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Consolidation of CS and US Representations in Associative Fear Conditioning

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ABSTRACT: Much attention has been paid to the associative processes that are necessary to fuse together representations of the various components of an episodic memory. In the present study, we focus on the processes involved in the formation of lasting representations of the individual components that make up a fear-conditioning episode. In one-trial contextual fear conditioning experiments, weak conditioning to context occurs if the shock is delivered immediately following placement of the animal in a novel conditioning apparatus, a phenomenon known as the immediate shock deficit. We show that the immediate shock deficit in mice may be alleviated by pre-exposure to either the context or shock. In using this approach to temporally dissect a contextual fear-conditioning task into its constituent representational and associative processes, we are able to examine directly the processes that are important for formation of lasting representations of the context conditioned stimulus (CS) or unconditioned stimulus (US). Our data indicate that the formation of a lasting representation of the context or shock engages protein synthesis-dependent processes. Furthermore, genetic disruption of cAMP-responsive element binding protein (CREB), a transcription factor that regulates the synthesis of new proteins required for long-term memory, disrupts the formation of lasting context memories. We go on to show that the stress hormone epinephrine modulates the consolidation of a context memory, and reverses consolidation deficits in the CREB-deficient mice. Finally we show that disrupting either NMDA or calcium/calmodulin-dependent kinase II (CaMKII) function impairs consolidation of context memories. Together, these data suggest that this approach is particularly suited for the characterization of molecular and cellular processes underlying the formation of stimulus representations. © **2004 Wiley-Liss, Inc.**

KEY WORDS: protein synthesis; hippocampus; CREB; NMDA; CaMKII; epinephrine

INTRODUCTION

In contextual fear conditioning, an association is formed between a distinctive place (conditioned stimulus [CS]) and an aversive event (unconditioned stimulus [US]) (Fanselow, 2000). It is possible to demonstrate that an animal has formed a specific CS-US association by showing that reexposure to the conditioned context, but not dissimilar contexts, evokes conditioned fear behavior such as freezing (Fanselow, 1980). Besides demonstrating that an animal remembers an aversive event (or the CS-US association), it is also possible to show that the animal forms independent memories for each of the component parts of the aversive event or episode. For example, prior experience with the context CS or the shock US may retard or facilitate subsequent conditioning (Fanselow, 1990; Fanselow et al., 1993; Kiernan and Westbrook, 1993; Kiernan et al., 1995; Rudy and O'Reilly, 1999; Wiltgen et al., 2001; Lattal and Abel, 2001b; Rudy et al., 2002). These observations indicate that animals likely form independent representations of each of the components of an event memory—the CS and US—in addition to the CS-US association itself (Pavlov, 1927; Guthrie, 1935; Konorski, 1967).

Most Pavlovian fear conditioning studies have focused on identifying the associative processes underlying the fusion of the various features of an event into a unified memory, i.e., the biological processes underlying the formation of lasting CS-US associations (LeDoux, 2000; Anagnostaras et al., 2001; Maren, 2001). Recent work, however, has begun to focus on the processes underlying the formation of representations of each of its constituent parts—the building blocks of an event memory (Fanselow, 1990; Rudy and O'Reilly, 1999, 2001; Rudy et al., 2002; Barrientos et al., 2002). In one-trial contextual fear conditioning experiments, weak conditioning to context occurs if the shock is delivered immediately after placement of the animal in a novel conditioning apparatus, a phenomenon known as the immediate shock deficit (Fanselow, 1986, 1990). In the present study, we show that the immediate shock deficit in mice may be alleviated by pre-exposure to either the context or shock. Therefore, the use of these pre-exposure procedures permits temporal isolation of the processes underlying the formation of independent CS and US representations. In a series of experiments, we show that these processes are protein synthesis dependent, since pretreatment with anisomycin blocks the facilitative effects of pre-exposure to the context or shock. In addition, disrupting either N-methyl-D-aspartate (NMDA) receptor, calcium/calmodulin-dependent kinase II (CaMKII) or cAMP-re-

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sponsive element binding protein (CREB) function blocks the facilitative effects of context pre-exposure, indicating that the formation of context memories engages each of these processes. Furthermore, systemic treatment with the stress hormone, epinephrine, enhances memory for context, and similar treatment alleviates context impairments in CREB-deficient mice.

MATERIALS AND METHODS

Subjects

Unless otherwise specified, we used the progeny from a cross between C57Bl/6NTacfBr (B6; Taconic Farms) and 129Sv/J (129; Jackson Laboratory) inbred mouse strains. For the $CREB^{\alpha\Delta}$ mice used in Experiments 3 and 5, we used the F2 progeny derived from a cross between $CREB^{\alpha\Delta}$ heterozygotes in the B6 background (>99%) and wild-type (WT) 129 mice. The α-CaMKII-T286 mice used in Experiment 7 were heterozygotes derived from 8–9 crosses into B6. Mice were weaned at 3 weeks of age and were subsequently genotyped using polymerase chain reaction (PCR) protocols as previously described (Bourtchuladze et al., 1994; Giese et al., 1998). All mice were group housed (2–5 mice per cage) and had continuous access to food and water. The vivarium was maintained on a 12:12 light/dark schedule, and all testing was carried out during the light phase of the cycle. At the commencement of testing mice were at least 8 weeks old. All experiments used approximately equal numbers of male and female mice. All animal care and testing procedures were approved by the Animal Research Committee at UCLA and were in accordance with the NIH Principles of Laboratory Animal Care.

Apparatus

Conditioning context

The conditioning context was located in a windowless room. All mice were tested individually. For each test, the mouse was transported to the test apparatus in a cage containing a mix of fresh wood shavings and wood shavings from its home cage. The conditioning context was housed in a sound-attenuated box (interior dimensions: 56 × 42 × 37 cm; length × width × height). Three of the four interior walls of the sound-attenuated chamber were painted white. The other wall consisted of black and yellow vertical striped pattern. A clear Plexiglas window allowed the mice to be continually observed. Background noise (68 dB) was provided by a fan located in one of the walls of the sound-attenuated chamber. The conditioning context (16 cm × 16 cm × 19 cm; length × width × height) was rectangular in shape and its walls were made of clear Plexiglas. The total floor area was 256 cm^2. On one of the walls there was a 24 V house light. The floor of the context consisted of a shock grid. Bars were 3 mm in diameter and 0.9 cm apart. Each bar was connected to a Master Shocker (model 82402SS), a device that delivers scrambled shocks. Between tests, the cage floor and interior of the conditioning context were cleaned with a 75% ethanol solution.

Shock pre-exposure (PE) context

The shock PE context was housed in a different room from that used for the conditioning context. The floor of the shock PE context was triangular in shape, with vertical Plexiglas walls. The sides of the triangle were 24 cm long, and the height of the context was 20 cm. The total floor area was 250 cm^2, similar to the conditioning context. An opaque blue material covered the exterior of two walls of the shock PE context. The other wall was left transparent to allow observation of the mice. The floor of the cage comprised a shock grid. Between tests, the cage floor and interior of the conditioning context were cleaned with a 1% acetic acid solution.

Automated freezing apparatus

In a subset of experiments, we used an automated freezing apparatus to examine contextual fear conditioning in mice. With this experimental setup, freezing is measured automatically using a National Institutes of Health (NIH) Image-based algorithm. For a full description of apparatus and methods, see Anagnostaras et al., (2000).

Behavior Measurement

Conditioning was assessed by "freezing" behavior. An animal was determined to be freezing when it adopted a motionless posture, refraining from all but respiratory movement (Fanselow, 1990). Freezing was assessed using a sampling method; 2-s observations were taken every 5 s. For an animal to be scored as freezing, it had to remain motionless for the entire 2-s observation. These observations were made by an experimenter who was blind to the experimental treatment and/or genotype of each mouse. Freezing data is presented as the percent time spent freezing. That is, the number of observations when freezing was observed divided by the total number of observations and multiplied by 100.

Drugs

All drugs were administered intraperitioneally (i.p.) in a volume of 10 ml/kg. Anisomycin (ANI; Sigma) was dissolved in phosphate-buffered saline (PBS) and pH-adjusted to 7.4. ANI injections (150 mg/kg) were given 30 min before pre-exposure or training. [±]-3-[2-Carboxypiperazin-4-yl]propanephosphonic acid (CPP; Sigma) was dissolved in PBS, and given 20 min before pre-exposure or training.

Epinephrine (EPI; Sigma) was dissolved in PBS. EPI injections (0, 0.05, 0.5 mg/kg) were given immediately following context pre-exposure.

