation at S133 is a minimal requirement for CBP recruitment to an induced promoter and for subsequent transcriptional activation via cyclic-AMP response element (CRE) (Chrivia et al., 1993). In addition, phosphorylated CREB (pCREB) has been implicated in activation of *c-Fos* gene in neurons (Ghosh et al., 1994; Ghosh and Greenberg, 1995; Ginty et al., 1994; Ginty et al., 1993). A deficiency in *c-Fos* activation in CBPHAT$^{PFC\text{-}hypo}$ mice (Figure 1C) provided *in vivo* evidence that CBPHAT-dependent epigenetic regulation was critical for the transcriptional activation of *c-Fos* gene expression in prefrontal neurons. If this interpretation is correct, then the neuronal activity-induced upstream signaling should not be affected by CBP HAT. To test this idea, we examined neuronal activity-induced CREB phosphorylation responses in CBPHAT$^{PFC\text{-}hypo}$ mice. CBPHAT$^{PFC\text{-}hypo}$ mice showed normal levels of neuronal activity-induced CREB phosphorylation. Expression of CBP HAT in mPFC neurons showed no effect on the neuronal activity-induced elevated levels of CREB phosphorylation (Figure 1D. t-test for levels of P-CREB: t-test: $t(10) = 1.12$, $p = 0.287$; Ctrl: $1.00 + 0.04$, $n = 6$; CBPHAT$^{PFC\text{-}hypo}$: $0.93 + 0.04$, $n = 6$).

To determine whether spatial memory depends on CBP HAT-mediated signaling in the mPFC, we tested the performance of CBPHAT$^{PFC\text{-}hypo}$ and control animals on the object-location task (Figure 2A). There was no effect of CBPHAT hypofunction on the total

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exploration time during either the training phase (Figure 2B left panel. Train - mean total exploration time. $t_{(12)} = 0.61$, $p = 0.280$; Ctrl: 19.21 s ± 2.70 s, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 13.38 s ± 2.23 s, n = 7) or the test phase following a 5 min delay (Figure 2B left panel. Test - mean total exploration time. $t_{(12)} = 0.75$, $p = 0.760$; Ctrl: 12.21 s ± 1.67 s, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 13.25 s ± 3.05 s, n = 7). CBPHAT$^{PFC\text{-}hypo}$ mice exhibited normal acquisition and short-term memory in the object-location task (Figure 2B right panel. Discrimination Index. $t_{(12)} = 0.175$, $p = 0.864$; Ctrl: 0.31 ± 0.06 %, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 0.32 ± 0.14%, n = 7). These data demonstrate that CBPHAT$^{PFC\text{-}hypo}$ mice expressing a repressor of histone acetylation in the mPFC were able to learn and form short-term memories lasting at least 5 min in the task. This also indicated that viral infection and stereotaxic surgery did not introduce any abnormalities to the prefrontal circuitry that would affect normal information acquisition or memory retrieval on the object-location task.

Next, we tested CBPHAT$^{PFC\text{-}hypo}$ mice on the object-location task following a 24 hr delay after initial training (Figure 2c). Total exploration times did not differ between groups in the train phase (Figure 2C left panel. Train - mean total exploration time. $t_{(12)} = 0.91$, $p = 0.200$; Ctrl: 22.37 s ± 2.40, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 29.25s ± 1.70 s, n = 7) or the test phase following a 24 hr delay (Figure 2C left panel. Test - mean total exploration time. $t_{(12)} = 1.12$, $p = 0.150$; Ctrl: 24.50 s ± 2.30, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 34.76 s ± 2.26 s, n = 7). Long-term memory for object-location is dependent on the integrity of CBP HAT–dependent mechanisms in neurons within the mPFC (Figure 2C right panel. Discrimination index. $t_{(12)} = 2.78$, $p = 0.017$; Ctrl: 0.33 ± 0.11, n = 7; CBPHAT$^{PFC\text{-}hypo}$: 0.03 ± 0.10, n = 7), suggesting that CBP-dependent signaling in the mPFC is recruited for learning spatial-object associations and supports specifically long-term memory for object-location, but is dispensable for short–term memory for object-location.

