The Dorsal Hippocampus Is Essential for Context Discrimination but Not for Contextual Conditioning

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The authors describe how (a) the timing of hippocampal lesions and (b) the behavioral-representational demands of the task affect the requirement for the hippocampus in contextual fear conditioning. Prior to training, lesions of the hippocampus greatly reduced contextual fear conditioning. In contrast, pretraining lesions of the hippocampus abolished context discrimination, a procedure in which mice are trained to discriminate between 2 similar chambers (shock context vs. no-shock context). Whereas either contextual- or cue-based strategies can be used to recognize an aversive context, discrimination between similar contexts is optimally acquired by contextual (hippocampal)-based strategies. In keeping with the lesion results, Nf1-/-/Nmdar1-/- mutant mice, which have spatial learning deficits, are impaired in context discrimination but not in contextual conditioning. Together, these data dissociate hippocampal and nonhippocampal contributions to contextual conditioning and they provide direct evidence that the hippocampus plays an essential role in the processing of contextual stimuli.

Contextual fear conditioning is a form of associative learning. Classically, animals learn to recognize a training environment (conditioned stimulus; CS) that has previously been paired with an aversive stimulus such as foot-shock (unconditioned stimulus; US). Contextual conditioning is then operationally defined as an increase in a range of conditioned responses, including autonomic (e.g., increased heart rate) and behavioral (e.g., freezing) changes, that are associated with fear (Fanselow, 1984). Freezing, a cessation of all bodily movement aside from respiration, has been the most widely measured conditioned response in this task (e.g., LeDoux, 1994). Contextual conditioning has been used to investigate the neural substrates mediating fear-motivated learning (Kim & Fanselow, 1992; Phillips & LeDoux, 1992; Selden, Everitt, Jarrard, & Robbins, 1991; Sutherland & McDonald, 1990). In addition, this form of conditioning has been extensively used to determine the impact of a number of mutations on hippocampal-dependent learning and memory (e.g., Silva et al., 1996).

Previous studies have suggested that the hippocampus plays a key role in contextual conditioning (Kim, Rison, & Fanselow, 1993; Phillips & LeDoux, 1992; Selden et al., 1991; Sutherland & McDonald, 1990). It is proposed that the hippocampus is required for the formation of a unified, polymodal representation of the cues present in the training environment (e.g., Nadel, Willner, & Kurz, 1985; Sutherland & Rudy, 1989), which is then associated with the US (e.g., Maren & Fanselow, 1995; Phillips & LeDoux, 1992). However, recent studies showing contextual conditioning in hippocampal-lesioned rats (Gisquet-Verrier & Doyère, 1997; Maren, Aharonov, & Fanselow, 1997; Phillips & LeDoux, 1994) and mice (Logue, Paylor, & Wehner, 1997) indicate that under certain conditions, contextual conditioning may be supported by nonhippocampal systems. For example, contextual conditioning may be mediated by associations between single cues in the training environment and the US.
Here we describe how (a) the timing of hippocampal lesions and (b) the behavioral–representational demands of the task affect the requirement for the hippocampus in contextual fear conditioning. The first series of experiments demonstrates that contextual conditioning can be mediated by both hippocampal and nonhippocampal neural systems and indicates that strategies mediated by nonhippocampal systems are normally suppressed. Because contextual conditioning may be mediated by either cue- or context-based strategies, in the second series of experiments, we use a context-discrimination procedure (e.g., Fanselow & Baackes, 1982) which is optimally solved by contextual-based strategies (McDonald, Koerner, & Sutherland, 1993). These experiments show that an intact hippocampus is essential for discriminating between similar contexts. In keeping with these results, N1j1+/Nmdar1−/− mutant mice, which have hippocampal-dependent spatial learning deficits (Silva et al., 1997), are impaired in context discrimination but not in contextual conditioning. Together, these data establish that the hippocampus is essential for processing contextual stimuli and indicate that context discrimination is a more sensitive measure of hippocampal dysfunction compared with the classical contextual conditioning procedure.

**Method**

**Subjects**

All experiments used equal numbers of male and female C57BL/6J mice (Taconic, Germantown, NY). The mice were group housed (2–5 mice per cage) and had continual access to food and water. The vivarium was maintained on a 12-hr light–dark cycle, and all testing was carried out during the light phase of the cycle.

Mice used in Experiments 1–3 were between 12 and 18 weeks old at the time of surgery. The mice used in Experiment 4 were F1 progeny of heterozygous mice (N1j1+/Nmdar1-/-) with 4 backcrosses into C57BL/6J background. The mice were genotyped with polymerase chain reaction protocols. Age- and gender-matched mutant mice and wild type (WT) controls were used. For the experiments described here, the experimenter was always unaware of the surgery (sham or lesion) or genotype of the subjects.

The Cold Spring Harbor Laboratory animal facility is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, and animals are maintained in accordance with the Animal Welfare Act and the National Institutes of Health (1986) guide.