General Behavioral Procedures

Immediate shock training

Each mouse was placed in the conditioning context and 5 s later, a 2-s, 0.75-mA shock was delivered via the cage floor bars. Following this shock, the mice remained in the context for a further 60 s. Each mouse was then removed and returned to its home cage.

Testing

For testing, each mouse was placed back in the context, and freezing was assessed over a 3-min period. During this period, no shocks were presented.

Specific Experimental Procedures

Experiment 1a: Effect of placement-to-shock interval on contextual conditioning

Each mouse was placed in the conditioning context. Following 5 s (n = 10), 30 s (n = 9), 120 s (n = 7) or 300 s (n = 8) a 2-s, 0.75-mA shock was delivered via the cage floor bars. Following this shock, the mouse remained in the context for a further 60 s. Mice were then tested 30 min later.

Experiment 1b: Reactivity to an immediate versus delayed shock

Mice (B6) were randomly assigned to an immediate shock (n = 10) or a delayed shock (n = 10) condition. Mice in either condition were placed in the conditioning context for a total of 90 s. Mice in the immediate condition received a 2-s, 0.5-mA footshock 5 s after placement in the conditioning context. In contrast, mice in the delayed condition received the footshock 60 s after placement in the conditioning context. Mice were then tested one day later. In this experiment, both training and testing were conducted in the automated freezing apparatus, and freezing and shock reactivity were assessed using a computer-assisted automated scoring system (Anagnostaras et al., 2000).

Experiment 1c: Effect of context pre-exposure on the immediate shock deficit

In this experiment, we tested whether pre-exposure to the conditioning context can protect mice against the immediate shock deficit. Mice received various amounts of context pre-exposure (PE groups): 30 s (n = 8), 120 s (n = 9), 300 s (n = 8), or 600 s (n = 7). Control groups of mice did not receive context pre-exposure (NPE groups). Rather, they were taken to the room adjacent to the room housing the conditioning context in transport cages for an equivalent time period: 30 s (n = 7), 120 s (n = 11), 300 s (n = 11), or 600 s (n = 7). One day following this both PE and NPE groups were trained with an immediate shock. Mice were tested 30 min later.

Experiment 1d: Effect of shock pre-exposure on the immediate shock deficit

In this experiment, we tested whether pre-exposure to shock can protect mice against the immediate shock deficit. Mice were placed in the shock PE context. Following a 5 s delay mice received either a 0.25-mA (n = 14) or 0.75-mA (n = 6) shock, or no shock (n = 12). Shocks were 2 s in duration. Following the delivery of the shock mice remained in shock PE context for a further 60 s, and they were then removed. Twenty-four h later, all groups of mice were fear conditioned with an immediate shock in the conditioning context. Thirty min after this, they were tested in the conditioning context. To control for generalization, 24 h following the completion of testing in the conditioning context, all groups of mice were tested in the original shock pre-exposure context. The duration of this test was 3 min.

Experiment 2a: Effect of anisomycin treatment on context pre-exposure in WT mice

In this experiment, we tested whether treatment with the protein synthesis inhibitor ANI (150 mg/kg, i.p.) blocks the facilitative effects of context pre-exposure on contextual fear conditioning with an immediate shock. Mice were pretreated with ANI (n = 7) or PBS (n = 7) before being pre-exposed to the conditioning context for 10 min. A control group of mice was injected with ANI (n = 7) or PBS (n = 7), but not pre-exposed to the conditioning context. Twenty-four h later, both groups were fear conditioned with an immediate shock in the conditioning context, and tested following a 30-min delay.

Experiment 2b: Effect of anisomycin treatment on shock pre-exposure in WT mice

In this experiment, we tested whether treatment with ANI blocks the facilitative effects of shock pre-exposure on contextual fear conditioning with an immediate shock. ANI-treated (n = 7) and PBS-treated (n = 7) mice were placed in the shock PE context and received a 2-s, 0.75-mA shock following a 5 s delay. They remained in this alternate context for a further 60 s and were then removed. A control group of mice were injected with ANI (n = 7) or PBS (n = 8), but were not placed in the shock PE context and did not receive a shock. Twenty-four h later, they were fear conditioned with an immediate shock in the conditioning context. Thirty min following this they were tested.

Experiment 2c: Effect of anisomycin treatment on context-shock learning in WT mice

In this experiment, we examined the effects of blocking protein synthesis during the training, rather than the pre-exposure, phase. Mice were pre-exposed to the context for 10 min, and then 24 h later trained with an immediate shock. Thirty min prior to training mice were pretreated with either the ANI or PBS. Separate groups of mice were tested 30 min (PBS = 11; ANI = 9) or 24 h (PBS = 9; ANI = 9) later.

Experiment 3: Effect of context pre-exposure in $CREB^{\alpha\Delta-/-}$ mice

In this experiment, we tested whether context pre-exposure would protect $CREB^{\alpha\Delta-/-}$ mice against the immediate shock deficit. Separate groups of WT and $CREB^{\alpha\Delta-/-}$ mice were pre-exposed to the conditioning context for 10 min (PE + Immediate group: WT = 10, $CREB^{\alpha\Delta-/-}$ = 10) or not (Immediate group: WT = 10, $CREB^{\alpha\Delta-/-}$ = 9). Twenty-four h later, they were fear conditioned with an immediate shock. Thirty min later, they were tested. An additional group of WT (n = 16) and $CREB^{\alpha\Delta-/-}$ (n = 16) mice was trained with a delayed shock. Each mouse was placed in the conditioning context. After 2 min they received a 2-s, 0.75-mA shock, and 60 s later were removed from the context. Thirty min later, they were tested.

Experiment 4: Effect of epinephrine treatment on context learning in WT mice

In this experiment, we examined the effect of systemic epinephrine treatment on contextual learning. Immediately after a 2 min context pre-exposure (PE group), WT mice were injected with PBS (n = 8), 0.05 mg/kg EPI (n = 9) or 0.5 mg/kg EPI (n = 8). Control groups of mice, not pre-exposed to the context (NPE group), were given PBS (n = 7), 0.05 mg/kg EPI (n = 7) or 0.5 mg/kg EPI (n = 7). Twenty-four h later, all groups of mice were fear conditioned with an immediate shock. Thirty min following this they were tested.