Discussion

CBPHAT$^{PFC\text{-}hypo}$ mice showed normal performance during a short-term memory test on the object-location task but long-term memory for object-location was disrupted. Our results are consistent with a specific requirement for the intrinsic histone acetyltransferase activity of CBP in the transcriptional signaling required for long-term memory consolidation but not for learning and short-term memory. CBP-mediated nucleosome acetylation is believed to have impacts on chromatin structure and mediates gene-specific removal of epigenetically controlled repression(Fischle et al., 2003; Jaenisch and Bird, 2003; Rosenfeld and Glass, 2001) followed by the induction of transient transcriptional changes associated with long-lasting forms of neuronal and behavioral plasticity. It is also possible that nuclear non-histone substrates for CBP acetyltransferase activity might be involved in epigenetic mechanisms controlling memory consolidation. Known non-histone substrates for nuclear acetyltransferases include CREB (Lu et al., 2003), proteins regulating chromatin remodeling and gene expression such as p53 (Gu and Roeder, 1997) and many others (Glozak et al., 2005; Kimura et al., 2005; Kouzarides, 2000; Sterner and Berger, 2000; Yang, 2004). Both CREB (Bourtchuladze et al., 1994; Dash et al., 1990; Josselyn et al., 2001; Kida et al., 2002; Pittenger et al., 2002; Yin et al., 1994) and CBP (Alarcon et al., 2004; Guan et al., 2002; Hirano et al., 2013; Korzus et al., 2004; Liu et al., 2013; Wood et al., 2005) have been implicated in memory consolidation in a variety of species. The molecular mechanism

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underlying long-term memory consolidation involves CREB phosphorylation at S133, a necessary step to recruit CBP followed by transcriptional activation (Chrivia et al., 1993). In addition, NMDA receptor-dependent phosphorylation of CBP (S301) is also required for NMDA-induced transcription(Impey et al., 2002). Thus, a CBP HAT-dependent mechanism is capable of integrating complex neuronal signals into changes in gene expression for prolonged periods of time required for memory consolidation. Under the circumstances when only a transcription factor is activated while CBP has eliminated HAT activity as it is in the case of CBPHAT$^{PFC\text{-}hypo}$ mutant, CBP acts as a repressor by preventing recruitment of other coactivators and blocking expression of those genes that require CBP–dependent acetylation underlying memory consolidation.

There is growing evidence that coinciding chromatin modulation based circuit plasticity in the mPFC and hippocampus may control learning and system level memory consolidation. We have recently demonstrated that contextual fear discrimination involves CBP HAT-dependent changes in the mPFC circuit (Vieira et al., 2014) in addition to previously described neurogenesis-based circuit plasticity in the hippocampus related to enhanced pattern separation (Aimone et al., 2011; Sahay et al., 2011a; Sahay et al., 2011b). In fact, environment-induced adult neurogenesis is extrinsically regulated by CBP function in mature hippocampal granule cells (Lopez-Atalaya et al., 2011). Furthermore, DNA-methylation based chromatin changes in the mPFC have been linked to hippocampus-dependent associative learning experience (Miller et al., 2010). In addition, an increase in histone acetylation in the mPFC (Stafford et al., 2012) and specific histone modifications around individual BDNF gene promoters in the mPFC (Bredy et al., 2007) were connected to extinction of conditioned fear, whereas intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction (Lattal et al., 2007). Evidence from the spatial memory task in this study indicates that nuclear protein acetylation-dependent mechanism in the mPFC complements a previously reported requirement for CBP function in the hippocampus for object-location memory (Barrett et al., 2011). These data suggest that parallel mechanisms of epigenetic regulation in both the mPFC and hippocampus support system level memory consolidation by interleaving memories into existing related memory network across differentially functioning modules (Preston and Eichenbaum, 2013).

In contrast to previous studies demonstrating normal performance on the object-location task in absence of the mPFC (Barker et al., 2007; Ennaceur et al., 1997), we observed a deficit on the object-location task during a long-term memory test (but not a short-term memory test) in the CBPHAT$^{PFC\text{-}hypo}$ mice. Nonselective excitotoxic lesions to prefrontal neurons induced by bilateral injections of N-methyl-D-aspartate (NMDA) did not result in abnormalities in the performance on the object-location task after 5 min or 15 min delays (Barker et al., 2007; Ennaceur et al., 1997). However, bilateral lesions to dopaminergic terminals within the infralimbic (IL) mPFC induced by infusions of 6-hydroxydopamine (6-OHDA) showed significant effect for treatment on the object-location task after 10 min delay (Nelson et al., 2011) indicating that selective catecholamine depletion within the IL can produce deficit in object-location memory. There are at least four possibilities for this discrepancy. First, learning systems appear to work in parallel; when one is disconnected, another may overcome the deficit. For example, the mPFC can compensate for absence of the dorsal hippocampus in contextual fear learning(Zelikowsky et al., 2013). In the