**Surgery**

For the lesion experiments (Experiments 1–3), mice were anesthetized with chloral hydrate (400 mg/kg) and placed in a standard stereotaxic instrument with a mouse adapter (Kopf Instruments, Tujunga, CA). Electrolytic lesions of the dorsal hippocampus (DH; n = 54) were made by means of stainless steel electrodes. With the exception of the tip, these electrodes were insulated with Parylene coating (0.5 MΩ impedance). Electrodes were connected to a Grass S-88 stimulator (Grass Instruments, West Warwick, RI), and lesions were made with 2.7 mA anodal current for 3 s. Electrodes were positioned in the following coordinates with respect to bregma: −1.8 P/L, 2.0 Z. Current return was by means of an electrode attached to the mouse’s tail. The procedure for sham surgeries (n = 48) was identical except that no current was passed. The mice were allowed to recover for 1 week after surgery.

**Histology**

After the completion of behavioral testing, DH-lesioned and sham-lesioned mice (Experiments 1–3) were anesthetized with chloral hydrate and perfused transcardially, first with 0.9% saline, then with 4% paraformaldehyde. Brains were removed and stored in a 30% sucrose, 4% paraformaldehyde solution overnight and then cut at −15°C with a cryostat. Fifty μm coronal sections were cut from tissue including the hippocampus. These sections were mounted on gelatin-coated slides and stained with cresyl violet. Lesions were examined under a light microscope and reconstructed on mouse brain atlas sections from Franklin and Paxinos (1997).

**Apparatus**

Three different conditioning chambers (A, B, and C) were used in these experiments, each located in different rooms. Chambers A and B were located in windowless, dimly lit rooms that were roughly equidistant (10 m) from the mouse vivarium. Chamber C was located within the mouse vivarium. Mice were tested individually. For each test, the mouse was carried to the test apparatus in a cage containing a mix of fresh wood shavings and wood shavings from its home cage. The characteristics of the conditioning chambers are described in detail below.

**Chamber A.** The conditioning chamber was housed in a sound-attenuated box (interior dimensions: 56 cm long × 42 cm wide × 37 cm high). 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 SPL) was provided by a fan located in one of the walls of the sound-attenuated chamber. The conditioning chamber (16 cm long × 16 cm wide × 19 cm high) was rectangular in shape, and its walls were made of clear Plexiglas. Total floor area was 256 cm². On one of the conditioning chamber walls, there was a 24-V housesight. The floor of the chamber consisted of an 18-bar shock grid. Bars were 3 mm in diameter and 0.9 cm apart. Each bar was connected to a Master Shocker (Model 82402SS, Lafayette Instruments, Lafayette, IN), a device that delivers scrambled shocks. Between tests, the cage floor and interior of the conditioning chamber were cleaned with a 75% ethanol solution.

**Chamber B.** The second conditioning chamber was housed in an identical sound-attenuated box. All four interior walls of the sound-attenuated chamber were painted white. A fan was located in one of the walls of the sound-attenuated chamber, but it was not turned on during either training or testing. The conditioning chamber consisted of a triangular base with vertical Plexiglas walls. The sides of the triangle were 24 cm long, and the height of the chamber was 20 cm. Total floor area was 250 cm², similar to Chamber A. The exteriors of two of the walls of the conditioning chamber were covered in an opaque blue material. The other wall was left transparent, to allow observation of the mice. The floor of the cage comprised a shock grid, identical to that in Chamber A. Between tests, the cage floor and interior of the conditioning chamber were cleaned with a lime-scented detergent (3% Rocal solution).

**Chamber C.** The third chamber was a clear plastic cage (27 cm long × 16 cm wide × 12 cm high) that was located in the brightly lit mouse vivarium. In contrast to Chambers A and B, where the floor consisted of a shock grid, the base of the cage was covered in a blue, absorbent, padded material. The total floor area was 432
cm². Previous studies have shown that contextual conditioning is indistinguishable after training in either small (250 cm²) or large (500 cm²) chambers (Marowitz, Borchucklade, & Silva, 1996). In addition, the back and side walls of the interior of the cage (apart from the front wall of the cage facing the observer) were covered in a blue, absorbent, padded material.

**Observation**

Conditioning was assessed by freezing. A mouse was determined to be freezing when it adopted a motionless posture, refraining from all but respiratory movement (e.g., Fanselow, 1990). Freezing was assessed with a sampling method, that is, 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 unaware of the experimental treatment (sham vs. lesion) or genotype (WT vs. Nfl+/−/Nndar1−/−) of each mouse. All tests were videotaped and, subsequently, a subset of these were rescorded by a second observer, again unaware of the experimental treatment of the mice. Scoring of freezing was highly correlated between observers (r = .96). Freezing data are presented as the percentage of time spent freezing (i.e., the number of observations when freezing was observed, divided by the total number of observations, and multiplied by 100).

**Experimental Procedures**

**Experiment 1: Effect of pretraining DH lesions on contextual conditioning.** Mice received either sham (n = 17) or DH (n = 17) lesions. After a 7-day recovery period, each mouse was placed into Conditioning Chamber A for a total of 180 s. After 120 s, a 30-s, 2.0-kHz, 85-dB (SPL) tone was presented. After 148 s, a 2-s, 0.75-mA shock was delivered via the cage floor bars. The tone and footshock coterminated at 150 s, and the mouse remained in the chamber for a further 30 s. The mouse was then removed and returned to its home cage. Seven days after this training session, each mouse was placed back in the chamber, and freezing was assessed over a 5-min period. During this period, the tone was not presented.