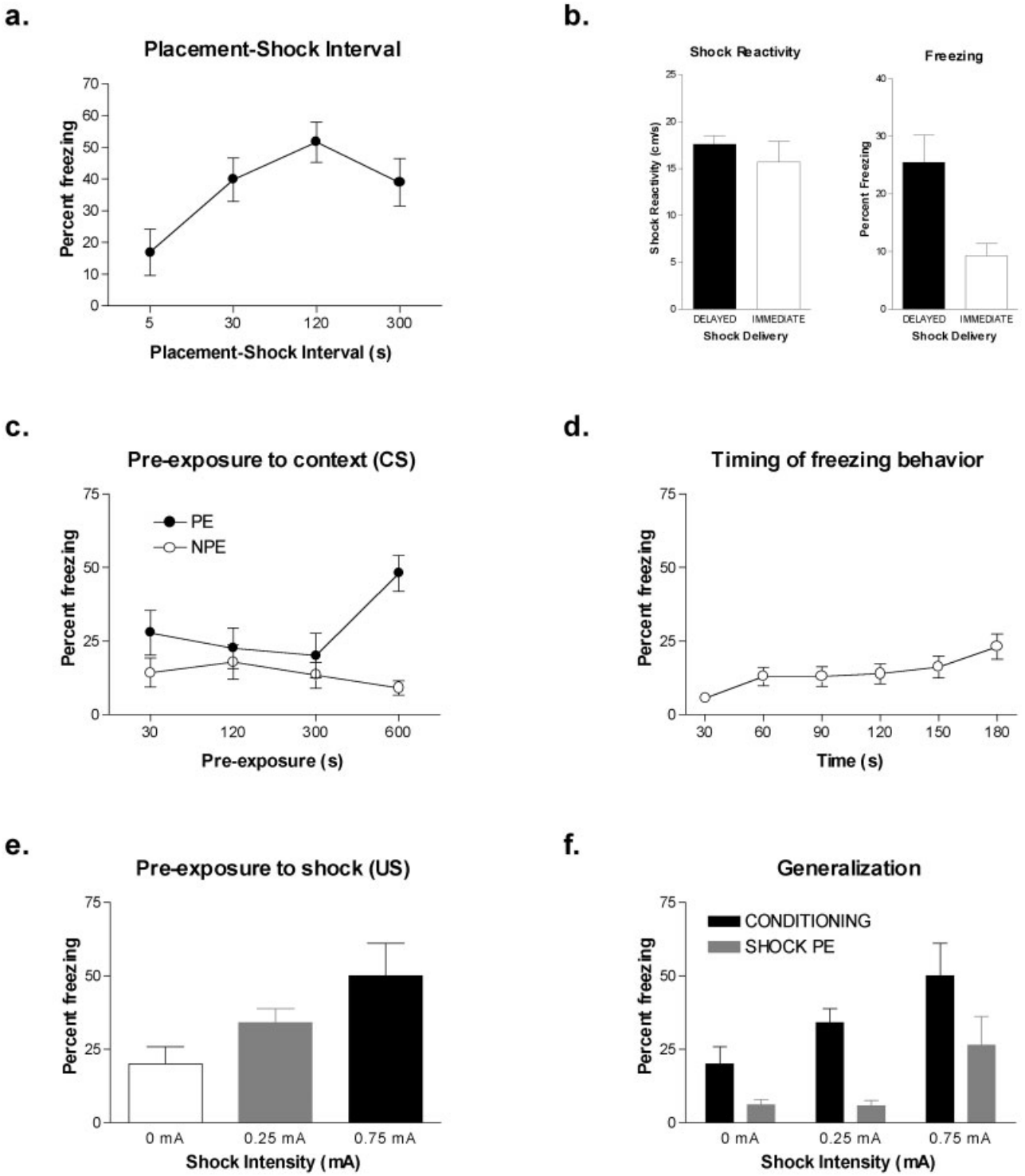


FIGURE 1

Experiment 5: Effect of epinephrine treatment on context learning in CREB$^{\alpha\Delta-/-}$ mice

In this experiment, we tested whether systemic epinephrine treatment would reverse contextual learning deficits in CREB$^{\alpha\Delta-/-}$ mice. All mice were pre-exposed to the conditioning context for 10 min. Immediately following this they were injected with PBS (WT = 9; CREB$^{\alpha\Delta-/-}$ = 7), 0.05 mg/kg EPI (WT = 11; CREB$^{\alpha\Delta-/-}$ = 7), or 0.5 mg/kg EPI (WT = 8; CREB$^{\alpha\Delta-/-}$ = 7). Twenty-four h later, all groups of mice were fear conditioned with an immediate shock. Thirty min later, they were tested.

Experiment 6: Effect of CPP treatment on context pre-exposure in WT mice

In this experiment, we tested whether treatment with CPP (0–10 mg/kg, i.p.) blocks the facilitative effects of context pre-exposure on contextual fear conditioning with an immediate shock. Mice (B6) were pretreated with PBS (n = 10), 5 mg/kg CPP (n = 9) or 10 mg/kg CPP (n = 6) before being pre-exposed to the conditioning context for 10 min. Twenty-four h later, mice were fear conditioned with an immediate shock in the conditioning context, and tested the following day.

Experiment 7: Effect of genetic disruption of CaMKII on context pre-exposure

In this experiment, we tested whether mice that are heterozygous for a point mutation at T286 (α-CaMKII-T286$^{+/-}$) exhibit impaired contextual processing. WT (n = 10) and α-CaMKII-T286$^{-/-}$ (n = 9) mice were trained and tested in an identical fashion to the mice in Experiment 6.

RESULTS

Immediate Shock Deficit: Effects of Context (CS) or Shock (US) Pre-exposure

In one-trial contextual conditioning, weak conditioning to context occurs if the shock is delivered immediately following placement of the animal in a novel conditioning apparatus. In this experiment, we varied the timing of the shock presentation during training to obtain a placement-shock function for mice, as has previously been described in rats (Fanselow, 1990). Mice were placed into the context and received a 2-s, 0.75-mA footshock at the following delays: 5, 30, 120, and 300 s. Following the shock, mice remained in the context for a further 60 s, and were tested 30 min later. The placement-shock interval during training influenced subsequent contextual fear ($F(3,30) = 4.28$, $P < 0.05$). Post hoc analyses indicated that freezing levels in mice trained with the shortest delay (5 s) were significantly lower than mice trained with either the 120 s or 300 s delay (Newman-Keuls; $P < 0.05$) (Fig. 1a). These data show that mice exhibit an immediate shock deficit as previously reported (Paylor et al., 1994; Kiyama et al., 1998; Milanovic et al., 1998; Lattal and Abel, 2001b; Stanciu et al., 2001; Wiltgen et al., 2001).

We next tested whether reduced levels of freezing in the mice trained with the shortest delay were due to reduced reactivity to the shock. Two groups of mice were trained. Mice received a single footshock either 5 s (immediate) or 60 s (delayed) following placement in the training context. Both groups of mice spent a total of 90 s in the training context, and were subsequently tested 24 h later. Shock reactivity was similar regardless of whether the shock was immediate or delayed ($F(1,18) < 1$) (Fig. 1b; left). Importantly, on subsequent testing, mice in the immediate shock group showed significantly lower levels of freezing compared to mice in the delayed shock group ($F(1,18) = 9.97$, $P < 0.05$) (Fig. 1b; right). This result indicates that the immediate shock deficit is not related to reduced reactivity to the delivery of an immediate shock. Furthermore, because mice in the immediate and delayed groups spent equivalent amounts of time in the context during training, these data show that it is the timing of the shock during training, rather than the total amount of time spent in the conditioning context, that determines subsequent levels of conditioned fear.

Context pre-exposure alleviates the immediate shock deficit in rats (Fanselow, 1990; Kiernan et al., 1995; Rudy and O'Reilly, 1999; Wiltgen et al., 2001; Barrientos et al., 2002; Rudy et al., 2002). We systematically varied the duration of context pre-exposure to determine the minimal amount of time required to alleviate the immediate shock deficit in mice. Mice were pre-exposed to the context for different durations (30, 120, 300, or 600 s) (pre-exposure groups; PE). One day following context pre-exposure, mice were trained with an immediate shock, and tested 30 min later.

FIGURE 1. Behavioral examination of the immediate shock deficit in mice. a: Effect of placement-shock interval on the development of contextual fear conditioning. During subsequent testing, freezing levels are higher in mice trained with longer placement-shock intervals. b: Shock reactivity is similar in mice trained with an immediate (placement-shock interval of 5 s) versus delayed (placement-shock interval of 60 s) shock (left). Despite this, in subsequent testing mice trained with the delayed shock exhibit significantly greater levels of freezing compared to those trained with an immediate shock (right). c: Effect of context pre-exposure (PE) vs. no pre-exposure (NPE) on contextual conditioning with an immediate shock. Extended pre-exposure to the context protects mice against the immediate shock deficit. Mice pre-exposed to the context for 10 min exhibited greater levels of freezing on subsequent tests compared to mice pre-exposed for shorter durations, or mice that were not pre-exposed. d: Distribution of freezing over time in mice exhibiting the immediate shock deficit. These test data are from mice in the NPE conditions in Experiment 1c. Freezing is not concentrated at the start of testing—that is, at the time the shock was delivered during training—as would be predicted by timing accounts of the immediate shock deficit. e: Prior experience with shock (delivered in an alternate context) facilitates contextual conditioning with an immediate shock. Mice receiving a 0.75-mA shock 24 h prior to conditioning, exhibited higher levels of freezing on subsequent testing. f: One day following testing in the conditioning context (Experiment 1d), mice were also tested in the shock PE context. Freezing in this context was lower in all groups, indicating that generalization from the shock PE context to the conditioning context cannot account for the facilitative effects of shock pre-exposure.

Control groups of mice were removed from the vivarium, but not placed in the context (no pre-exposure group; NPE). Only extended pre-exposure to the conditioning context alleviated the immediate shock deficit (exposure × time interaction; $F(3,60) = 3.06$, $P < 0.05$) (Fig. 1c). Post hoc analyses revealed that freezing levels were higher in the mice that were pre-exposed for 600 s compared to each of the other PE groups. In addition, freezing in the 600-s PE group was significantly higher than in the control 600-s NPE group ($P < 0.05$). Although not tested here, the facilitative effects of context pre-exposure are specific to the to-be-conditioned context as pre-exposure to an alternate context fails to reverse the immediate shock deficit in rats (Kiernan et al., 1995; Rudy et al., 2002) and mice (Lattal and Abel, 2001b).

The reduction in freezing levels following immediate shock training might be because mice tend to concentrate freezing bouts towards the beginning of the test session—that is, at a time corresponding to shock delivery during training. Examination of freezing in the mice that were not pre-exposed to the context (NPE groups) reveals that this is not the case. Collapsing across each of these control groups, freezing tended to increase, rather than decrease, as a function of time during testing ($F(5,175) = 5.02$, $P < 0.05$) (Fig. 1d). It is unclear why freezing increases over time, but this analysis is nonetheless inconsistent with timing accounts of the immediate shock deficit (Bevins and Ayres, 1995; Gallistel and Gibbon, 2000).

