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Long-Term Memory Underlying Hippocampus-Dependent Social Recognition in Mice

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ABSTRACT: The ability to learn and remember individuals is critical for the stability of social groups. Social recognition reflects the ability of mice to identify and remember conspecifics. Social recognition is assessed as a decrease in spontaneous investigation behaviors observed in a mouse reexposed to a familiar conspecific. Our results demonstrate that group-housed mice show social memory for a familiar juvenile when tested immediately, 30 min, 24 h, 3 days, and 7 days after a single 2-min-long interaction. Interestingly, chronic social isolation disrupts long-term, but not 30-min, social memory. Even a 24-h period of isolation disrupts long-term social memory, a result that may explain why previous investigators only observed short-term social memory in individually housed rodents. Although it has no obvious configural, relational, or spatial characteristics, here we show that social memory shares characteristics of other hippocampus-dependent memories. Ibotenic acid lesions of the hippocampus disrupt social recognition at 30 min, but not immediately after training. Furthermore, long-term, but not short-term social memory is dependent on protein synthesis and cyclic AMP responsive element binding protein (CREB) function. These results outline behavioral, systems, and molecular determinants of social recognition in mice, and they suggest that it is a powerful paradigm to investigate hippocampal learning and memory. *Hippocampus 2000;10:47–56.*

KEY WORDS: CREB; mutant; isolation; protein synthesis; social learning

INTRODUCTION

Social recognition is critical for the structure and stability of the networks of relationships that define societies. For animals such as mice, recognition of conspecifics may be important for maintaining social hierarchy and for mate choice (Berry and Bronson, 1992; Jiming et al., 1994). In the laboratory, social recognition can be studied in rodents by placing a juvenile in a cage with an adult. The adult will spontaneously investigate the younger animal, and the duration of this investigation reflects the familiarity between the two animals (Thor and Holloway, 1982; Bluthe et al., 1993; Dluzen and Kreutzberg, 1993). A repeated exposure to the same juvenile results in a decrease in investigation time. Thus, the difference in investigation times between the first and second exposure can be used as an index of social memory. Previous studies have reported that social memory does not last longer than 1 h in individually housed mice and rats (Thor and Holloway, 1982; Sekiguchi et al., 1991b; Bluthe et al., 1993).

Olfaction plays a key role in social recognition in rodents, since either chemically induced anosmia or removal of the vomeronasal organ blocks individual recognition (Matochik, 1988; Bluthe and Dantzer, 1993). Additionally, soiled bedding or urine samples from a juvenile can be used as cues in the recognition process (Sawyer et al., 1984; Popik et al., 1991).

Social memory is strongly modulated by sexually dimorphic vasopressinergic neurons that originate in the bed nucleus of the stria terminalis and the medial amygdala and that project to forebrain structures such as the hippocampus, the lateral septum, and the olfactory bulb (Caffe et al., 1987). Systemic, intracerebroventricular, septal, or olfactory bulb injections of arginine-vasopressin (AVP) can substantially lengthen the duration of social memory in male or female rats and mice (Le Moal et al., 1987; Dantzer et al., 1988; Bluthe and Dantzer, 1990; Sekiguchi et al., 1991a; Bluthe et al., 1993; Engelmann and Landgraf, 1994; Dluzen et al., 1998; Everts and Koolhaas, 1999). In contrast, anti-AVP serum injected either centrally or into the dorsal or lateral septum can block recognition only in male animals (Dantzer et al., 1988; Bluthe et al., 1990, 1993; Bluthe and Dantzer, 1993; Engelmann and Landgraf, 1994; van Wimersma Greidanus and Maigret, 1996; Everts and Koolhaas, 1997, 1999). Furthermore, septal lesions, but not lesions of the basolateral amygdala, impair social recognition in rats (Bluthe et al., 1993; Maaswinkel et al., 1996).

The hippocampus is known to have a role in social recognition in humans (Corkin, 1984). Two findings suggest that this structure is also involved in social memory in rodents. First, anti-AVP serum injected into either the dorsal or ventral hippocampus blocks social recognition in rats (van Wimersma Greidanus and Maigret, 1996). Second, transections of the fimbria also

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disrupt this behavior in rats (Maaswinkel et al., 1996). Additionally, the hippocampus is known to be involved in other nonspatial, olfactory-dependent tasks such as the social transmission of food preferences, odor-paired associates, and odor-guided nonmatching to sample (Winocur, 1990; Bunsey and Eichenbaum, 1995, 1996; Wood et al., 1999).

In contrast to other hippocampus-dependent forms of memory, previous studies suggested that juvenile recognition in mice is short-lived. Here, we show that mice have the ability to form long-term social memories. The object of the present study was to characterize the properties of this form of memory. A considerable amount of evidence from a variety of studies suggests that de novo protein synthesis is required for the formation of long-term memory (Flexner et al., 1963; Davis and Squire, 1984; Matthies, 1989). Furthermore, the formation of long-term memory in several different hippocampus-dependent learning tasks has been found to involve cyclic AMP responsive element binding protein (CREB)-mediated gene transcription (Bourtchuladze et al., 1994; Kogan et al., 1997). Therefore, we examined protein synthesis inhibitor-treated mice and $CREB^{\alpha\Delta-}$ mutant mice to determine if long-term juvenile social recognition shares some of the same properties as other forms of memory.

MATERIALS AND METHODS

Mice

Adult mice from a C57BL/6J genetic background were purchased from Jackson Laboratory (Bar Harbor, ME). Male juvenile mice of the same background were obtained from our own breeding pairs. The $CREB^{\alpha\Delta-}$ mutant mice (mice lacking the α and Δ isoforms of CREB) used for these studies were originally generated in the laboratory of Gunther Schutz (Hummler et al., 1994). As we described in our previous publications, the viability of the $CREB^{\alpha\Delta-}$ mutation in the C57B1/6 background is low (Kogan et al., 1997). Therefore, we used F2 homozygotes derived from a cross between $CREB^{\alpha\Delta-}$ heterozygotes in the C57B1/6 background and WT 129svj mice. The same background was also used for the wild-type (WT) mice that were used in the hippocampal-lesion experiments. The mice were genotyped with polymerase chain reaction (PCR) analysis of tail DNA samples.