**Experiment 2A: Effect of posttraining DH lesions on contextual conditioning.** In Experiment 2A, we tested the effects of posttraining DH lesions on contextual fear conditioning. To control for potential differential effects of DH lesions on contextual fear conditioning using background versus foreground training procedures (Phillips & LeDoux, 1994), mice were randomly assigned to two groups. In Group 1, a 30-s tone CS was paired with the shock during training. In Group 2, no tone was presented during training.

In Group 1, each mouse was placed into Conditioning Chamber A for a total of 180 s. After 120 s, a 30-s, 2.0-kHz, 85-dB (SPL) tone was presented. After 148 s, a 2-s, 0.75-mA shock was delivered via the cage floor bars. The tone and footshock coterminated at 150 s, and the mouse remained in the chamber for a further 30 s. The mouse was then removed and returned to its home cage. The training for Group 2 was identical except that the tone CS was not presented. One day after this training session, mice received sham lesions (Group 1, n = 12; Group 2, n = 6) or DH lesions (Group 1, n = 8; Group 2, n = 7). Seven days after training, each mouse was placed back in the chamber, and freezing was assessed over a 5-min period. During this period, the tone was not presented.

**Experiment 2B: Contextual conditioning in DH-lesioned mice retrained in a second context.** Two weeks after the completion of Experiment 2A, mice were retrained in a second context (Chamber B). For Group 1 (initially trained in the presence of a tone CS), no tone was presented during training. For Group 2 (not previously trained with a tone CS), a 30-s tone CS was paired with the shock during training. The training procedures were then identical to those used in Experiment 2A. Seven days after training, each mouse was placed back in the chamber, and freezing was assessed over a 5-min period. During this period, no tone CS was presented.

**Experiment 3A: Effect of pretraining DH lesions on context discrimination.** This experiment consisted of three stages: preexposure (1 day), training (1 day), and testing (2 days). On each of the 4 days, each mouse was placed in Chamber A in the morning and Chamber B in the afternoon (or vice versa). On the 1st day, sham-lesioned (n = 13) and DH-lesioned (n = 16) mice were preexposed to both chambers (A and B) for a total of 10 min each. During this period, no footshocks were delivered. The next day, mice were trained in both Chambers A and B. During training, mice were placed in the chambers for a total of 3 min. In Chamber A, a 2-s, 0.75-mA shock was delivered via the cage floor bars after 148 s. The mice remained in the chamber for a further 30 s after the offset of the US and were then removed. Training was identical in Chamber B, except that no shocks were delivered. On the 2 test days posttraining, mice were placed in Chambers A and B for 3 min each. Just as during training, mice received a footshock after 148 s in Chamber A but not in Chamber B. Freezing data from these 2 days are presented from the first 148 s preceding the shock delivery. For training and testing, the order (Chamber A vs. Chamber B) was counterbalanced across groups (sham vs. lesion controls). In the present study, Chamber A was always paired with shock, and chamber B was always used as the no-shock context. In two previous experiments, we have found that similar levels of discrimination occur whether Chamber A or Chamber B is used as the shock context (ns > 10; Giese, Frankland, & Silva, 1998). The protocol for this experiment was based on a similar discrimination protocol designed for rats (Fanselow & Baackes, 1982; McDonald et al., 1995).

**Experiment 3B: Effect of extended training on context discrimination in DH-lesioned mice.** A subset of the animals in Experiment 3A received extended training and testing in Chambers A and B. Sham-lesioned (n = 10) and DH-lesioned (n = 11) mice were tested for an additional 2 days after the completion of Experiment 3A. As before, on each of these days, mice received a footshock in Chamber A but not in Chamber B. Freezing was scored for the entire 180 s, but only data from the 148 s preceding shock delivery are presented.

**Experiment 3C: Specificity of conditioned freezing.** Four days after the completion of Experiment 3B, a subset of mice (sham control, n = 7; DH-lesioned, n = 9) were tested in Chambers A and C. The novel context (Chamber C) differed in all regards from both Chambers A and B. Freezing was assessed over a 150-s period, and during these sessions, no footshocks were delivered.

**Experiment 4A: Contextual conditioning in Nfl+/−/Nndar1−/− mutant mice.** WT (n = 8) and Nfl+/−/Nndar1−/− (n = 9) mice were trained and tested using a protocol similar to that used in Experiment 1. Briefly, each mouse was placed into Conditioning Chamber A for a total of 180 s. After 120 s, a 30-s, 2.0-kHz, 85-dB (SPL) tone was presented. After 148 s, a 2-s, 0.75-mA shock was delivered via the cage floor bars. The tone and footshock coterminated at 150 s, and the mouse remained in the chamber for a further 30 s. One day after this training session, each mouse was placed back in the chamber, and freezing was assessed over a 5-min period. During this period, the tone was not presented.

**Experiment 4B: Context discrimination in Nfl+/−/Nndar1−/− mutant mice.** A second set of WT (n = 22) and Nfl+/−/Nndar1−/− (n = 21) mice were tested in the context-discrimination procedure described above (Experiment 3A). The protocol was
identical except that the mice were tested for 1 (rather than 2) days after preexposure and initial training.

Statistical Analysis

Analysis of variance (ANOVA) was performed on the freezing data from Experiments 1–4. In each of these ANOVAs, treatment (sham vs. lesion; Experiments 1–3) or genotype (WT vs. Nf1<sup>−/−</sup>/Nmdar1<sup>+/−</sup>; Experiment 4) was a between-subjects variable. For Experiments 1, 2A, 2B, and 4A, time was a within-subjects variable. For Experiment 3, day, context and time were within-subjects variables. For Experiment 4B, context and time were within-subjects variables.