Since extended context pre-exposure alleviated the immediate shock deficit, we next tested whether pre-exposure to the shock US produces similar effects. Mice received a shock (2-s duration; 0.25-mA or 0.75 mA) 5 s following placement in an alternate distinctive (shock PE) context. One day later, mice were trained in the conditioning context using immediate shock procedures, and tested 30 min later. Pre-exposure to shock in the alternate context facilitated contextual conditioning following training with immediate shock procedures ($F(2,29) = 4.38$, $P < 0.05$) (Fig. 1e). Freezing during subsequent testing was significantly higher in mice that were pre-exposed to the high shock (0.75 mA) compared to mice not receiving shock pre-exposure (0 mA) ($P < 0.05$).

These data indicate that pre-exposure to the shock US facilitates subsequent fear conditioning, as does pre-exposure to the context CS. The pre-exposure shock was delivered immediately to minimize conditioning to the shock pre-exposure context. Nevertheless, it is possible that generalization from the shock pre-exposure context to the conditioning context might account for the facilitated conditioning in the shock-pre-exposed mice. To examine this, following testing in the conditioning context, mice were also tested in the shock pre-exposure context. Mice froze less in shock PE context compared to the conditioning context ($F(1,29) = 38.3$, $P < 0.05$) (Fig. 1f), a finding that makes it unlikely that generalization occurred from the shock PE context to the conditioning context. Because mice were tested in the shock pre-exposure context after they were tested in the conditioning context, it should be noted that extinction could account for reduced freezing in the shock pre-exposure context. However, multiple exposures to the conditioning context following training do not produce the same magnitude decrement in conditioned freezing (data not shown).

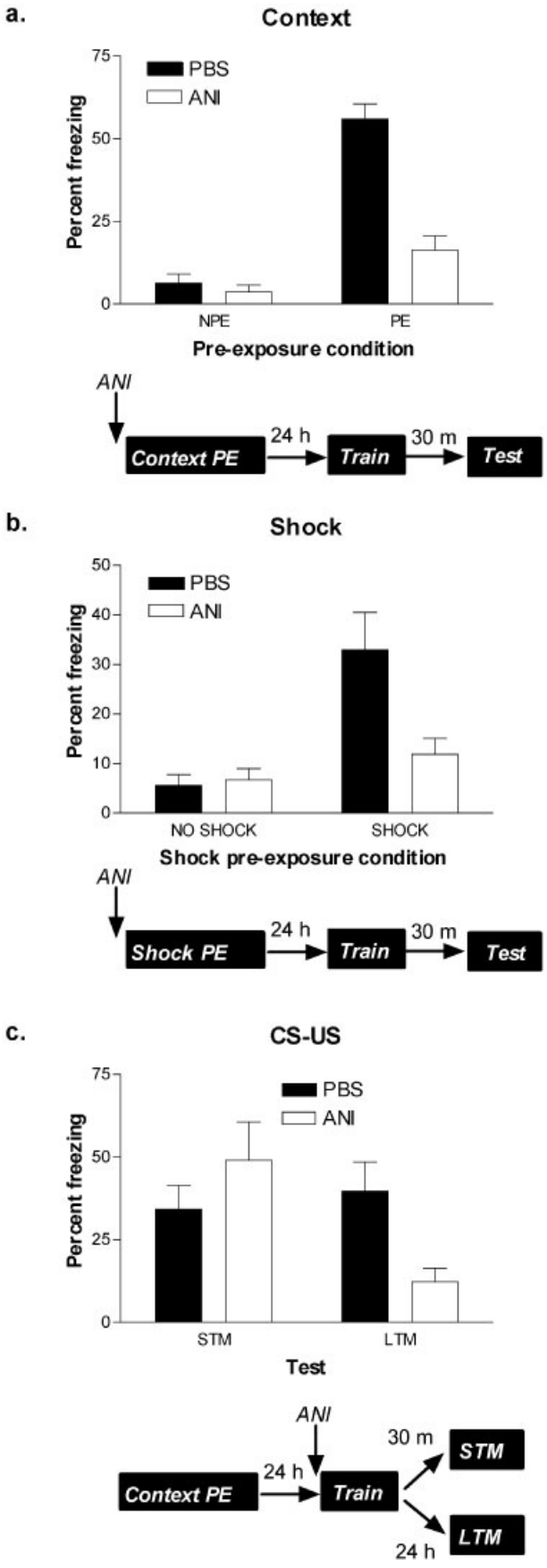
Facilitative Effects of Context (CS) or Shock (US) Pre-exposure Are Blocked by Protein Synthesis Inhibition

Contextual fear conditioning is thought to require an animal to form a representation of the training context (CS), the shock (US) as well as a context-shock (CS-US) association (Fanselow, 2000; Rudy et al., 2002). The above experiments dissociated CS and US representational processes underlying Pavlovian fear conditioning, and suggest that these procedures can be used to directly examine mechanisms underlying the formation of CS and US representations, independent of CS-US associations.

Protein synthesis is essential for the formation of long-term memories (Davis and Squire, 1984). We therefore asked whether the formation of either lasting CS (context) or US (shock) representations depends on protein synthesis. To test whether the formation of a context representation is protein synthesis dependent, we pre-exposed mice to the training context for 10 min. Mice were pretreated with either the protein synthesis inhibitor ANI or PBS. One day later, they were trained using immediate shock procedures, and tested 30 min later. A group of control mice were treated identically, except that they were not pre-exposed to the training context. Disrupting protein synthesis specifically attenuated the effects of context pre-exposure on contextual conditioning (exposure × drug interaction; $F(1,24) = 26.8$, $P < 0.05$) (Fig. 2a). In mice pre-exposed to the context, ANI pretreatment significantly reduced freezing levels compared to PBS-treated controls ($P < 0.05$).

To test whether the formation of a shock representation is protein synthesis dependent, we pre-exposed mice to a 0.75-mA shock in the alternate (shock PE) context. Mice were pretreated with ANI or PBS. One day later, they were trained using immediate shock procedures, and tested 30 min later. A group of control mice were treated identically, except that they were not pre-exposed to shock. Disrupting protein synthesis specifically blocked the facilitative effects of shock pre-exposure on contextual conditioning (exposure × drug interaction; $F(1,25) = 6.66$, $P < 0.05$) (Fig. 2b). In mice pre-exposed to the shock, ANI pretreatment significantly reduced freezing levels in the subsequent test compared to PBS-treated controls ($P < 0.05$).

Therefore, the facilitative effects of pre-exposure to the context CS or shock US are blocked by protein synthesis inhibition. In the next experiment we examined the effects of blocking protein synthesis during the training, rather than the pre-exposure, phase. Mice were pre-exposed to the context and 24 h later trained with immediate shock procedures. Prior to training, mice were treated with either ANI or PBS. To examine the effects of ANI treatment on both short- and long-term memory, separate groups of mice were then tested 30 min or 24 h later. As expected, mice pretreated with PBS exhibited robust conditioning whether tested 30 min or 24 h following training. In contrast, mice pretreated with ANI prior to training exhibited normal memory 30 min following training, but impaired memory when tested 24 h following training (drug × test delay interaction; $F(1,34) = 6.43$, $P < 0.05$) (Fig. 2c). Post hoc analyses showed that conditioned freezing levels were reduced in ANI-treated mice compared to the PBS-treated mice in



the test 24 h following training ($P < 0.05$). Consistent with a large literature, these data indicate that disruption of protein synthesis blocks the formation of long-term (but not short-term) memory (Davis and Squire, 1984). While it is tempting to conclude that these data show that protein synthesis inhibition blocks the formation of lasting CS-US associations, this experiment does not rule out the possibility that protein synthesis inhibition blocks the formation of a lasting US memory at the time of training. For example, an intact US memory may be necessary for the retrieval of the CS-US association during subsequent testing.

Facilitative Effects of Context Pre-Exposure Are Blocked in Mice With a Targeted Disruption of CREB Function

Studies in a wide variety of species have shown that the synthesis of proteins necessary for long-term memory formation is regulated, at least in part, at the transcriptional level by CREB (Yin and Tully, 1996; Silva et al., 1998; Alberini, 1999; Kandel and Pittenger, 1999). Just as in studies examining the effects of protein synthesis inhibition on memory formation, a unifying feature of these studies is that manipulating CREB function affects only long-term memory (i.e., tested at 24 h), and not short-term memory (i.e., tested at 1 h or less). Accordingly, we have previously shown that mice with a targeted disruption of the α and Δ CREB isoforms ($CREB^{\alpha\Delta-/-}$ mice) have normal short-term, but impaired long-term, memory for contextual fear conditioning (Bourtchuladze et al., 1994; Kogan et al., 1997).