Naive mice were used in all experiments. Except for the social isolation experiments, mice were group-housed (2–5 animals/cage) with same-sex littermates. Experiments were done with adult male mice 4–8 months old and male juveniles less than 5 weeks of age. Juveniles were used as the stimulus animals to minimize aggression (Terranova et al., 1998). Within most experiments, the juveniles were from the same litter and a particular juvenile was used for a maximum of four interactions with different adults. The mice were kept on a 12:12-h light-dark cycle, and the experiments were always conducted during the light phase of the cycle. With the exception of testing times, the mice had ad libitum access to food and water. Animals were maintained in accordance with the applicable portions of the Animal Welfare Act and the Department of Health and Human Services *Guide to the Care and Use of Laboratory Animals.*

Social Recognition

For social recognition experiments, we used a modification of the procedure described by Thor and Holloway (1982). Adult male mice were placed into individual cages immediately prior to the experimental sessions in an observation room under dim light and allowed to habituate to the new environment for 15 min. The cages used were identical to those in which the animals were normally housed (plastic, 27 cm long $\times$ 16 cm wide $\times$ 12 cm high).

A male juvenile mouse was placed into a cage with an adult for an initial interaction trial of 2 min. The interval between the initial and test trials depended on the particular experiment. Following the intertrial delay, either the same or a novel juvenile was placed back into the adult's cage for a 2-min test trial. The social investigation of the juvenile by the adult mouse was observed continuously by a trained observer who timed the duration of investigation behavior with a hand-held stopwatch. Behaviors that were scored as social investigation were as previously described and included the following: direct contact with the juvenile while inspecting any part of the body surface (including grooming, licking, and pawing), sniffing of the mouth, ears, tail, ano-genital area, and close following (within 1 cm) of the juvenile (Thor and Holloway, 1982). Adults were required to investigate the juveniles for a minimum of 24 s during the initial trial (i.e., 20% of trial time), or they were retested once with another juvenile. Trials with initial investigation times not meeting these criteria were excluded from the analysis. Any aggressive encounter between animals was immediate cause for terminating the experiment and excluding data from an adult from the analysis.

Hippocampal Lesions and Histology

The mice used as surgical subjects were wild-type animals, 2–6 months old. Bilateral lesions of the hippocampus were made by multiple injections of ibotenic acid (Sigma, St. Louis, MO). Ibotenic acid was dissolved in phosphate-buffered saline (pH 7.4) at a concentration of 5 mg/ml. Mice were anesthetized with chloral hydrate (200 mg/kg, ip). In addition, mice were pretreated with diazepam (5 mg/kg, ip) to prevent hippocampal seizure activity induced by ibotenic acid injections. The animals were placed in a stereotaxic instrument, and craniotomy was performed to expose the brain directly above the injection coordinates. A series of injections was made using a 1-μl Hamilton syringe (VWR Science) connected to a 32-gauge cannula via polyethylene tubing. A total of 20 injections was made according to the stereotaxic coordinates described in Table 1 (Franklin and Paxinos, 1997). For sham lesions, the procedures followed were identical except that no microinjections were made. Mice were allowed to recover for at least 3 weeks prior to training.

At the completion of social recognition testing, mice were overdosed with chloral hydrate and perfused transcardially with 10% formaldehyde. Their brains were removed and postfixed in

TABLE 1.

Stereotaxic Coordinates With Respect to Bergma Used for Microinjections of Ibotenic Acid Into Bilateral Hippocampi in Mice

Anteroposterior (mm)	Medial-lateral (mm)	Dorsal-ventral (mm)	Volume (μl)
−1.0	0.8	2.0	0.06
−1.6	1.0	1.7	0.07
−2.0	1.0	1.8	0.06
−2.0	2.0	1.8	0.07
−2.5	1.6	1.8	0.07
−2.5	2.4	2.0	0.07
−3.0	2.5	2.0	0.08
−3.0	3.0	3.0	0.07
−3.5	3.0	2.6	0.08
−3.5	3.0	1.0	0.06

10% formaldehyde solution, followed by a 30% sucrose solution. Coronal sections (50 μm) were then cut though the rostral-caudal extent of the hippocampus. These sections were stained for thionin and mounted on slides for subsequent histological verification of the lesions under light microscopy.

Protein Synthesis Inhibition

To test the effect of protein synthesis inhibition on social recognition, we used anisomycin (Sigma Chemical Co.). Anisomycin was dissolved in saline by adding 1 N HCl. The pH was adjusted to 7.4 by adding NaOH. Mice were injected 30 min prior to the initial interaction period either with the anisomycin solution (150 mg/kg of body weight, subcutaneously) or with an equivalent volume of saline. This dosage of anisomycin was previously found to be effective at inhibiting approximately 90% of cerebral protein synthesis and impairing fear-conditioning memory (Davis and Squire, 1984; Bourtchouladze et al., 1998).

Data Analysis

The experiments were carried out blind to the treatments tested and genotype of the mice. To evaluate differences in social recognition between groups of mice, we calculated "recognition ratios," which equaled the value of the investigation duration for the test trial divided by the sum of the initial and test trial investigation durations. A recognition ratio of 0.50 indicates that there was no difference between the initial and test investigations and therefore there was an absence of recognition. For analysis of data we used paired *t*-test for two samples for within-group comparisons, or single-factor analysis of variance (ANOVA) for between-group comparisons. All values are expressed as mean ± SEM.