Results

Histological Analysis

A total of 102 mice were used in the neuroanatomical lesion studies (Experiments 1–3). Of these, 54 received electrolytic lesions of the DH and 48 received sham surgeries. Histological analysis revealed that 48 out of 54 mice had bilateral lesions of the DH. Data from these mice were included in subsequent statistical analyses. The criterion for inclusion was evidence of substantial damage to the CA1, CA2, and CA3 subregions of the dorsal part of the hippocampus. Mice were excluded from analyses if the lesions included substantial damage to neighboring extrahippocampal structures. Figure 1 shows reconstructions of the smallest and largest DH lesions for each of the three experiments. In some cases, these lesions also included damage to the innermost portion of the dentate gyrus (n = 7) or ventral regions of the hippocampus, including CA1 (n = 5).

Some lesions (n = 11) included damage to neocortical areas dorsal to the hippocampus. This damage was limited to the ventralmost regions of the somatosensory 1 trunk region and posterior parietal association area, immediately surrounding the electrode track. The extent of neocortical damage was similar in sham-lesioned mice. In addition, in 3 mice, there was evidence of limited damage to areas just dorsal to the hippocampus. This damage was restricted to the dorsalmost regions of the dorsal lateral geniculate nucleus and the lateral posterior thalamic nucleus, accounting for less than 5% of the total volume of these structures.

Data from 6 mice were not included in statistical analyses. These mice sustained either no damage to the hippocampus, substantial damage to overlying neocortical areas in addition to damage to the DH, or unilateral damage to the DH.

Experiment 1: Pretraining DH Lesions Do Not Abolish Contextual Conditioning

Pretraining DH lesions resulted in a reduction but not a complete block of contextual fear conditioning (Figure 2). One week after surgery, mice were trained with a single footshock, and 1 week later, they were tested in the same chamber in which they were trained. In this test, sham-lesioned mice (n = 17) spent more time freezing compared with DH-lesioned mice (n = 17, 56.4% ± 6.6% vs. 40.7% ± 7.1%, respectively). A mixed measures ANOVA, with treatment (two levels: sham vs. lesion) as a between-subjects variable and time (five levels: 1, 2, 3, 4, and 5 min) as a within-subjects variable, was performed on the freezing scores for sham-lesioned and DH-lesioned mice. There was a main effect of treatment, F(1, 32) = 4.35, p < .05,

![Figure 1](image-url)  
Figure 1. Reconstructions of dorsal hippocampus lesions. The largest (gray shaded area) and smallest (black shaded area) lesions of the dorsal hippocampus are shown for each experiment (1–3) on coronal sections from Franklin and Paxinos (1997). Numbers to the left indicate the anterior–posterior coordinate of sections with respect to bregma.
Pretraining Lesions

![Graph showing the effect of pretraining dorsal hippocampus (DH) lesions on contextual conditioning. Percentage of time spent freezing is plotted for each minute of the 5-min test for sham-lesioned (open squares; n = 17) and DH-lesioned (solid squares; n = 17) mice. Pretraining DH lesions attenuated conditioned freezing.](image)

reflecting greater levels of conditioned freezing in sham-lesioned mice. In addition, there was a main effect of time, $F(4, 128) = 4.80, p < .01$, but no significant interaction between treatment and time, $F(4, 128) = 0.47, p > .05$.

**Experiment 2A: Posttraining DH Lesions Block Contextual Conditioning**

In Experiment 2A, we tested the effects of posttraining DH lesions on contextual conditioning. During training, either a tone was paired with the shock (Group 1), or no tone was presented (Group 2). One day after training, both groups of mice received either sham lesions or DH lesions. One week after training, these mice were tested for contextual conditioning. In contrast to Experiment 1, these posttraining DH lesions greatly reduced contextual fear conditioning, regardless of whether a tone CS was presented during training (Figures 3A and 3B). Across the 5-min test, sham-lesioned mice spent substantially more time freezing compared with DH-lesioned mice (Group 1: 38.6% ± 4.8% vs. 9.2% ± 4.9%; Group 2: 45.6% ± 12.2% vs. 11.2% ± 6.0%). ANOVA showed that there was no effect of group ($F < 1$) or Group × Treatment interactions ($F < 1$), so the data from Groups 1 and 2 were combined and analyzed together. A mixed ANOVA, with treatment (two levels: sham vs. lesion) as a between-subjects variable and time (five levels: 1, 2, 3, 4, and 5 min) as a within-subjects variable, was performed on the freezing scores. There was a main effect of treatment, $F(1, 31) = 22.94, p < .001$, indicating that DH lesions greatly reduced conditioned freezing. There was no significant effect of time, $F(4, 124) = 1.83, p > .05$, or significant interaction between treatment and time, $F(4, 124) = 0.83, p > .05$. Although freezing levels in DH-lesioned mice were greatly reduced, contextual conditioning was not completely blocked because freezing levels during testing were still significantly greater than during initial training, $F(1, 14) = 7.47, p < .05$.