Disruptions of processes underlying the formation of lasting context (CS), shock (US) representations, or stable CS-US associations, may account for these deficits in long-term contextual fear memory. In the next experiment we examined whether normal CREB function is required for the formation of lasting context representations (Fig. 3). We first showed that $CREB^{\alpha\Delta-/-}$ mice exhibit normal short-term memory (30 min) when trained with a delayed shock ($F(1,30) < 1$, $P > 0.05$). These data indicate that short-lived processes required for the expression of contextual fear memories 30 min following training—for example, the formation of CS (context), US (shock) representations, and CS-US (context-shock) associations—are unaffected by the $CREB^{\alpha\Delta-/-}$ mutation. When trained with an immediate shock, however, both $CREB^{\alpha\Delta-/-}$

FIGURE 2. Effect of protein synthesis inhibition on the formation of lasting conditioned stimulus (CS) and unconditioned stimulus (US) representations, and on lasting CS-US associations. a: Mice were either pre-exposed to the conditioning context (PE) or not pre-exposed (NPE). ANI-treatment (open bars) specifically blocked the facilitative effects of context pre-exposure compared to phosphate-buffered saline (PBS)-treated controls (closed bars). b: Mice received shock pre-exposure (shock) or not (no shock). ANI-treatment (open bars) specifically blocked the facilitative effects of shock pre-exposure compared to PBS-treated controls (closed bars). c: Mice were pre-exposed to the conditioning context for 10 min. One day later, they were trained with immediate shock procedures. Pre-training ANI-treatment (open bars) blocked long-term memory (tested 24 h following training), but not short-term memory (tested 30 min following training). PBS-treated mice (closed bars) exhibited similar levels of freezing at 30 min and 24 h.

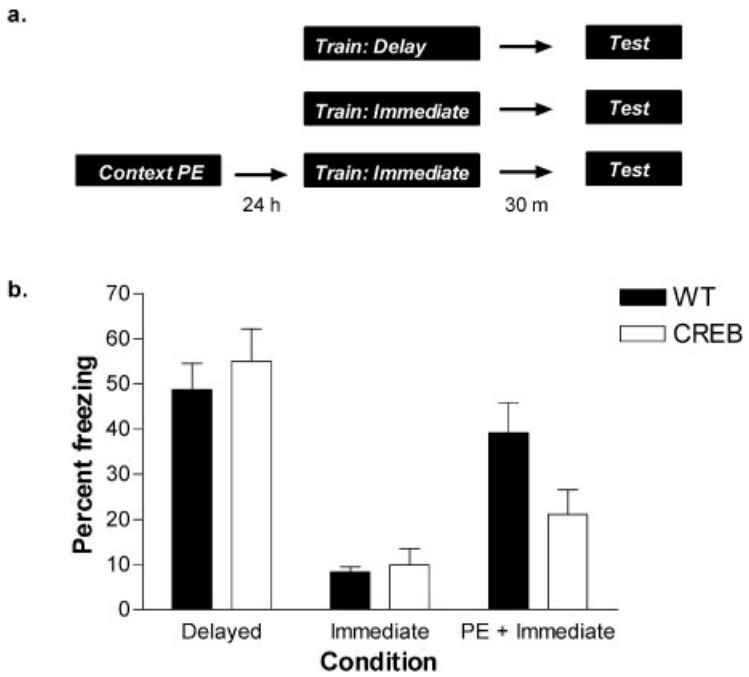


FIGURE 3. Context pre-exposed (PE) does not alleviate the immediate shock deficit in cAMP-responsive element binding protein (CREB)$^{\alpha\Delta-/-}$ mice. a: The three training conditions for wild-type (WT) and CREB$^{\alpha\Delta-/-}$ mice. b: When trained with a delayed shock, WT (closed bars) and CREB$^{\alpha\Delta-/-}$ (open bars) mice showed normal short-term (30-min) contextual fear memory. However, if the shock is delivered immediately during training (rather than after a delay) both WT and CREB$^{\alpha\Delta-/-}$ mice exhibit reduced levels of conditioned freezing on test, or an immediate shock deficit. Pre-exposure to the context protected WT, but not CREB$^{\alpha\Delta-/-}$, mice against this immediate shock deficit.

and WT control mice exhibited reduced levels of conditioned fear when tested 30 min later. In WT mice, this immediate shock deficit was rescued by pre-exposure to the conditioning context 24 h prior to training. In contrast, context pre-exposure failed to rescue the immediate shock deficit in CREB$^{\alpha\Delta-/-}$ mice (genotype $\times$ exposure interaction: $F(1,35) = 4.43$, $P < 0.05$). Critically, there was no difference in levels of freezing between CREB$^{\alpha\Delta-/-}$ mice that were pre-exposed (PE + immediate group) and those that were not (immediate group) ($P > 0.05$). Furthermore, pre-exposed WT mice exhibited significantly more freezing compared to pre-exposed CREB$^{\alpha\Delta-/-}$ mice ($P < 0.05$).

In these experiments, we used only a short delay (30 min) between training and testing. Because memory is normal at these short delays in CREB$^{\alpha\Delta-/-}$ mice, this design allows us to examine the impact of the CREB$^{\alpha\Delta-/-}$ mutation on contextual processing: that is, on processes necessary to form and maintain a context memory during the 24 period between pre-exposure and training. Therefore, these data indicate that the targeted disruption of CREB function in the CREB$^{\alpha\Delta}$ mice impairs the formation of a lasting representation of context.

Context Memories Are Enhanced by Epinephrine

Memories for emotionally charged events tend to be stronger and more persistent (Cahill and McGaugh, 1996; McGaugh and Roozendaal, 2002). A large number of studies have shown that the activation of adrenal stress hormones, such as epinephrine, facilitates memory consolidation via central β-adrenergic mechanisms (McGaugh, 2002). To test whether memory for context may be modulated in a similar manner, we examined the impact of epinephrine treatment on the effectiveness of context pre-exposure. Since we expected epinephrine treatment to enhance memory, we used a pre-exposure duration (2 min) that does not normally alleviate the immediate shock deficit (see Fig. 1c). Consistent with our earlier experiment, a 2 min PE was insufficient to alleviate the immediate shock deficit: the PBS-treated mice, regardless of whether or not they had been pre-exposed to the context, showed similarly low levels of freezing when tested. However, mice treated with epinephrine immediately following the 2 min context pre-exposure showed increased contextual fear conditioning. Importantly, the facilitative effects of epinephrine were limited to mice that were pre-exposed to the context (dose $\times$ exposure interaction: $F(2,40) = 3.45$, $P < 0.05$) (Fig. 4). This indicates that epinephrine does not produce nonspecific facilitation of conditioning; rather the facilitative effects are contingent on pre-exposure to the to-be-conditioned context.

Context Deficits in CREB$^{\alpha\Delta-/-}$ Mice Are Reversed by Epinephrine Treatment

The memory-enhancing effects of epinephrine are mediated centrally by β-adrenergic receptors (Liang et al., 1986). Since the activation of β-adrenergic receptors is coupled to cAMP/PKA signaling, and CREB-dependent transcription is reduced, but not eliminated, in the CREB$^{\alpha\Delta}$ mice (Blendy et al., 1996), we tested whether epinephrine treatment would reverse deficits in contextual

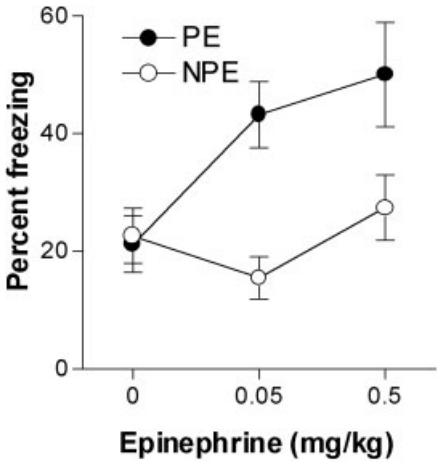


FIGURE 4. Effect of epinephrine treatment on consolidation of context memories. Wild-type (WT) mice were pre-exposed (PE) or not pre-exposed (NPE) to the conditioning context for 2 min. Immediately following this, mice were treated with epinephrine. Twenty-four h later, they were trained with immediate shock procedures and then tested after a 30 min delay. Epinephrine dose-dependently facilitated conditioning only in the pre-exposed mice.

processing in these mutants (see Fig. 3). To do this, CREB$^{\alpha\Delta-/-}$ mice and their WT controls were pre-exposed to the conditioning context for 10 min. Immediately following this, mice were injected with epinephrine or PBS. One day later, they were trained using immediate shock procedures, and tested 30 min later. Epinephrine treatment given immediately following context pre-exposure facilitated subsequent conditioning in the CREB$^{\alpha\Delta-/-}$ mice (Fig. 5). For the highest dose of epinephrine (0.5 mg/kg), WT and CREB$^{\alpha\Delta-/-}$ mice exhibited equivalent levels of freezing on test (Planned comparison; $P = 0.49$). However, for lower doses of epinephrine (0 or 0.05 mg/kg) WT mice froze significantly more than CREB$^{\alpha\Delta-/-}$ mice on test (Planned comparisons; $P < 0.05$). These data suggest deficits in forming a lasting context representation in CREB$^{\alpha\Delta-/-}$ mice can be partially reversed by treatment with epinephrine, most likely via activation of residual CREB-dependent processes.