RESULTS

Long-Term Social Memory in Mice

Previous studies found that for individually housed mice and rats, social recognition memory dissipates between 1–2 h (Thor and Holloway, 1982; Sekiguchi et al., 1991b; Bluthe et al., 1993). In contrast, the mice that we tested were group-housed. Separate groups of mice were tested 1, 3, or 7 days after the initial interaction. In the interim between the initial and the test trials, the animals were returned to their home cages. At all three time points tested there were significant reductions in the duration of

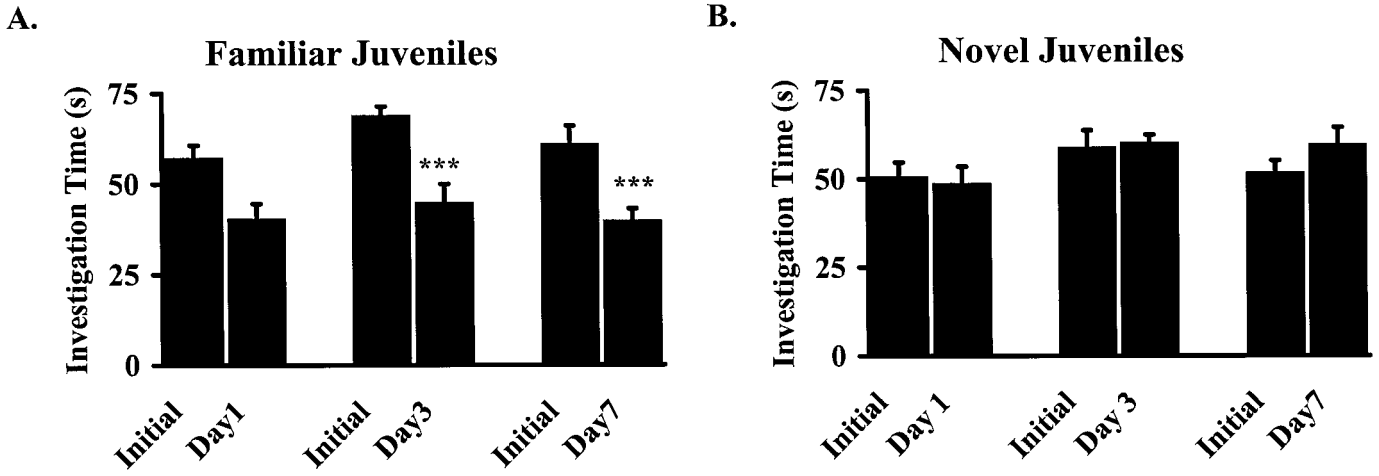


FIGURE 1. Long-term social memory in adult male mice. Social recognition was tested in two successive 2-min interaction trials with intertrial intervals of 1, 3, or 7 days. Mean investigation duration is shown for initial and test trials. A: Familiar juveniles were exposed to adult mice at test trials. There was significant reduction in investigation duration, indicating long-term social memory at each test interval (n = 12, 10, and 10 for days 1, 3, and 7, respectively; $^{***}P < 0.001$, compared to Initial). B: Novel juveniles were exposed to adult mice at test trial (n = 8, 9, and 8 for days 1, 3, and 7, respectively).

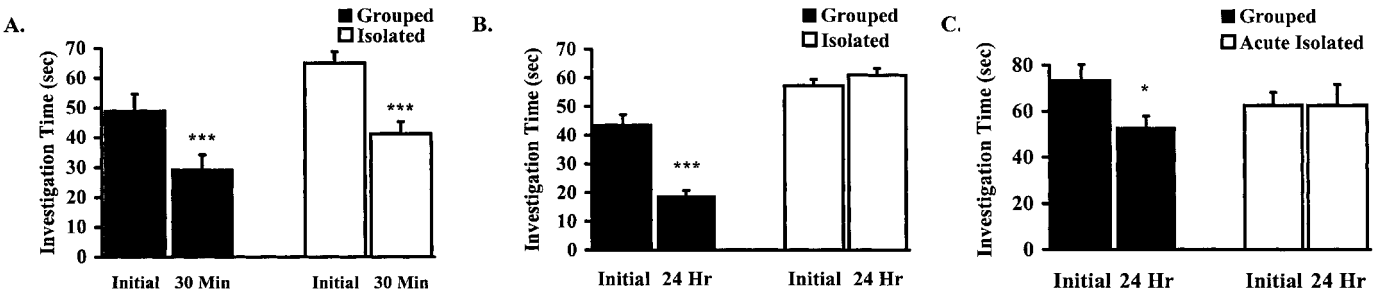


FIGURE 2. Long-term social memory is impaired in isolated mice. A: Familiar juveniles were exposed to the grouped or chronically isolated mice 30 min after initial interaction. There was significant reduction in investigation duration, indicating intact social memory in both groups (Grouped, n = 10; Isolated, n = 16; $^{***}P < 0.01$, compared to Initial). B: Familiar juveniles were exposed to grouped or chronically isolated mice 24 h after initial interaction. There was significant reduction in investigation duration only for grouped mice (Grouped, n = 11; Isolated, n = 15; $^{***}P < 0.001$, compared to Initial). C: Familiar juveniles were exposed to grouped or acutely isolated mice 24 h after the initial interaction. There was significant reduction in investigation duration, indicating intact social memory only for grouped mice (Grouped, n = 7; Isolated, n = 6; $^{*}P < 0.05$, compared to Initial).

investigation times. This indicates that the adults were able to recognize the familiar juveniles independent of the intertest intervals ($t(11) = 5.00$, $P < 0.001$, $t(9) = 5.23$, $P < 0.001$, and $t(9) = 4.55$, $P < 0.001$ for days 1, 3, and 7, respectively) (Fig. 1A). Our analysis showed that the recognition ratios calculated for each of the time points that were tested with familiar juveniles (0.41, 0.39, and 0.40 for 1, 3, and 7 days, respectively) were indistinguishable ($F(2, 29) = 0.78$, $P = 0.47$), consistent with the observation that the degree of recognition did not vary across test periods (Table 2).