**Experiment 2B: Normal Contextual Conditioning in DH-Lesioned Mice After Retraining in a Second Context**

Following Experiment 2A, mice were subsequently retrained in a second context (Chamber B). As before, mice received one shock during training and were tested for contextual fear conditioning 1 week later. In contrast with Experiment 2A, DH-lesioned mice showed normal or nearly normal contextual conditioning, regardless of whether a tone CS was presented during training (Figures 3C and 3D). Across the 5-min test, sham-lesioned mice spent more time freezing compared to DH-lesioned mice in Group 1 (41.5% ± 5.0% vs. 28.8% ± 4.6%) and equivalent levels of freezing in Group 2 (30.8% ± 6.2% vs. 30.0% ± 6.9%). ANOVA showed that there was no effect of group ($F < 1$) or Group × Treatment interactions ($F < 1$), so the data from the Groups 1 and 2 were combined and analyzed together. A mixed measures ANOVA, with treatment (two levels: sham vs. lesion) as a between-subjects variable and time (five levels: 1, 2, 3, 4, and 5 min) as a within-subjects variable, was performed on the freezing scores. There was no significant effect of treatment, $F(1, 31) = 2.32, p > .05$, indicating that levels of conditioned freezing in sham-lesioned mice were not significantly different from DH-lesioned mice. There was a main effect of time, $F(4, 124) = 6.74, p < .001$, but no significant interaction between treatment and time, $F(4, 124) = 1.05, p > .05$.

**Differential Effects of Pre- and Posttraining DH Lesions on Contextual Fear Conditioning**

The differential effects of pre- versus posttraining DH lesions on contextual fear conditioning were assessed by comparing between experiments (Experiment 1 vs. Experiment 2A) and within experiments (Experiment 2A vs. 2B). Both these analyses show that post- but not pretraining lesions lead to a substantial reduction in levels of conditioned freezing.

**Experiment 1 versus 2A.** In Experiment 2A, the groups tested did not differ ($F < 1$), so their data were combined. We performed an ANOVA, with experiment (1 vs. 2A) and treatment (sham vs. lesion) as between-subjects variables, on the freezing scores. There was a main effect of experiment, $F(1, 63) = 30.16, p < .001$, reflecting higher overall levels of freezing in Experiment 1, and a main effect of treatment, $F(1, 63) = 24.08, p < .001$, reflecting higher overall levels of freezing in the sham-lesioned mice. Most important, there was a significant Experiment × Treatment interaction, $F(1, 63) = 4.02, p < .05$, indicating that pre- and posttraining lesions differentially affected levels of conditioned freezing. In keeping with this, post hoc analyses ($p < .05$; Newman–Keuls test) showed that freezing levels in mice that received pretraining DH lesions (Experiment 1) were greater than those in mice that received posttraining DH lesions (Experiment 2A). This indicates that posttrain-
Figure 3. Data for Group 1 (sham n = 12; lesion n = 8) and Group 2 (sham n = 6; lesion n = 7) in Experiment 2. A and B: the effects of posttraining dorsal hippocampal (DH) lesions on contextual conditioning for mice trained with a tone (Group 1) or without a tone (Group 2). Percentage of time spent freezing is plotted for each minute of the 5-min test for sham-lesioned (open squares) and DH-lesioned (solid squares) mice. Posttraining DH lesions greatly reduced conditioned freezing in both groups. C and D: In Experiment 2B, both groups of mice were subsequently retrained in a second context (Chamber B). The percentage of time spent freezing is plotted for each minute of the 5-min test for sham-lesioned (open squares) and DH-lesioned (solid squares) mice for Group 1 (trained without tone) and Group 2 (trained with tone). The percentage of time spent freezing did not differ significantly between sham- and DH-lesioned mice. E and F: Summary of the data from Groups 1 and 2 in Experiments 2A and 2B. The levels of conditioned freezing are plotted for sham-lesioned (open bars) and DH-lesioned (solid bars) mice trained in Context A (Experiment 2A; posttraining) and retrained in Context B (Experiment 2B; retraining). Although posttraining lesions greatly reduced contextual conditioning in Experiment 2A, these mice showed normal, or nearly normal, contextual conditioning when retrained in Context B (Experiment 2B). Levels of conditioned freezing were significantly greater in DH-lesioned mice in Experiment 2B compared with Experiment 2A in both Groups 1 and 2 (p < .05; Neuman–Keuls test).

Experiment 2A versus 2B. In Experiment 2, the effects of pre- versus posttraining DH lesions on contextual conditioning were assessed in the same mice (Figures 3E and 3F). Because there was no difference between Groups 1 and 2
(F < 1), data were combined. A mixed measures ANOVA, with treatment (two levels: sham vs. lesion) as a between-subjects variable and experiment (two levels: training in Context A vs. training in Context B) as a within-subjects variable, was performed on the freezing scores for sham- and DH-lesioned mice. There were main effects of experiment, F(1, 31) = 8.11, p < .01, reflecting greater overall levels of freezing in Experiment 2B compared with 2A and treatment, F(1, 31) = 13.59, p < .001, reflecting greater overall levels of freezing in sham controls. Most important, there was a significant Treatment × Experiment interaction, F(1, 27) = 15.10, p < .001. This confirms that posttraining (Experiment 2A) and pretraining (Experiment 2B) DH lesions differentially affect contextual fear conditioning. Post hoc analyses (p < .05; Newman–Keuls test) showed that freezing levels in the DH-lesion group were greater in Experiment 2B than in Experiment 2A, indicating that posttraining lesions lead to greater reductions in contextual fear conditioning than do pretraining lesions. In addition, freezing levels were greater in the sham group compared with the DH-lesion group in both Experiments 2A and 2B. Levels of freezing in the sham-lesion group did not differ across experiments.