Facilitative Effects of Context Pre-exposure Are Blocked by Pharmacological Blockade of NMDA Receptors and Genetic Disruption of α-CaMKII

The above series of experiments indicate that examination of the facilitative effects context pre-exposure on the immediate shock deficit may be an effective approach for the identification of molecular events underlying the formation of contextual memories. To characterize further the utility of this approach, we extended our analyses to examine the effects of two treatments (pharmacological and genetic) known to disrupt contextual fear conditioning and hippocampal long-term potentiation.

The activation of NMDA receptors and subsequent autophosphorylation of α-CaMKII at T286 are known to play key roles in hippocampal-dependent behavioral and synaptic plasticity (Martin et al., 2000; Lisman et al., 2002). Therefore, to test whether normal NMDA receptor function is required for the formation of context representations, we tested whether the NMDA antagonist, CPP, blocks the facilitative effect of context pre-exposure. Mice were pre-exposed to the conditioning context for 10 min following pretreatment with CPP (0–10 mg/kg). Twenty-four h later, they were trained using immediate shock procedures, and then tested the next day. Pharmacological disruption of NMDA receptor function blocked the facilitative effects of context pre-exposure on contextual conditioning ($F(2,22) = 3.89$, $P < 0.05$) (Fig. 6a). Similarly, a heterozygous point mutation at T286 (α-CaMKII-T286$^{+/-}$) blocked the facilitative effects of context pre-exposure on contextual conditioning ($F(1,17) = 6.92$, $P < 0.05$) (Fig. 6b). These data suggest that this approach can be broadly applied. Furthermore, since α-CaMKII-T286$^{+/-}$ mice exhibit normal contextual fear conditioning using standard procedures (Ohno et al., 2001), context pre-exposure approaches may represent a more sensitive behavioral assay for the detection of contextual processing deficits in mice.

DISCUSSION

In this study, we used behavioral procedures to dissect a Pavlovian fear-conditioning task into its constituent representational and associative components. Using this approach, we focused in particular on those processes that are important for the formation of lasting representations of the context CS or shock US. Our data indicate that the formation of lasting context (and shock) representations requires the activation of NMDA receptors, autophosphorylation of CaMKII at T286, CREB-dependent transcription and protein-synthesis. Furthermore, we show that stress hormones, such as epinephrine, modulate the consolidation of the

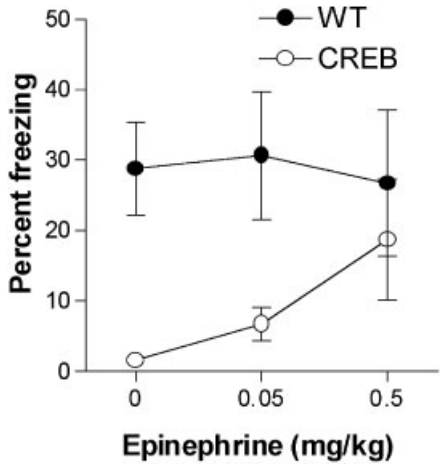


FIGURE 5. Context deficits in cAMP-responsive element binding protein (CREB)$^{\alpha\Delta-/-}$ mice are reversed by epinephrine treatment. Wild-type (WT) and CREB$^{\alpha\Delta-/-}$ mice were pre-exposed to the conditioning context for 10 min. Twenty-four h later, they were trained with immediate shock procedures and then tested after a 30-min delay. Epinephrine treatment immediately following context pre-exposure dose-dependently alleviated conditioning deficits in the CREB$^{\alpha\Delta-/-}$ mice.

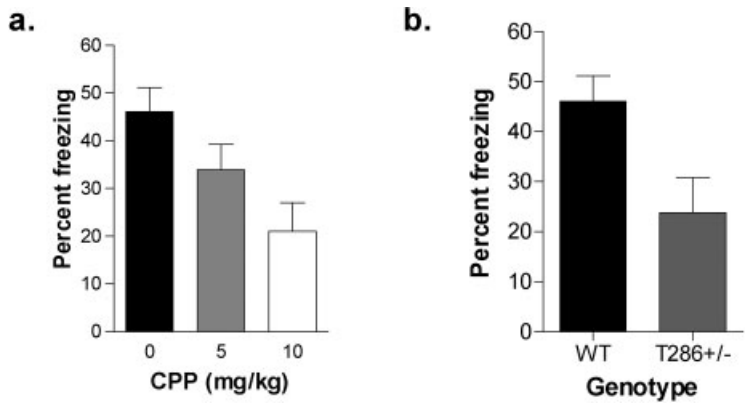


FIGURE 6. Disrupting either N-methyl-D-aspartate (NMDA) receptor or calcium/calmodulin-dependent kinase II (CaMKII) function blocks the facilitative effects of context pre-exposure. a: Wild-type (WT) mice were pre-exposed to the conditioning context for 10 min. CPP treatment dose-dependently blocked the facilitative effects of context pre-exposure compared to phosphate-buffered saline (PBS)-treated controls. b: WT and α-CaMKII-T286$^{+/-}$ mice were pre-exposed to the conditioning context for 10 min. The facilitative effects of context pre-exposure were attenuated in the α-CaMKII-T286$^{+/-}$ mice.

context memory. Finally, we show that the failure to successfully consolidate context in CREB$^{\alpha\Delta-/-}$ mice is alleviated by epinephrine. Together, our data indicate that the mechanisms responsible for forming lasting representations of various features of an event overlap with the associative processes responsible for fusing together these individual representations into a unified episode. Furthermore, they suggest that these procedures may be especially effective for identifying molecular and cellular processes underlying the formation of lasting stimulus representations.

In one-trial contextual fear conditioning experiments, weak conditioning to context occurs if the shock is delivered immediately upon placement of the animal in the conditioning apparatus, a phenomenon known as the immediate shock deficit (Fanselow, 1986, 1990). We found that pre-exposing mice to either the context or the shock protected mice against the immediate shock deficit. Deficits in contextual conditioning following training with an immediate shock have been attributed to failures of either CS (Fanselow, 1990) or US (Lattal and Abel, 2001b) processing. That is, either the short delay between placement in the context and shock delivery, or other factors such as stress-related deficits in sensory processing associated with handling, interfere with the animal's ability to process effectively (1) the context, or (2) the shock. Our data suggest that failures in either CS or US processing may contribute to the effect since prior experience with either alleviates the immediate shock deficit. Regardless of the mechanism, the impact of either context or shock pre-exposure on the immediate shock deficit indicates that mice can readily form a lasting memory of the context or the shock (Fanselow and Gale, 2003), independent of a pairing between the two. Therefore, we were able to use these pre-exposure procedures to effectively isolate processes underlying the formation of context or shock memories.

Previous studies have shown that protein synthesis inhibition blocks the formation of long-term contextual fear conditioning memories (Abel et al., 1997; Bourtchouladze et al., 1998; Schafe et al., 1999; Stiedl et al., 1999). However, using standard contextual conditioning procedures it is not possible to determine whether these deficits are due to a block of the formation of a lasting representation of the context CS or shock US, or a lasting memory for the CS-US association. We found that protein synthesis is required for the establishment of lasting memories for each of the to-be-associated elements: the context and the shock. These data are consistent with a recent study that found that intra-hippocampal infusions of anisomycin block the facilitative effects of context pre-exposure on contextual conditioning (Barrientos et al., 2002). Furthermore, our data parallel similar findings in other paradigms (e.g., conditioned taste aversion, latent inhibition) showing that the formation of lasting CS and US representations depend on protein synthesis (Berman and Dudai, 2001; Rosenblum et al., 1993; Schauz and Koch, 2000).