As a control, we also retrained and then tested the adult mice with a novel juvenile at the same time points mentioned above. The results show that for all intervals tested, there was no significant reduction in the investigation duration with novel juveniles ($t(7) = 0.37$, $P = 0.36$, $t(8) = -0.30$, $P = 0.37$, $t(7) = 0.37$, $P = 0.36$ for days 1, 3, and 7, respectively) (Fig. 1B). This control shows that reduction of investigation time is specific to familiar juveniles. Overall, these results demonstrate that group-housed mice are able to form a robust long-term memory for social recognition.

Social Isolation

Since previous studies with individually-housed animals reported only short-term memory for social recognition (not greater than 1 h), we tested whether social isolation would disrupt long-term memory in our mice. We first performed a chronic isolation experiment in which adult mice were housed in individual cages for a period of 3 weeks prior to the start of the experiment. This experiment was conducted using the same protocol described above, except that we used 30-min and 24-h time points for testing. Both group-housed and socially isolated mice demonstrated intact short-term memory for social recognition when tested with familiar juveniles 30 min after the initial interaction ($t(10) = 7.04$, $P < 0.001$, and $t(15) = 7.71$, $P < 0.001$ for grouped and isolated mice, respectively) (Fig. 2A). In contrast, chronically isolated mice showed no social recognition memory when tested 24 h after the initial interaction ($t(14) = -1.375$, $P = 0.095$). Group-housed mice, however, revealed a significant reduction in investigation time of familiar juveniles ($t(10) = 7.04$, $P < 0.001$), demonstrating that they had intact long-term memory (Fig. 2B). The recognition ratios calculated for the two housing conditions at 24 h with familiar juveniles (0.30 and 0.52 for grouped and isolated, respectively) were significantly different ($F(1, 24) = 72.14$, $P < 0.001$), indicating that the degree of recognition memory was affected by chronic isolation (Table 2).

Next, we tested the effects of acute isolation, in which adult mice were housed in individual cages for a period of 24 h prior to the start of the experiment. This experiment was conducted using the same protocol described above. The mice were only tested 24 h after the initial interaction. As with the chronically isolated mice, acutely isolated mice showed no social recognition memory when tested 24 h after the initial interaction ($t(5) = 0.00$, $P = 0.50$). As before, group-housed mice showed a significant reduction in investigation time of familiar juveniles ($t(7) = 3.03$, $P < 0.05$), indicating that they had intact long-term recognition memory (Fig. 2C). The recognition ratios calculated for the two housing conditions at 24 h (0.40 and 0.50 for grouped and isolated, respectively) were significantly different ($F(1, 11) = 4.9$, $P < 0.05$), indicating that the degree of recognition memory was also affected by acute social isolation (Table 2).

Hippocampal Lesions

We next investigated the impact of hippocampal lesions on social recognition memory in mice. The extent of cell loss was examined under light microscopy across coronal sections covering the full rostral-caudal extent of the hippocampus. Lesions were characterized by cell loss, leading to an absence of labeling of cell layers in dorsal and ventral hippocampus. In cases where cells layers were labeled, the width of the cell layer was substantially reduced compared to sham-operated controls. In addition, the ventricular space of the dorsal third ventricle and lateral ventricle were in most cases enlarged, indicating a reduction in overall tissue volume of the neighboring hippocampus in lesioned mice. In all mice, cell loss was evident in both the dorsal and ventral hippocampus. In most cases (7/8), there was some sparing of tissue

TABLE 2.

Recognition Ratios for Social Memory†

Experiment	Juvenile	Trial interval	Recognition ratio (n)
Long-term memory			
Day 1	Familiar	24 h	0.41 ± 0.02* (12)
	Novel	24 h	0.48 ± 0.03 (8)
Day 3	Familiar	3 days	0.39 ± 0.02*** (10)
	Novel	3 days	0.51 ± 0.02 (9)
Day 7	Familiar	7 days	0.40 ± 0.02** (10)
	Novel	7 days	0.53 ± 0.03 (10)
Social isolation			
Chronic isolation	Familiar	30 min	0.37 ± 0.02 (16)
	Familiar	24 h	0.52 ± 0.01*** (15)
Group-housed	Familiar	30 min	0.35 ± 0.02 (10)
	Familiar	24 h	0.30 ± 0.03 (11)
Acute isolation	Familiar	24 h	0.50 ± 0.03* (6)
Group-housed	Familiar	24 h	0.40 ± 0.03 (7)
Lesions			
Hippocampus lesions	Familiar	30 min	0.48 ± 0.03 (8)
	Novel	30 min	0.45 ± 0.04 (8)
	Familiar	Immediate	0.25 ± 0.05** (7)
	Novel	Immediate	0.46 ± 0.04 (7)
Sham-operated	Familiar	30 min	0.40 ± 0.03* (10)
	Novel	30 min	0.49 ± 0.03 (6)
	Familiar	Immediate	0.35 ± 0.04* (9)
	Novel	Immediate	0.52 ± 0.06 (7)
Protein synthesis			
Anisomycin-treated	Familiar	30 min	0.37 ± 0.05 (9)
	Familiar	24 h	0.53 ± 0.03* (11)
Saline-treated	Familiar	30 min	0.37 ± 0.03 (9)
	Familiar	24 h	0.45 ± 0.02 (10)
CREB			
$CREB^{\alpha\Delta-}$ mutant mice	Familiar	30 min	0.41 ± 0.04* (9)
	Novel	30 min	0.51 ± 0.02 (10)
	Familiar	24 h	0.51 ± 0.02** (7)
Wild-type mice	Familiar	30 min	0.41 ± 0.04* (8)
	Novel	30 min	0.48 ± 0.02 (9)
	Familiar	24 h	0.39 ± 0.03 (7)