Experiment 3A: Pretraining DH Lesions Block Context Discrimination

In Experiment 3A, sham-lesioned (n = 13) and DH-lesioned (n = 16) mice were tested in a context-discrimination paradigm (Fanselow & Baackes, 1982; McDonald et al., 1995). In this task, mice were trained to discriminate between two similar contexts, one in which they were shocked and the other in which they received no shock. The two chambers (Context A vs. B) comprised both unique cues and common cues. Freezing was assessed on each of 2 days after initial training in Context A (paired with shock) and Context B (not paired with shock). The data presented in Figure 4A show that context discrimination was abolished in mice receiving pretraining DH lesions. Sham-lesioned mice froze more in the context associated with shock (Context A) compared with the no-shock context (Context B) on both Posttraining Day 1 (43.3% ± 4.4% vs. 28.2% ± 4.9%) and Posttraining Day 2 (53.9% ± 4.0% vs. 36.2% ± 3.3%). In contrast, DH-lesioned mice showed roughly equal levels of conditioned freezing in both contexts on Posttraining Day 1 (33.1% ± 4.0% vs. 30.6% ± 4.0%) and Posttraining Day 2 (48.8% ± 4.9% vs. 45.4% ± 5.4%). A mixed measures ANOVA, with treatment (two levels: sham vs. lesion) as a between-subjects variable and day (two levels: Posttraining Day 1 vs. Posttraining Day 2), context (two levels: Context A vs. Context B) and time (five levels: 30, 60, 90, 120, and 150 s) as within-subjects variables, was performed on the freezing scores. There was a significant main effect of day, F(1, 27) = 20.05, p < .001, reflecting greater levels of conditioned freezing with increased training. There was also a significant main effect of context, F(1, 19) = 26.25, p < .001, reflecting discrimination between contexts. Planned comparisons showed that freezing levels were greater in Context A compared with Context B across all posttraining days for sham-lesioned mice (ps < .05). In contrast, freezing was only greater in Context A compared with Context B on Posttraining Days 3 and 4 for DH-lesioned mice (ps < .05).

Experiment 3C: DH-Lesioned Mice Can Discriminate Between Trained Context and a Novel Chamber

In Experiment 3C, we tested whether the DH-lesioned mice exhibit generalized freezing in a novel context or whether the conditioned freezing observed in Experiments 3A and 3B was specific to the cues present during training in Chambers A and B. After context-discrimination training in the two similar contexts (Chambers A and B), sham- and DH-lesioned mice were tested in Context A and in a novel context (Chamber C). Cues in Chamber C had minimal overlap with those in Chambers A and B. Figure 4C shows that both sham-lesioned (n = 7) and DH-lesioned (n = 9) mice exhibited high levels of conditioned freezing in the shock context (Chamber A) and nearly no freezing in the novel context (Chamber C). Sham-lesioned mice froze 73.8% ± 4.5% of the time in Context A versus 8.6% ± 3.6%
Figure 4. A: Effect of pretraining dorsal hippocampal (DH) lesions on context discrimination. Percentage of time spent freezing in Context A (shock context; solid bars) versus Context B (no-shock context; open bars) shown for each of 2 posttraining days for sham-lesioned mice (left panel) and DH-lesioned mice (right panel). Sham-lesioned control mice \( (n = 13) \) showed clear discrimination between contexts \( (*p < .05; \) Neuman–Keuls test). In contrast, DH-lesioned mice \( (n = 16) \) exhibited equal levels of freezing in both contexts. B: The effect of extended training on context discrimination. Percentage of time spent freezing is shown for sham-lesioned control mice (left panel; \( n = 10) \) and DH-lesioned mice (right panel; \( n = 11) \) in Context A (shock context; solid circles) and Context B (no-shock context; open circles) over 4 posttraining days. Sham-lesioned control mice show clear context discrimination on each test day \( (*p < .05) \). In contrast, although DH-lesioned mice did not discriminate between contexts on Days 1 and 2, they eventually exhibited context discrimination on Days 3 and 4 \( (*p < .05) \). C: Specificity of conditioned freezing in sham-lesioned and DH-lesioned control mice. After training in Contexts A and B, sham-lesioned control mice (left panel; \( n = 7) \) and DH-lesioned mice (right panel; \( n = 9) \) were tested in Context A and a novel context, C. Both sham- and DH-lesioned mice exhibited robust levels of conditioned freezing in Context A (shock context; solid bars) and nearly no freezing in Context C (novel context; shaded bars; \( *p < .05) \).
of the time in Context C; DH-lesioned mice froze 61.5% ± 5.6% of the time in Context A versus 0.7% ± 0.7% of the time in Context C. We performed a mixed measures ANOVA on the freezing scores, with treatment (two levels: sham vs. lesion) as a between-subjects variable and context (two levels: Context A vs. Context C) and time (five levels: 30, 60, 90, 120, and 150 s) as within-subjects variables. There was a significant main effect of treatment, \( F(1, 14) = 6.69, p < .05 \), reflecting greater levels of freezing overall in the sham-lesioned mice. There was also a significant main effect of context, \( F(1, 14) = 219.16, p < .001 \), reflecting different levels of freezing in Context A compared with Context C. Planned comparisons showed that both sham- and DH-lesioned mice froze significantly more in Context A than in Context C (ps < .05).