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CBPHAT$^{PFC\text{-}hypo}$, the mPFC network is present during learning and able to encode information for short-term use (which eliminates compensatory mechanism) but unable to encode long-term memory. In addition, selective depletion of dopaminergic innervations in the infralimic subdivision of the mPFC without interruption of other components of mPFC circuits resulted in a deficit in the object-location task (Nelson et al., 2011) demonstrating again that when the mPFC circuit is present, compensatory mechanisms may not be available. Noteworthy, the dopaminergic system in the mPFC controls working memory (Goldman-Rakic, 1998; Robbins, 2000) and long-term object-location memory (Nagai et al., 2007). Thus, it is likely that other brain circuits may compensate for the absence of the mPFC in learning on the object-location task. Second, our object-location task differs form the task described in (Barker et al., 2007). While others used a testing box with wooden walls, we used visual cues attached to the transparent walls and were visible to the testing chamber. Thus, our object-location task provides more topographical cues in a more complex context and may involve different rule learning. Spatial memory (i.e. detecting the change of spatial location of a familiar object) allows for selection a novel stimulus (i.e. object spatial relationship in context) from one that has been encountered previously. While, excitotoxic mPFC lesions or its inactivation with lidocaine do not produce any effect on the performance in the object-location task, they yield a strong deficit in a version of the spatial memory task referred to as object-in-place (Barker et al., 2007; Ennaceur et al., 1997; Hannesson et al., 2004). Thus, the discrepancy between this study and Barker et al, 2007 may arise from differences in task demands such as the requirement for specific rule learning. Third, it is possible that the mPFC is only required for long-term memory in the object-location task but not for short-term memory. A specific role for the mPFC for strategic control over memory retrieval processes has been proposed before (Depue, 2012; Preston and Eichenbaum, 2013). In addition, inactivation of the mPFC resulted in delay-specific context fear memory deficit, specifically the mPFC was required for remote memory recall but not for recent memories (Bontempi et al., 1999; Frankland et al., 2004; Maviel et al., 2004; Wiltgen et al., 2004). However, it is quite unlikely that cortical reorganization of memories would occur within 24 hr.

These data demonstrate that the CBPHAT$^{PFC\text{-}hypo}$ mice provide an effective model to test the role of the mPFC circuitry in spatial memory consolidation by showing a delay-dependent effect. CBPHAT$^{PFC\text{-}hypo}$ mice displayed normal information acquisition and normal short-term memory; however, long-term memory for object-location was disrupted. These data suggest that spatial memory involves CBP-dependent encoding of specific information into mPFC neural networks, that is required for appropriate behavioral response following a 24 hr delay, in addition to previously described neural plasticity associated with hippocampus-dependent spatial learning.

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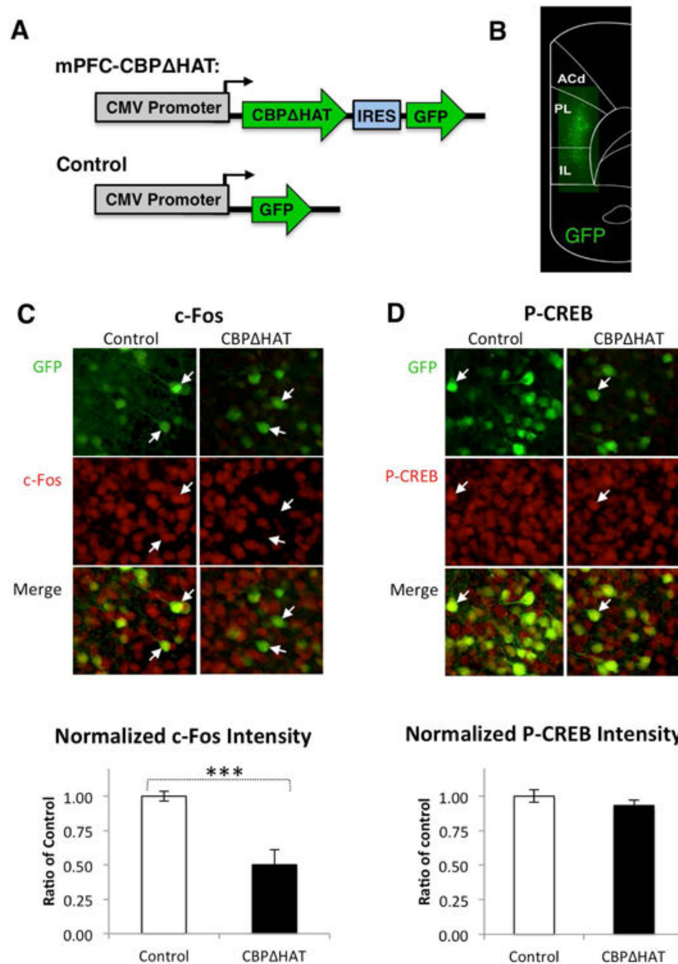

Figure 1.