†A recognition ratio equal to 0.50 indicates that there was no difference between initial and test investigations, indicating an absence of recognition. Recognition ratios are compared between groups tested with familiar and novel juveniles at the same test interval. In cases of isolated, protein synthesis inhibitor-treated, and $CREB^{\alpha\delta-}$ mutant mice tested at 24 h, comparisons are between groups tested with familiar juveniles at the same test interval.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

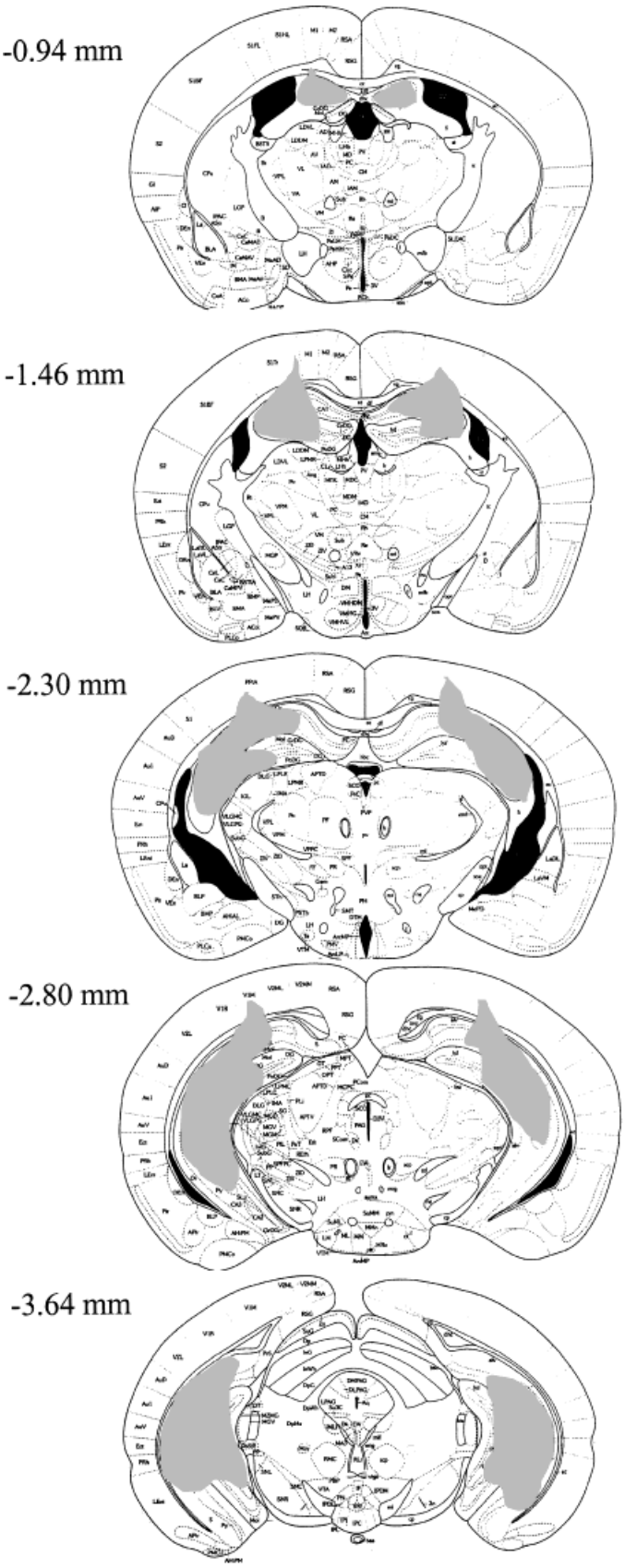
in the most medial portion of the dorsal hippocampus (including the dentate gyrus). In some cases (3/8), there was some sparing of tissue in the most ventro-caudal part of the ventral hippocampus. In addition to hippocampal cell loss, there was evidence of limited damage to the neocortex immediately above the hippocampus, most likely associated with the insertion of injection cannulae through this tissue. In three cases there was some cell loss evident in the subiculum. There was little or no damage in thalamic areas, immediately ventral to the dorsal hippocampus (Fig. 3).

The social recognition experiments were conducted using the same protocol as used above, except that we examined the recognition memory of the mice with one group of juveniles at 30 min and with another immediately posttraining. Hippocampal-lesioned mice were impaired at social recognition when tested at 30 min ($t(7) = 0.68$, $P = 0.26$), while sham-operated mice had intact 30-min memory as indicated by a significant reduction in the duration of the investigation during the test trial ($t(9) = 2.82$, $P < 0.01$) (Fig. 4A). Control experiments conducted with novel juveniles during the test trials indicated that the reduction in investigation observed in the sham mice was specific to the familiar juveniles ($t(7) = 0.89$, $P = 0.20$, and $t(5) = 0.83$, $P = 0.22$ for lesioned and sham mice, respectively) (Table 2).

When mice were tested for social recognition memory immediately following training (within 30 s of the initial interaction), both hippocampal-lesioned and sham-operated mice had intact memory. They both showed significant reductions in investigation duration with the familiar juvenile ($t(6) = 7.37$, $P < 0.001$, and $t(8) = 3.22$, $P < 0.01$ for lesioned and sham mice, respectively) (Fig. 4B). These results show that like other hippocampus-dependent forms of memory, immediate memory for social recognition is not sensitive to hippocampal lesions (Bunsey and Eichenbaum, 1995). Control experiments with novel juveniles presented during test trials immediately after training indicated the specificity of memory for familiar juveniles ($t(6) = 0.97$, $P = 0.19$, and $t(6) = -0.21$, $P = 0.42$ for lesioned and sham mice, respectively) (Table 2).

