Supporting Information

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SI Text

Water Maze. The water-maze task is adapted from that described by Packard and McGaugh in rats (1), but is described in detail because it has several novel features.

Animals were trained to escape from a circular pool of room-temperature water (25°C-27°C, made opaque by the addition of non-toxic white latex paint) by locating an escape platform (12 cm × 12.5 cm) hidden 1 cm below the surface, as in the Morris water maze (2, 3). The pool was 164 cm in diameter and located in the middle of a square room with high-contrast spatial cues on all walls (Fig. S1*A*). The platform was marked by one of three interchangeable visible cues. Cues were plastic cylinders (11 cm high, 2.5 cm in diameter), painted neutral gray, with black and white vertical stripes or black and white horizontal stripes (stripe width, ≈1 cm).

The first 5 days consisted of shaping to the task, which we found in pilot studies to be necessary for efficient learning during the second, two-cue phase (Fig. S1*E*; described later). Animals were not handled before day 1. On all experimental days, animals were moved into the water-maze room and allowed to habituate for at least 30 min before the first training trial. On day 1, the platform was marked with the gray cylinder; animals were placed on the platform for 30 seconds and then returned to the home cage. Animals that left the platform within 30 seconds were guided back to it twice, but then returned to the home cage if they continued to leave the platform. This procedure was repeated a total of four times for each animal—once in each quadrant—with 15 to 20 min between exposures.

Days 2 through 5 consisted of one-cue shaping trials. The platform was marked with the gray cylinder and placed in the center of one quadrant (NE, SE, NW, SW; see Fig. S1*A*); each animal was exposed to the platform once in each quadrant, in pseudo-random order, with 15 to 20 min between trials. On each trial, mice were placed in the water, facing away from the wall, at a cardinal point opposite the escape platform (e.g., when the platform was in the NE quadrant, the starting location could be either S or W). They were allowed to search for the escape platform for 120 seconds; if they had not found it after this period, they were gently guided to the escape platform. Mice were allowed to remain on the escape platform for 15 seconds, after which they were returned to their home cage. Animals that left the escape platform during this 15-second period were guided back to it once, but returned to their home cage if they left a second time. Following these shaping trials, animals were left in the vivarium for 2 days.

Two-cue training was performed on days 8 to 12 or 8 to 14 (also called days 1 to 5 or 1 to 7 of the two-cue phase; Fig.S1*E*); on each of these days, animals had four training trials separated by 15 to 20 min. In a two-cue trial, the escape platform was again placed in the center of a quadrant; it was marked by the vertically striped or horizontally striped cue (i.e., the goal cue). The other striped cue (i.e., the lure cue) was placed in an adjacent quadrant, mounted on a stand that held it at precisely the same elevation as the goal cue but did not permit the animal to escape from the water. On each trial the mouse was placed in the pool, facing the pool center, at the cardinal point opposite the two cues, such that it was equidistant from the two. It was allowed to search for the escape platform for 120 seconds, then allowed to rest on the platform for 15 seconds before being returned to the home cage.

In the cued task, the platform had an equal probability of being in each of the four quadrants but was reliably associated with one of the two cues (i.e., the goal cue was always either horizontally or vertically striped). Whether the goal was horizontally or vertically striped was held constant for each animal across all days of training but was counterbalanced across animals in each experimental group. These constraints allow for eight possible combinations of starting location, goal location, and lure location (Fig. S1*B*). Each animal was trained on each of these eight possible configurations once over each 2 days of training, in a pseudo-random order, such that the same location was never reinforced twice in a row and the relationship of the goal to the starting location (i.e., left or right) was never the same more than twice in a row.

In the spatial task, the platform had an equal probability of being associated with either of the striped cues on any given trial but was always in the same location. The lure therefore had an equal probability of being in either of the two adjacent quadrants but was never found in the opposite quadrant; similarly, the starting location was always one of the two cardinal points opposite the goal quadrant. These constraints allow for four possible configurations of starting location, goal location, lure location, and goal cue (Fig. S1*C*). Each animal was trained on each of these four combinations on each day of training, in a random order.

Learning was assayed using a probe trial, administered in place of a training trial on the fourth trial of days 3, 5, and 7 (except where otherwise specified in the text). In a probe trial, cue location was determined exactly as in a regular training trial but no escape platform was present; both cues were placed on stands, and therefore no escape from the pool was possible. Extra-maze cues were identical to those present in a training trial. The mouse's search pattern was recorded by an overhead digital camera for 60 seconds, after which the mouse was removed from the pool and returned to its home cage. Systematic bias toward the goal cue relative to the lure cue was interpreted as evidence of learning in both cued and spatial tasks (Fig. S1*D*). Search pattern was digitized and analyzed using Ethovision (Noldus). Cue bias was quantified in several ways: (*i*) time spent in the quadrant containing the goal or lure (*ii*) integrated proximity to the goal and lure over the 60-second probe trial; and (*iii*) occupancy in a circular zone centered on the goal or lure, with a diameter of twice the width of the platform.

Experiments in Figs. 1 *B* and *C* and 2, which were designed to document a deficit in cued learning (which sometimes was not apparent in pilot experiments until 7 days of training) had 7 days of two-cue training. The experiment in Fig. 1*E*, which was designed to replicate enhanced spatial learning early in two-cue training, had 5 days of two-cue training. The experiment in Fig. 3, which was designed to observe impaired spatial learning and enhanced cued learning, likewise had 5 days of two-cue training.