CBP HAT transgene expressed in mPFC neurons (CBP HAT$^{PFC\text{-}hypo}$ mice) blocks expression of *c-Fos*, but not CREB phosphorylation.

(**A**) Viral-mediated delivery to the mPFC. Long-term expression HSV1 viruses carrying CBP HAT (HSV-1/CBP HAT-IRES2-EGFP) or eGFP as the control (HSV-1/-EGFP) were injected into the mouse mPFC. To determine the pattern of GFP-tagged virus expression, the imaged tissue was compared to a standard mouse atlas and areas of maximal GFP expression were labeled as injection sites. (**B**) A representative image (50-micrometer z-stack) of mPFC viral infection showed the precision of our viral-targeting procedures. Green, GFP. PL, prelimbic cortex. IL, infralimbic cortex. ACd, dorsal anterior cingulate. (**C-D**) Expressed CBP HAT in the mPFC blocks expression of *c-Fos ((***C**), but not p-CREB (**D**), in the mPFC in CBP HAT$^{PFC\text{-}hypo}$ mice. To determine the biochemical effects of viral infection with CBP HAT, the brains of infected animals were compared to controls in a standard IHC analysis. (**C**, *top*) Cells expressing viral CBP HAT showed significantly lower levels of c-Fos compared to GFP control cells. (**C**, *bottom*) Graph showing normalized c-Fos intensity in the CBP HAT$^{PFC\text{-}hypo}$ and control mice (**D,** *top*) CBP HAT viral infection had no effect on the expression of phosphorylated CREB in CBP HAT$^{PFC\text{-}hypo}$ and control mice. (**D**, *bottom*) Graph showing normalized P-CREB intensity in the

CBP HAT$^{PFC\text{-}hypo}$ and control mice. Representative images show GFP (in green) and c-Fos or p-CREB (in red). The asterisks indicate statistical significance: ***, $p < 0.001$.

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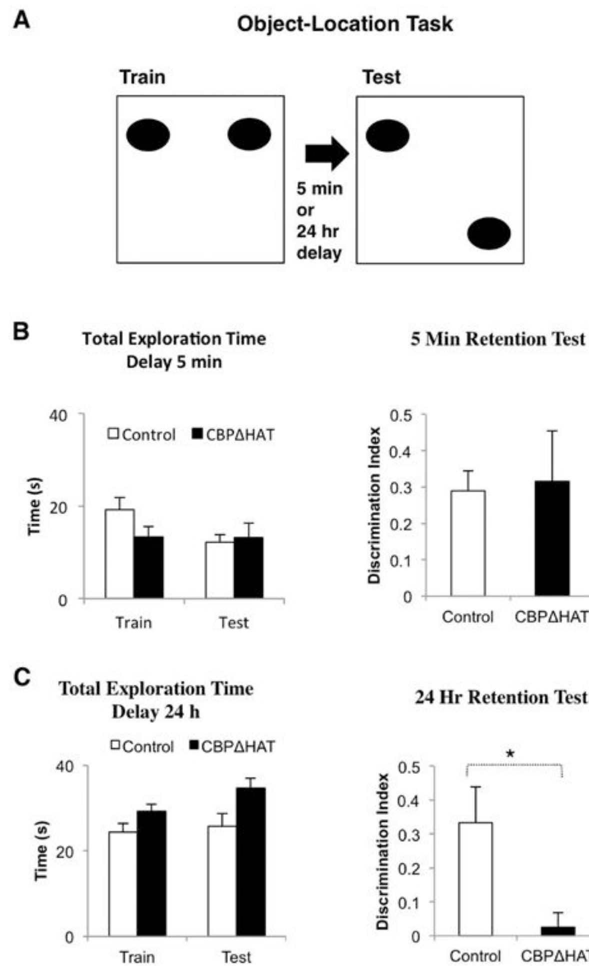

Figure 2.

Mice expressing CBP HAT in the mPFC showed a specific deficit in long-term memory for object-location.

(**A**) Diagram of the object-location task. Mice are trained on object-location task and tested after 5 min- or 24 hr-delay. (**B**) CBP HAT$^{PFC\text{-}hypo}$ and control mice showed similar levels of exploration during training and testing, as well as similar short-term memory for object-location after a 5 min delay. (**C**) CBP HAT$^{PFC\text{-}hypo}$ and control mice showed the same total exploration time during training and retention test but CBP HAT$^{PFC\text{-}hypo}$ mice exhibit deficit in long-term memory for object-location after a 24 hr delay. The asterisks indicate statistical significance: *, $p < 0.05$.