Protein Synthesis Inhibition

Our observations of long-term social recognition memory in mice prompted us to investigate further the underlying mechanisms of this form of memory. A well-established property of long-term memory is its dependence on de novo protein synthesis (Davis and Squire, 1984). Thus, we tested whether 24-h memory for social recognition is dependent on protein synthesis. We injected mice with either the protein synthesis inhibitor, anisomycin, or saline, and trained the animals using the protocol described above. When examined at 30 min posttraining, both anisomycin- and saline-treated animals showed a significant reduction in the investigation duration of a familiar juvenile, indicating intact short-term memory ($t(8) = 2.37$, $P < 0.05$, and $t(8) = 4.09$, $P < 0.01$ for anisomycin- and saline-treated, respectively) (Fig. 5A). In contrast, when tested at 24 h posttraining, the anisomycin-treated mice showed no reduction in investigation duration, while the saline-treated mice showed a significant reduction, indicating intact long-term memory ($t(10) = -1.24$, $P = 0.12$, and



$t(9) = 2.76$, $P < 0.05$. for anisomycin- and saline-treated, respectively) (Fig. 5B). The recognition ratios calculated at 24 h (0.45 and 0.53 for saline- and anisomycin-treated, respectively) were significantly different ($F(1, 19) = 5.85$, $P < 0.05$), indicating that the degree of long-term recognition memory was impaired by protein synthesis inhibition (Table 2). These results demonstrate that long-term, but not short-term social recognition memory in mice is dependent on protein synthesis.

CREB$^{\alpha\Delta-}$ Mice

Manipulations of a cyclic AMP- and calcium-dependent transcription factor (CREB) can alter the expression of long-term memory (Yin and Tully, 1996; Abel and Kandel, 1998; Silva et al., 1998). We previously showed that mice deficient in the alpha and delta isoforms of the CREB transcription factor (CREB$^{\alpha\Delta-}$ mice) are impaired in long-term, but not short-term memory (Bourtchuladze et al., 1994; Kogan et al., 1997). To test whether the CREB$^{\alpha\Delta-}$ mutation disrupts long-term social recognition memory, we trained and tested CREB$^{\alpha\Delta-}$ mice using the protocol described above.

Both wild-type (WT) and CREB$^{\alpha\Delta-}$ mice showed intact short-term memory for a familiar juvenile when tested at 30 min posttraining ($t(7) = 2.12$, $P < 0.05$, and $t(8) = 2.68$, $P < 0.05$ for WT and CREB$^{\alpha\Delta-}$ mice, respectively (Fig. 6A). Control experiments with novel juveniles indicated the specificity of the 30-min memory ($t(8) = 1.46$, $P = 0.09$, and $t(9) = -0.68$, $P = 0.26$ for WT and CREB$^{\alpha\Delta-}$ mice, respectively) (Table 2). In contrast, while WT mice demonstrated intact long-term memory, CREB$^{\alpha\Delta-}$ mice showed no recognition memory when tested 24 h after training ($t(6) = 3.44$, $P < 0.01$, and $t(6) = -1.13$, $P = 0.15$ for WT and CREB$^{\alpha\Delta-}$ mice, respectively) (Fig. 6B). The recognition ratios calculated at 24 h (0.39 and 0.51 for WT and CREB$^{\alpha\Delta-}$ mice, respectively) were significant different ($F(1, 12) = 10.7$, $P < 0.01$), indicating that the degree of long-term recognition memory was impaired by the CREB$^{\alpha\Delta-}$ mutation (Table 2). Altogether, these results show that the CREB$^{\alpha\Delta-}$ mutation disrupted long-, but not short-term memory for social recognition.

DISCUSSION

The results of our study demonstrate that mice can form robust long-term social memories for juvenile conspecifics. We conducted several experiments to characterize some of the properties of this ethologically relevant form of memory.

FIGURE 3. Reconstruction of a representative hippocampal lesion, shown on a series of coronal sections from Franklin and Paxinos (1997). The anterior-posterior coordinate (with respect to bregma) is shown on the left for each session. Cell loss or tissue damage was evident in the gray areas.

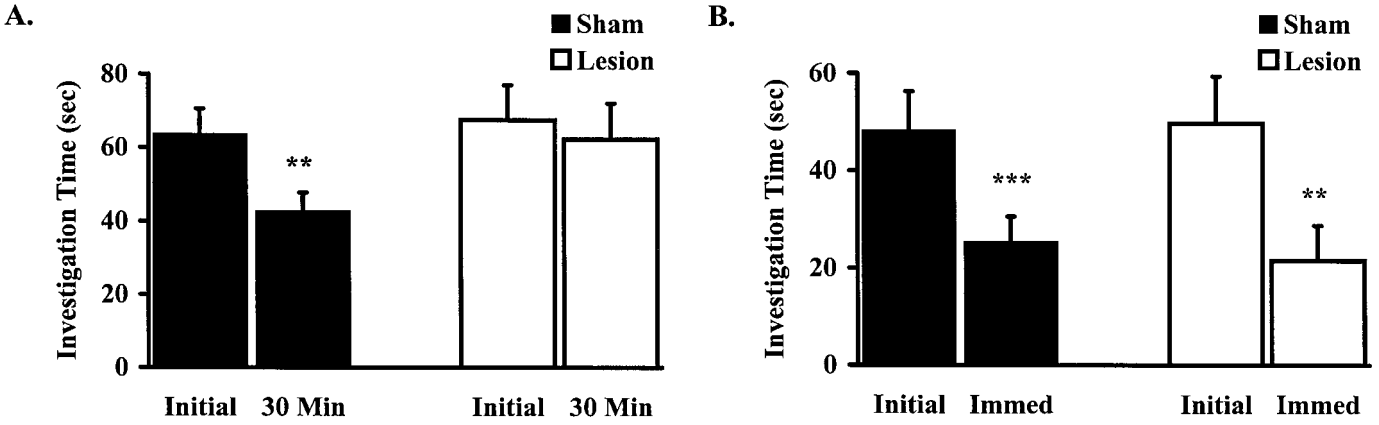


FIGURE 4. Social memory is impaired in hippocampus-lesioned mice. A: Familiar juveniles were exposed to sham-operated or lesioned mice 30 min after initial interaction. There was significant reduction in investigation duration only in the sham group (Sham, n = 10; Lesion, n = 8; $^{**}P < 0.01$, compared to Initial). B: Familiar juveniles were exposed to sham-operated or lesioned mice immediately after initial interaction. Both groups of mice had intact social memory (Sham, n = 9; Lesioned, n = 7; $^{**}P < 0.01$ and $^{***}P < 0.001$, compared to Initial).