The synthesis of most proteins is mediated by activity-regulated transcription factors (Shaywitz and Greenberg, 1999). Studies in a wide variety of species have shown that the synthesis of proteins necessary for long-term memory formation are regulated, at least in part, by the transcription factor CREB (Alberini, 1999; Kandel and Pittenger, 1999; Silva et al., 1998; Yin and Tully, 1996). Previously we have found that CREB$^{\alpha\Delta}$ mutant mice exhibit impaired long-term memory (24 h), but normal short-term memory ($\leq$1 h) in contextual fear conditioning (Bourtchuladze et al., 1994; Kogan et al., 1997; Falls et al., 2000). By using pre-exposure procedures to examine context memory in isolation, our current data indicate that CREB$^{\alpha\Delta-/-}$ mice are unable to form a lasting representation of the context. Therefore, inhibiting protein synthesis and disrupting CREB function produce similar effects: both manipulations block the development of lasting representations of place, demonstrating that CREB plays a critical role in the transcriptional activation required for these processes.

The hippocampus plays a central role in the representation of contexts or places (Frankland et al., 1998; Anagnostaras et al., 1999, 2001; Fanselow, 2000; O'Reilly and Rudy, 2001). Therefore, the inability to form lasting representations of place is consistent with observations that the stability of newly formed hippocampal place cells, as well as the formation of lasting spatial memories, is disrupted by protein synthesis inhibitors (Meiri and Rosenblum, 1998; Agnihotri et al., 2001; Lattal and Abel, 2001a)

and compromised in $CREB^{\alpha\Delta-/-}$ mice (Bourtchuladze et al., 1994; Kogan et al., 1997; Cho et al., 1998). Importantly, it has recently been shown that either hippocampal lesions or intra-hippocampal infusions of anisomycin block the facilitative effects of context pre-exposure on contextual conditioning (Barrientos et al., 2002; Rudy et al., 2002), indicating that protein synthesis in the hippocampus is required for the formation of stable representations of place. We extend these findings to show that the formation of lasting context memories also requires the activation of NMDA receptors, α-CaMKII and CREB. These data, together with those of Rudy and colleagues (Rudy and O'Reilly, 1999; Rudy and O'Reilly, 2001; Barrientos et al., 2002; Rudy et al., 2002), suggest that context pre-exposure procedures may be a particularly effective method for identifying hippocampal molecular and cellular processes associated with the formation of lasting representations of place. Indeed, studying context in isolation, rather than in aversively-motivated situations such as the water maze and contextual fear conditioning, may be a more appropriate behavioral correlate for place cell studies.

Using standard one-trial contextual fear conditioning procedures the optimal placement-shock interval was 150 s, although a much shorter interval (i.e., 30 s) appeared to be sufficient for conditioning to occur. In stark contrast, much longer context pre-exposure durations were necessary to alleviate the immediate shock deficit: only a pre-exposure lasting 600 s was sufficient to reverse the immediate shock deficit. Therefore, in the absence of an aversive reinforcer, such as shock, longer duration exposures are required to form a lasting memory for the context. Emotionally arousing stimuli, such as shock delivery, facilitate memory consolidation by activating adrenal stress hormones (McGaugh and Roozendaal, 2002). The activation of adrenal stress hormones following shock delivery therefore allows animals to selectively remember more emotionally charged events, at the expense of less important ones (Cahill and McGaugh, 1996). We tested this idea by giving mice a 2 min pre-exposure to the context 24 h before training them using immediate shock procedures. Under normal conditions this 2 min pre-exposure has no effect on subsequent conditioning. However, epinephrine treatment, given immediately following pre-exposure, makes this 2 min pre-exposure as effective as a 10 min pre-exposure in alleviating the immediate shock deficit. This way, a relatively neutral experience—one that by itself would not elicit conditioned responding—is made effective, presumably by activation of the adrenal stress hormone system. That is, mimicking the effects of shock with epinephrine treatment ensures that the context memory is fully consolidated even after a relatively brief exposure. A previous study found that epinephrine administration following contextual fear conditioning (using standard procedures) was ineffective (Lee et al., 2001). However, the present context pre-exposure procedures may provide a more sensitive method for detection of modulatory influences on contextual memories since short duration pre-exposures (in combination with immediate shock training) produce near-baseline levels of freezing on test.

Epinephrine also facilitated the consolidation of a context memory in $CREB^{\alpha\Delta-/-}$ mice. CREB function is reduced, rather than eliminated, in $CREB^{\alpha\Delta-/-}$ mice. Therefore, epinephrine may activate residual CREB via activation of β-adrenergic receptors that are coupled to cAMP signaling (Kobayashi and Yasoshima, 2001). This idea recalls an older literature showing that the amnestic effects of protein synthesis inhibitors given prior to training may be attenuated by posttraining administration of stimulants, including epinephrine (Martinez et al., 1981). Furthermore, the finding that epinephrine was effective when given following context pre-exposure suggests the following. First, that the efficiency of CREB-mediated consolidation may be regulated during a brief time-window following initial learning. Second, the context impairments in $CREB^{\alpha\Delta-/-}$ mice are due to a failure to consolidate, rather than encode, contextual information. This conclusion is consistent with recent reports (Kida et al., 2002; Pittenger et al., 2002). To test these ideas further it will be necessary to show that CREB activity is elevated in trained $CREB^{\alpha\Delta-/-}$ mice following epinephrine treatment. Previous studies suggested that the memory deficits of the $CREB^{\alpha\Delta-/-}$ mice can be partially alleviated when the mutation is crossed into some strains of mice (Gass et al., 1998). Our data suggest that one possibility is that the upregulation of genes (either controlling the synthesis or the effects of epinephrine) in these mouse strains is responsible for the partial rescue of the memory deficits caused by the $CREB^{\alpha\Delta-/-}$ mutation.

A CS or US representation must incorporate a large amount of information. Not only must the underlying neural networks represent both the sensory and emotional features of a given stimulus, but they must also encode complex relational information. For example, the temporal relationship of the stimulus with other CS and US must also be encoded. Furthermore, the stimulus representation is presumably dynamic in nature, with new instances or experience leading to the integration of relevant information into these networks (O'Reilly and Rudy, 2001). The formation of episodic memories is then thought to involve the rapid and automatic fusion of dynamic, information-laden representations into a unified memory (Martin et al., 2000; Morris et al., 2003). The use of immediate shock approaches make it possible to examine the molecular and cellular processes underlying the formation of CS and US representations in isolation. Our data show that the building of CS (and US) representations requires NMDA receptor activation, autophosphorylation of CaMKII at T286, CREB-dependent transcription and protein synthesis. Previous molecular and cellular studies of memory have focused on associative processes in fear conditioning. The present data suggest similar approaches can be used to understand the molecular and cellular bases of stimulus representations.

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REFERENCES

Abel T, Nguyen PV, Barad M, Deuel TAS, Kandel ER, Bourtchouladze R. 1997. Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. Cell 88:615–626.

Agnihotri NT, Hawkins RD, Kandel ER, Kentros C. 2001. Protein synthesis inhibition selectively abolishes the long-term stability of new hippocampal place cell maps. Soc Neurosci Abs 27:836.

Alberini CM. 1999. Genes to remember. J Exp Biol 202:2887–2891.

Anagnostaras SG, Gale GD, Fanselow MS. 2001. Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 11:8–17.

Anagnostaras SG, Josselyn SA, Frankland PW, Silva AJ. 2000. Computer-assisted behavioral assessment of Pavlovian fear conditioning in mice. Learn Mem 7:58–72.

Anagnostaras SG, Maren S, Fanselow MS. 1999. Temporally graded retrograde amnesia of contextual fear after hippocampal damage in rats: within-subjects examination. J Neurosci 19:1106–1114.

Barrientos RM, O'Reilly RC, Rudy JW. 2002. Memory for context is impaired by injecting anisomycin into dorsal hippocampus following context exploration. Behav Brain Res 134:299–306.

Berman DE, Dudai Y. 2001. Memory extinction, learning anew, and learning the new: dissociations in the molecular machinery of learning in cortex. Science 291:2417–2419.

Bevins RA, Ayres JJB. 1995. One-trial context fear conditioning as a function of the interstimulus interval. Anim Learn Behav 23:400–410.

Blendy JA, Kaestner KH, Schmid W, Gass P, Schutz G. 1996. Targeting of the CREB gene leads to up-regulation of a novel CREB mRNA isoform. EMBO J 15:1098–1106.

Bourtchouladze R, Abel T, Berman N, Gordon R, Lapidus K, Kandel ER. 1998. Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. Learn Mem 5:365–374.

Bourtchuladze R, Frenguelli B, Blendy J, Cioffi D, Schutz G, Silva AJ. 1994. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79:59–68.

Cahill L, McGaugh JL. 1996. Modulation of memory storage. Curr Opin Neurobiol 6:237–242.