**Experiment 4A: Normal Contextual Conditioning in Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) Mice**

Figure 5A shows contextual conditioning data for WT mice and Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) mice. There were no differences in preshock freezing during training (\( F < 1 \)). When these mice were subsequently tested, WT (\( n = 8 \)) and Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) (\( n = 9 \)) mice showed similar levels of conditioned freezing after training with one shock: WT mice spent 37.0% ± 8.3% of the time freezing, whereas Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) mice spent 36.3% ± 4.9% of the time freezing. We performed a mixed measures ANOVA on the freezing scores, with genotype (two levels: WT vs. Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\)) as a between-subjects variable and time (five levels: 1, 2, 3, 4, and 5 min) as a within-subjects variable. There was no main effect of genotype, \( F(1, 15) < 1 \), on freezing, indicating that mutants and WT mice showed roughly equal levels of conditioned freezing. There was a significant main effect of time, \( F(4, 60) = 3.23, p < .05 \), on freezing, indicating that the likelihood of freezing changed across the test time.

**Experiment 4B: Context Discrimination Impaired in Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) Mice**

In Experiment 4B, WT (\( n = 22 \)) and Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) (\( n = 21 \)) mice were trained in the context-discrimination paradigm. Freezing was assessed 1 day after initial training in Chamber A (paired with shock) and Chamber B (not paired with shock). The data presented in Figure 5B show that context discrimination was abolished in Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) mutant mice. WT control mice froze more in the context associated with shock (Context A; 36.2% ± 3.3%) compared with the no-shock context (Context B; 26.4% ± 2.6%). In contrast, Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\) mutant mice showed roughly equal levels of conditioned freezing in both Context A (27.1% ± 3.1%) and Context B (25.1% ± 3.8%). We performed a mixed measures ANOVA on the freezing scores, with genotype (two levels: WT vs. Nfl\(^{+/−}\)/Nmdar1\(^{+/−}\)) as a between-subjects variable and context (two levels: Context A vs. Context B) and time (five levels: 30, 60, 90, 120, and 150 s) as within-subjects variables. There was a significant main effect of context, \( F(1, 41) = 7.03, p < .05 \), reflecting greater overall levels of conditioned freezing in the shock context (Context A) compared with the no-shock context (Context B). There was a significant effect of time, \( F(4, 164) = 9.47, p < .001 \), indicating that the likelihood of freezing changed across the test time. Planned comparisons showed that WT control mice (\( p < .05 \)) froze more in Context A compared with Context B.

**Discussion**

The role of the hippocampus in contextual fear conditioning has been controversial. Lesions of the hippocampus have been found to either block (Chen, Kim, Thompson, & Tonegawa, 1996; Good & Honey, 1997; Kim & Fanselow,
contextual fear (whereas pretraining lesions did not), these data demonstrate that strategies mediated by nonhippocampal systems are normally suppressed when the hippocampus is functional during training. When the same mice from Experiment 2A were subsequently retrained (either with or without a tone CS) in a second context, they showed nearly normal, or normal, contextual conditioning (Experiment 2B). Therefore, in keeping with Experiment 1, this result suggests that in the absence of hippocampal suppression during training, nonhippocampal systems can support (albeit less efficiently) contextual conditioning. These conclusions support an earlier study that provided evidence that spatial strategies mediated by hippocampal systems suppress the learning of a conditioned cue preference in the radial arm maze (McDonald & White, 1995).

It has been proposed that the hippocampus is required for the formation of a polymodal representation of context (Nadel et al., 1985; Sutherland & Rudy, 1989), which can then be associated with the US (e.g., Maren & Fanselow, 1995; Rogan & LeDoux, 1996). That is, the hippocampus is required for the formation of a unified representation of the visual, olfactory, tactile, and auditory cues present in the training environment. However, conditioning also may be possible by forming associations between single cues within the context and the US. If the hippocampus normally mediates contextual processing, then the present data suggest that in the absence of the hippocampus, contextual conditioning may be mediated by associations between single cues in the context and the US. This interpretation supports the prediction that hippocampal lesions should make contextual strategies inoperable and force animals to use cue-based strategies (Nadel & Willner, 1980, p. 226). Although cue-based strategies may be used in contextual conditioning, our results show that they are not optimal. This may be because single cues from the aversive context can occur in other neutral contexts, thus decreasing their predictive value. In contrast, a context is defined by a unique combination of cues, and exactly the same combination is unlikely to occur elsewhere by chance. Therefore, in general, contextual CSs should have greater predictive value than single cues from within that context.