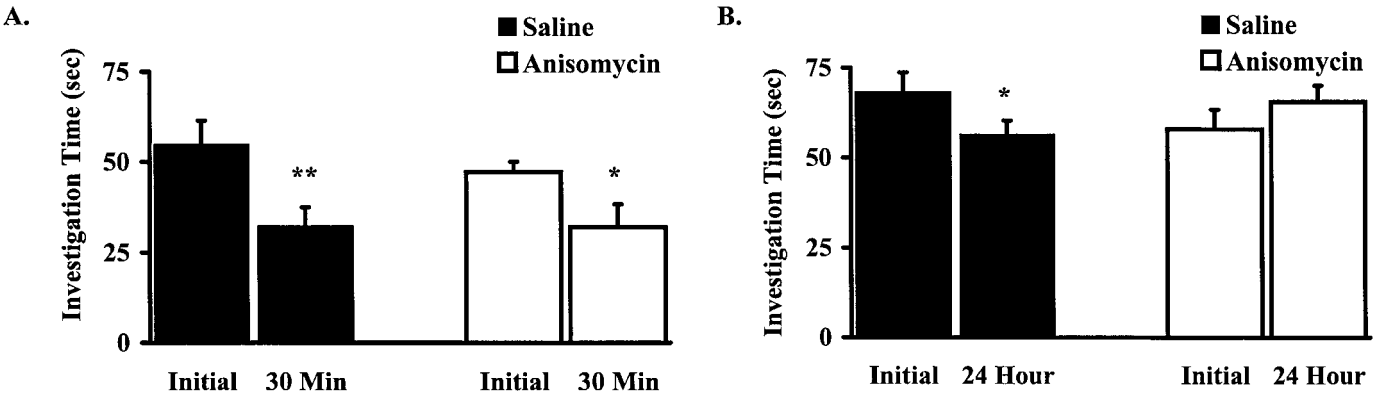


FIGURE 5. Long-term social memory is impaired by a protein synthesis inhibitor. A: Familiar juveniles were exposed to saline- or anisomycin-treated mice 30 min after initial interaction. There was significant social recognition for both groups (Saline, n = 9; Anisomycin, n = 9; $^{*}P < 0.05$ and $^{**}P < 0.01$, compared to Initial). B: Familiar juveniles were exposed to saline- or anisomycin-treated mice 24 h after initial interaction. There was significant social recognition only for saline-treated mice (Saline, n = 10; Anisomycin, n = 11; $^{*}P < 0.05$, compared to Initial).

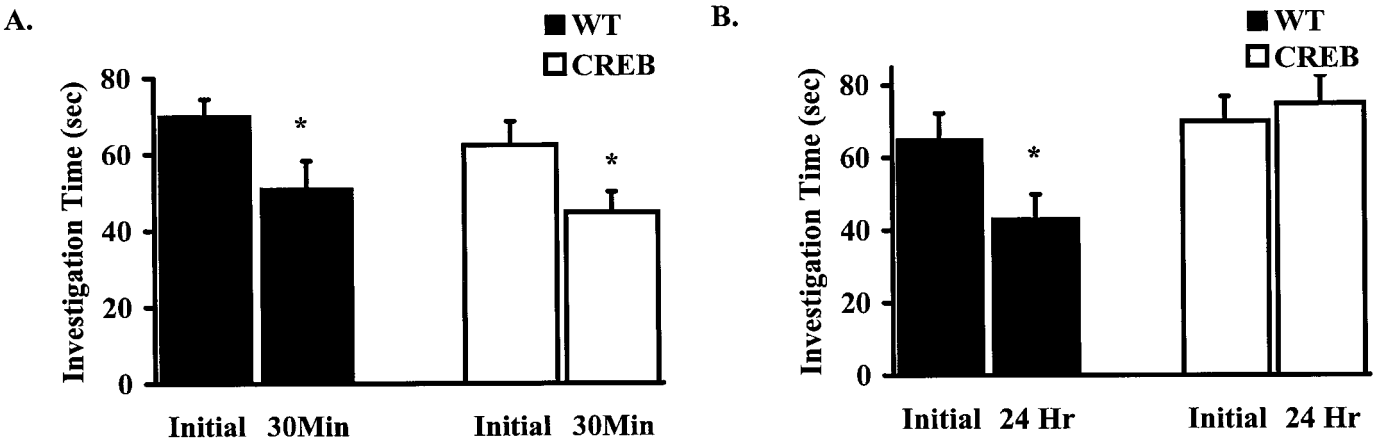


FIGURE 6. Long-term social memory is impaired in CREB$^{\alpha\Delta-}$ mutant mice. A: Familiar juveniles were exposed to WT or CREB$^{\alpha\Delta-}$ mutant mice 30 min after initial interaction. There was significant reduction in investigation duration, indicating intact social recognition in both groups (WT, n = 8; CREB, n = 9; $^{*}P < 0.05$, compared to Initial). B: Familiar juveniles were exposed to WT or CREB$^{\alpha\Delta-}$ mutant mice 24 h after initial interaction. There was significant reduction in investigation duration only for WT mice (WT, n = 7; CREB, n = 7; $^{*}P < 0.01$, compared to Initial).