Cho YH, Giese KP, Tanila H, Silva AJ, Eichenbaum H. 1998. Abnormal hippocampal spatial representations in alphaCaMKIIT286A and CREBalphaDELTA^{-} mice. Science 279:867–869.

Davis HP, Squire LR. 1984. Protein synthesis and memory: a review. Psychol Bull 96:518–559.

Falls WA, Kogan JH, Silva AJ, Willott JF, Carlson S, Turner JG. 2000. Fear-potentiated startle, but not prepulse inhibition of startle, is impaired in CREBalphadelta$^{-/-}$ mutant mice. Behav Neurosci 114: 998–1004.

Fanselow MS. 1980. Conditional and unconditional components of post-shock freezing. Integr Physiol Behav Sci 15:177–182.

Fanselow MS. 1986. Associative vs topographical accounts of the immediate shock freezing deficit in rats: implications for the response selection rules governing species-specific defensive reactions. Learn Motiv 17:16–39.

Fanselow MS. 1990. Factors governing one-trial contextual conditioning. Anim Learn Behav 18:264–270.

Fanselow MS. 2000. Contextual fear, gestalt memories, and the hippocampus. Behav Brain Res 110:73–81.

Fanselow MS, Decola JP, Young SL. 1993. Mechanisms responsible for reduced contextual conditioning with massed unsignaled unconditional stimuli. J Exp Psychol Anim Behav Process 19:121–137.

Fanselow MS, Gale GD. 2003. The amygdala, fear, and memory. Ann NY Acad Sci 985:125–134.

Frankland PW, Cestari V, Filipkowski R, McDonald RJ, Silva AJ. 1998. The dorsal hippocampus is essential for context discrimination but not for contextual conditioning. Behav Neurosci 112:863–874.

Gallistel CR, Gibbon J. 2000. Time, rate, and conditioning. Psychol Rev 107:289–344.

Gass P, Wolfer DP, Balschun D, Rudolph D, Frey U, Lipp H-P, Schuetz G. 1998. Deficits in memory tasks of mice with CREB mutations depend on gene dosage. Learn Mem 5:274–288.

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ. 1998. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. Science 279:870–873.

Guthrie ER. 1935. The psychology of learning. New York: Harper. 258 p.

Kandel ER, Pittenger C. 1999. The past, the future and the biology of memory storage. Philos Trans R Soc Lond B Biol Sci 354:2027–2052.

Kida S, Josselyn SA, Pena de Ortiz S, Kogan JH, Chevere I, Masushige S, Silva AJ. 2002. CREB required for the stability of new and reactivated fear memories. Nature Neurosci 5:348–355.

Kiernan MJ, Westbrook RF. 1993. Effects of exposure to a to-be-shocked environment upon the rat's freezing response: evidence for facilitation, latent inhibition, and perceptual learning. Q J Exp Psychol B 46:271–288.

Kiernan MJ, Westbrook RF, Cranney J. 1995. Immediate shock, passive avoidance and potentiated startle: implications for the unconditioned response to shock. Anim Learn Behav 23:22–30.

Kiyama Y, Manabe T, Sakimura K, Kawakami F, Mori H, Mishina M. 1998. Increased thresholds for long-term potentiation and contextual learning in mice lacking the NMDA-type glutamate receptor epsilon1 subunit. J Neurosci 18:6704–6712.

Kobayashi K, Yasoshima Y. 2001. The central noradrenaline system and memory consolidation. Neuroscientist 7:371–376.

Kogan JH, Frankland PW, Blendy JA, Coblentz J, Marowitz Z, Schuetz G, Silva AJ. 1997. Spaced training induces normal long-term memory in CREB mutant mice. Curr Biol 7:1–11.

Konorski J. 1967. Integrative activity of the brain; an interdisciplinary approach. Chicago: University of Chicago Press. p xii.

Lattal KM, Abel T. 2001a. Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. J Neurosci 21:5773–5780.

Lattal KM, Abel T. 2001b. An immediate-shock freezing deficit with discrete cues: a possible role for unconditioned stimulus processing mechanisms. J Exp Psychol Anim Behav Process 27:394–406.

LeDoux JE. 2000. Emotion circuits in the brain. Annu Rev Neurosci 23:155–184.

Lee HJ, Berger SY, Stiedl O, Spiess J, Kim JJ. 2001. Post-training injections of catecholaminergic drugs do not modulate fear conditioning in rats and mice. Neurosci Lett 303:123–126.

Liang KC, Juler RG, McGaugh JL. 1986. Modulating effects of posttraining epinephrine on memory: involvement of the amygdala noradrenergic system. Brain Res 368:125–133.

Lisman J, Schulman H, Cline H. 2002. The molecular basis of CaMKII function in synaptic and behavioural memory. Nat Rev Neurosci 3:175–190.

Maren S. 2001. Neurobiology of pavlovian fear conditioning. Annu Rev Neurosci 24:897–931.

Martin SJ, Grimwood PD, Morris RGM. 2000. Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23: 649–711.

Martinez JL, Jr., Jensen RA, McGaugh JL. 1981. Attenuation of experimentally-induced amnesia. Prog Neurobiol 16:155–186.

McGaugh JL. 2002. Memory consolidation and the amygdala: a systems perspective. Trends Neurosci 25:456–461.

McGaugh JL, Roozendaal B. 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr Opin Neurobiol 12:205–210.

Meiri N, Rosenblum K. 1998. Lateral ventricle injection of the protein synthesis inhibitor anisomycin impairs long-term memory in a spatial memory task. Brain Res 789:48–55.

Milanovic S, Radulovic J, Laban O, Stiedl O, Henn F, Spiess J. 1998. Production of the Fos protein after contextual fear conditioning of C57BL/6N mice. Brain Res 784:37–47.

Morris RG, Moser EI, Riedel G, Martin SJ, Sandin J, Day M, O'Carroll C. 2003. Elements of a neurobiological theory of the hippocampus: the role of activity-dependent synaptic plasticity in memory. Philos Trans R Soc Lond B Biol Sci 358:773–786.

Ohno M, Frankland PW, Chen AP, Costa RM, Silva AJ. 2001. Inducible, pharmacogenetic approaches to the study of learning and memory. Nat Neurosci 4:1238–1243.

O'Reilly RC, Rudy JW. 2001. Conjunctive representations in learning and memory: principles of cortical and hippocampal function. Psychol Rev 108:311–345.

Pavlov IP. 1927. Conditioned reflexes; an investigation of the physiological activity of the cerebral cortex. Translated and edited by G.V. Anrep. London: Oxford University Press.

Paylor R, Tracy R, Wehner J, Rudy JW. 1994. DBA/2 and C57BL/6 mice differ in contextual fear but not auditory fear conditioning. Behav Neurosci 108:810–817.

Pittenger C, Huang YY, Paletzki RF, Bourtchouladze R, Scanlin H, Vronskaya S, Kandel ER. 2002. Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. Neuron 34:447–462.

Rosenblum K, Meiri N, Dudai Y. 1993. Taste memory: the role of protein synthesis in gustatory cortex. Behav Neural Biol 59:49–56.

Rudy JW, Barrientos RM, O'Reilly RC. 2002. Hippocampal formation supports conditioning to memory of a context. Behav Neurosci 116: 530–538.

Rudy JW, O'Reilly RC. 1999. Contextual fear conditioning, conjunctive representations, pattern completion, and the hippocampus. Behav Neurosci 113:867–880.

Rudy JW, O'Reilly RC. 2001. Conjunctive representations, the hippocampus, and contextual fear conditioning. Cognit Affect Behav Neurosci 1:66–82.

Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE. 1999. Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 6:97–110.

Schauz C, Koch M. 2000. Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. Learn Mem 7:393–399.

Shaywitz AJ, Greenberg ME. 1999. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. Annu Rev Biochem 68:821–861.

Silva AJ, Kogan JH, Frankland PW, Kida S. 1998. CREB and memory. Annu Rev Neurosci 21:127–148.

Stanciu M, Radulovic J, Spiess J. 2001. Phosphorylated cAMP response element binding protein in the mouse brain after fear conditioning: relationship to Fos production. Mol Brain Res 94:15–24.

Stiedl O, Palve M, Radulovic J, Birkenfeld K, Spiess J. 1999. Differential impairment of auditory and contextual fear conditioning by protein synthesis inhibition in C57BL/6N mice. Behav Neurosci 113:496–506.

Wiltgen BJ, Sanders MJ, Behne NS, Fanselow MS. 2001. Sex differences, context preexposure, and the immediate shock deficit in Pavlovian context conditioning with mice. Behav Neurosci 115:26–32.

Yin JCP, Tully T. 1996. CREB and the formation of long-term memory. Curr Opin Neurobiol 6:264–268.