Context Discrimination

Because contextual conditioning may be mediated by either contextual- or cue-based strategies, we tested hippocampal-lesioned mice in a task (Fanselow & Baackes, 1982) that should be optimally solved by contextual-based strategies (McDonald et al., 1995). In context discrimination, mice were trained to discriminate between two similar chambers, one in which they were shocked and the other in which they were not. A key aspect of the design of this task was that the two chambers consisted of both unique cues and shared cues, which led to some specific predictions. If mice use contextual-based strategies, then they should discriminate between the shock and no-shock contexts. If mice use cue-based strategies, then discrimination should occur only if associations are made between unique cues and the occurrence of shock. In contrast, reliance on single associa-

Contextual Conditioning

In Experiment 1, pretraining lesions of the dorsal hippocampus attenuated, but failed to abolish, contextual conditioning. This result suggests that in the absence of the hippocampus, other neural systems can support context conditioning. These data are consistent with recent reports in rats (Gisquet-Verrier & Doyère, 1997; Maren et al., 1997) and mice (Cho et al., in press; Logue et al., 1997). For example, Cho et al. made pretraining ibotenic acid lesions that destroyed approximately 80% of hippocampal neurons. Although these lesions produced severe spatial learning deficits in the Morris water maze, consistent with the effects of hippocampal damage on water maze performance in rats (Morris, Garrud, Rawlins, & O'Keefe, 1982; Sutherland, Kolb, & Wishaw, 1982), they did not block contextual conditioning. Therefore, two different lesion techniques (ibotenic acid and electrolytic), using identical apparatus and procedures, yielded similar results. This indicates that pretraining damage to either hippocampal neurons or hippocampal fibers of passage does not abolish contextual conditioning. However, note that our results show evidence for attenuated conditioned freezing in DH-lesioned mice, indicating that nonhippocampal systems may be less efficient at context recognition.

In contrast, in Experiment 2A, we showed that posttraining DH lesions greatly reduce contextual fear (regardless of whether a tone CS was paired with shock during training). This indicates that although the hippocampus is not essential for contextual conditioning, it is normally involved in processing contextual information. This dissociation between the effects of pre- versus posttraining hippocampal lesions is consistent with a recent report in rats (Maren et al., 1997). In addition, this result provides evidence for a functional interaction between hippocampal and nonhippocampal systems during contextual conditioning. Because posttraining lesions of the hippocampus greatly reduced
tions between shared (and potentially salient; e.g., shock grid) cues and the occurrence of shock should lead to equal levels of conditioned freezing in both chambers.

In contrast to the contextual conditioning experiments, pretraining lesions of the DH disrupted context discrimination. Whereas sham control mice exhibited greater levels of freezing in the chamber associated with shock versus the no-shock chamber, DH-lesioned mice showed robust, but equal, levels of freezing in both chambers. This is consistent with the idea that in the absence of the hippocampus, the animal is biased toward cue-based strategies (Nadel & Willner, 1980). Therefore, although nonhippocampal systems (mediating cue-based learning) can support contextual conditioning, they are inefficient at discriminating between two similar contexts.

Discrimination in sham-lesioned mice may then be mediated by contextual-based strategies, or cue-based strategies in which associations are made between unique cues and the occurrence of shock. The latter seems unlikely because that would mean that the effect of the DH lesion would be to bias mice toward focusing on common (rather than unique) cues in the training contexts. Therefore, these data provide strong evidence that the hippocampus is essential for contextual processing (Nadel et al., 1985).

DH-lesioned mice were eventually able to discriminate between contexts with additional training trials. This indicates that although cue-based strategies are inefficient, they can ultimately lead to discrimination between two contexts if associations are made between the US and unique (rather than shared) single cues. This result confirms that learning about context, rather than single cues within the context, results in more efficient context discrimination. This conclusion underscores the idea that contextual CSs, rather than single cues within the context, have greater predictive value.

After training, both sham- and DH-lesioned mice showed essentially no freezing in a novel chamber which shared no cues with those present in the training environments. Because DH-lesioned mice exhibited no freezing in this novel context, these data indicate that (a) hippocampal lesions do not result in generalized fear to novel places and (b) conditioned freezing in DH-lesioned mice is specific to the cues present during training.

**Nf1^+/−/Nmdar1^+/− Mice and Contextual Fear Conditioning**

**Nf1^+/−/Nmdar1^+/−** mice are impaired in the hidden, but not visible, platform version of the Morris water maze (Silva et al., 1997), suggesting that they are especially impaired in hippocampal-based learning (Morris et al., 1982; Sutherland et al., 1982). In keeping with a hippocampal-dependent learning impairment, we showed that these mutants exhibit normal contextual conditioning but are impaired in context discrimination. Therefore, the parallels between the anatomical and genetic lesion experiments suggest that a functional disruption of the hippocampus impairs context discrimination but not contextual conditioning. Together, these results indicate that context discrimination, rather than contextual conditioning, may be a more sensitive behavioral assay to detect hippocampal-related learning impairments.

**Conclusions**

Taken together, these data suggest the following conclusions. First, contextual conditioning may be mediated by both hippocampal and nonhippocampal systems, although nonhippocampal systems are less efficient. Second, when the hippocampus is normally functioning, contextual conditioning strategies mediated by nonhippocampal systems are suppressed. Third, although the hippocampus is not essential for contextual conditioning, it is crucial for context discrimination. Finally, in keeping with the lesion results, Nf1^+/−/Nmdar1^+/− mutant mice with hippocampal-dependent learning deficits are impaired in context discrimination but not in contextual conditioning, suggesting that context discrimination is a more sensitive measure of hippocampal dysfunction. Together, our results dissociate hippocampal and nonhippocampal contributions to contextual fear conditioning, and they provide direct evidence that the hippocampus plays an essential role in the processing of contextual stimuli. Although nonhippocampal systems can support recognition of an aversive context, they are inefficient at discriminating between two similar contexts.

**References**


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