The protocols that we used in this study were based on those previously used to investigate social recognition in rats and mice (Thor and Holloway, 1982; Bluthe et al., 1993). We placed a juvenile mouse into a cage with an adult, who invariably spent some period of time socially investigating the younger animal. When at a later time the same juvenile was placed back with the adult, the older animal spent significantly less time examining the younger familiar mouse. No such reduction in investigation time was observed when another novel juvenile was introduced during the test trial, suggesting that this decrease does not reflect a general habituation to social stimuli. We found that adult mice can even distinguish between inbred littermates that are essentially genetically identical, suggesting that they have distinct olfactory signatures that adults can use to identify and remember them as individuals. One likely contributor to the olfactory signature may be antigens of the major histocampatibility complex (MHC), which are secreted in the urine of mice (Yamaguchi et al., 1981; Singh et al., 1987). Untrained mice have the ability to discriminate odors in urine from other mice that differ genetically at only one MHC locus (Penn and Potts, 1998).

The most striking finding of our study was that adult male mice form social memories that can last for at least 7 days. Furthermore, this robust form of memory can be induced with interaction times as short as 2 min. Previous studies reported short-term social memory that lasted only between 30 min and 2 h (Thor and Holloway, 1982; Dantzer et al., 1987; Sekiguchi et al., 1991b; Bluthe et al., 1993). Notably, in each of the earlier studies the animals were individually housed before the experiments, while we routinely group-housed our mice. It appears likely that this was the significant factor in determining the ability of our mice to form long-term memories, since social recognition tested 24 h after training was impaired in isolated animals. Even 1 day of isolation was sufficient to disrupt social recognition at 24 h.

Our findings underscore the importance of maintained social interactions on cognitive function. There are well-documented effects of social isolation on mice, that include profound changes in social behavior, increased aggression, altered activity levels, elevated anxiety responses, and impairments in learning and memory (Essman, 1966; Morrison, 1969; Valzelli and Garattini, 1972; Valzelli, 1973; Brain, 1975; O'Donnell et al., 1981). For example, social isolation impairs performance in conditioned place preference, contextual fear conditioning, and spatial learning in the radial arm maze (Juraska et al., 1984; Pacteau et al., 1989; Rudy, 1996; Coudereau et al., 1997). Furthermore, individual housing has been shown to cause changes in prepulse inhibition and hippocampal serotonergic function in rats (Domeney and Feldon, 1998; Rilke et al., 1998). Treatment with desglycinamide-(Arg^8)-vasopressin allowed individually housed rats to recognize juveniles 24 h after their initial interaction, suggesting that individual housing could be impairing long-term social recognition by reducing brain levels of vasopressin (Sekiguchi et al., 1991a).

Our finding that social recognition is dependent on hippocampal function further adds to the growing evidence that the hippocampus is not limited to processing spatial information (Eichenbaum, 1996). The hippocampus has been shown to be critical for other nonspatial, olfactory-dependent learning paradigms such as social transmission of the food preference task, odor-paired associates task, and odor-guided continuous nonmatching to sample task (Winocur, 1990; Bunsey and Eichenbaum, 1995, 1996; Wood et al., 1999). The hippocampus is required for social memory, perhaps because this structure is involved in integrating the complex stimuli necessary for the recognition process (Sutherland and Rudy, 1989; Eichenbaum, 1996). The chemosensory cues used in social recognition probably involve both volatile and nonvolatile components that are sensed by the olfactory and vomeronasal systems, respectively (Sawyer et al., 1984; Matochik, 1988; Bluthe and Dantzer, 1993; Tirindelli et al., 1998). The main olfactory epithelia send projections to the olfactory bulb, which in turn projects to various cortical regions including the hippocampus (Scalia and Winans, 1975; Tirindelli et al., 1998). On the other hand, the vomeronasal organ communicates with the accessory olfactory bulb, which sends projections via the accessory olfactory tract to various regions, including the medial amygdala and the bed nucleus of the stria terminalis (Scalia and Winans, 1975; Tirindelli et al., 1998). These latter brain regions are the primary sources of the hippocampal vasopressin that strongly modulates social memory (Caffe et al., 1987; van Wimersma Greidanus and Maigret, 1996). Thus, social recognition involves multiple signals that converge on the hippocampus through distinct sensory pathways. The hippocampus may encode the unique configuration of features that form a polymodal representation of an individual mouse.

A considerable amount of evidence shows that de novo protein synthesis is essential for the induction of long-, but not short-term memory (Davis and Squire, 1984; Matthies, 1989). The results reported here show that long-term social memory has the same dependence on protein synthesis. We have also shown that this form of memory is also CREB-dependent. Studies in *Aplysia, Drosophila,* rats, and mice showed that CREB-mediated transcription is a requirement for the induction of long-term memory (Dash et al., 1990; Alberini et al., 1994; Bourtchuladze et al., 1994; Yin et al., 1994; Bartsch et al., 1995; Guzowski and McGaugh, 1997; Kogan et al., 1997; Lamprecht et al., 1997; Silva et al., 1998). CREB may be a gain control device that regulates the expression of genes necessary for memory consolidation (Silva et al., 1998). Additionally, CREB appears to regulate both the number and timing of the training trials required for long-term memory formation (Yin et al., 1994; Kogan et al., 1997; Silva et al., 1998). We previously demonstrated that CREB$^{\alpha\Delta-}$ mutant mice have intact short-term, but impaired long-term memory in several hippocampus-dependent tasks (Bourtchuladze et al., 1994; Kogan et al., 1997). Our finding that long-term social memory is also dependent on CREB function parallels our previous findings with other hippocampus-dependent tasks, including the social transmission of food preference, water maze, and contextual fear conditioning (Kogan et al., 1997).

Social recognition is ideally suited to study the molecular basis of learning and memory in mutant mice. First, no shaping of the animals is necessary, and the learning can be accomplished in a single, brief training trial that results in robust long-term memory. This allows the study of the time-course of discrete phases of

memory consolidation. Second, it is possible to test the same subject multiple times, which allows for within-subject comparisons under different experimental conditions. Finally, social memory requires the hippocampus in both mice and humans, which suggests that social recognition studies in mice may be relevant to the study of human memory mechanisms (Corkin, 1984). Consequently, social recognition is an ideal paradigm for studying learning and memory.

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