Validation of Lesions. For immunohistochemical documentation of lesions, brains were rapidly dissected and fixed overnight in 4% paraformaldehyde/PBS solution at 4°C. After fixation, brains were equilibrated with 30% sucrose and sliced on a microtome at 40 μm; slices were stored in cryoprotectant solution (30% glycerin, 30% ethylene glycol, 0.2 × PBS solution) at 4°C. Floating sections were washed three times for 10 min with PBS solution, blocked with PBS/0.3% Triton/2% goat serum (Sigma) for 1 hour with gentle shaking, and then immunostained overnight for GFAP (rabbit polyclonal ant-GFAP IgG, G9269, 1:500; Sigma) and NeuN (mouse monoclonal ant-NeuN IgG, MAB377; 1:1,000; Chemicon International) in PBS/0.3% Triton. The following day, slices were rinsed twice in PBS/0.3% Triton and

twice in PBS solution, stained for 1 h with secondary antibodies (FITC goat anti-rabbit IgG 1:300; rhodamine goat anti-mouse IgG 1:300) in PBS/0.3% Triton/2% goat serum, washed again three times in PBS/0.3% Triton, and mounted on glass slides. GFAP and NeuN immunoreactivity were visualized on an upright Nikon fluorescent microscope.

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2. Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate antagonist, AP5. *Nature* 319:774–776.
3. Vorhees CV, Williams MT (2006) Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protocols* 1:848–858.
4. Gallagher M, Burwell R, Burchinal M (1993) Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. *Behav Neurosci* 107:618–626.

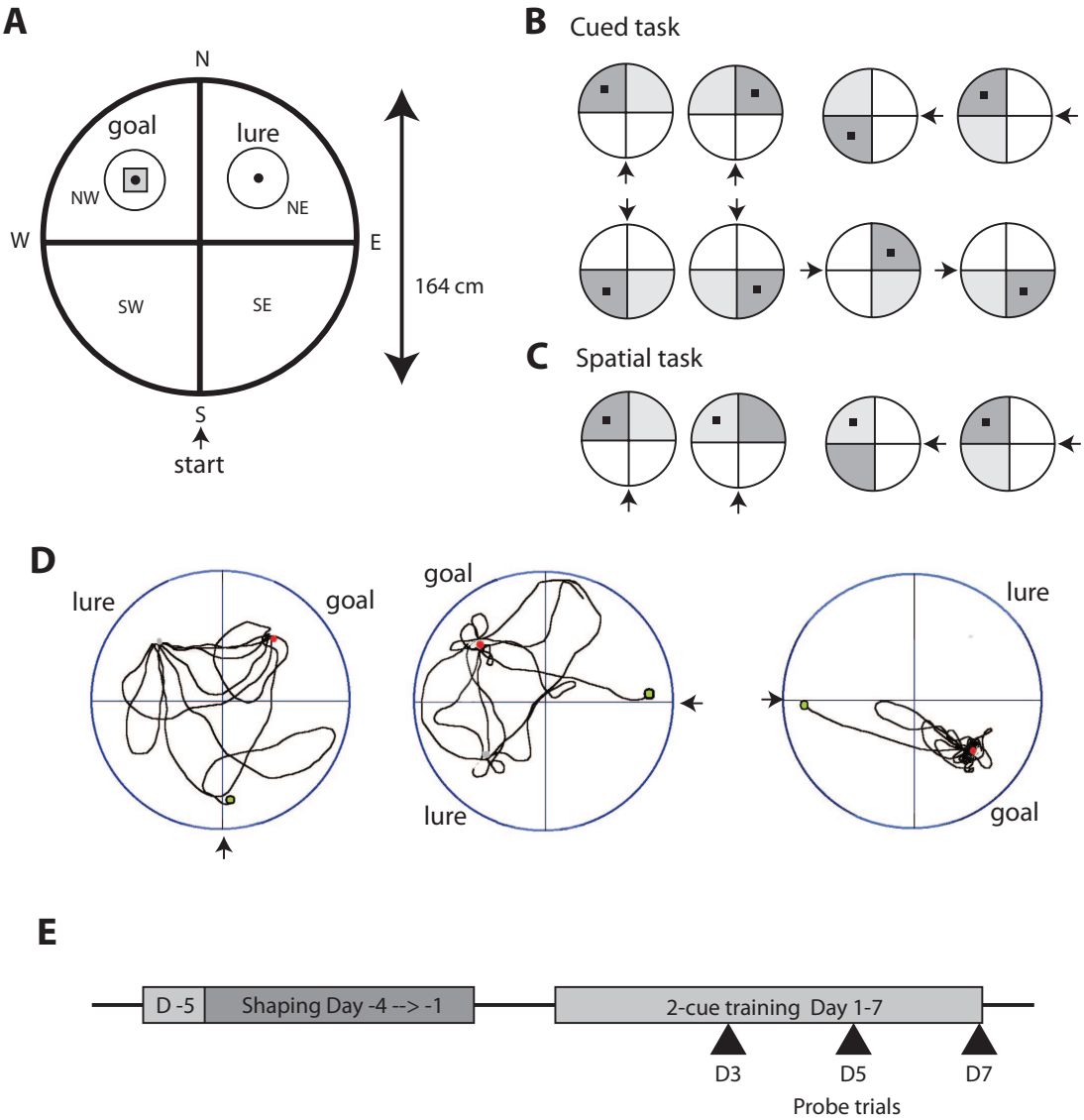

Fig. S1. A two-cue water maze task in mice. (*A*) Layout of the apparatus. A 164-cm-diameter pool is placed in a room (10′ × 10′) with bold spatial cues present on all walls. During training trials, an escape platform (12 cm × 12 cm) is placed 1 cm below the surface of the opacified water in the center of the goal quadrant (the NW quadrant in this illustrative example; platform shown to scale). The platform is marked by a visible cue (a cylinder 11 cm tall and 2.5 cm in diameter), painted either gray, in the shaping phase, or with bold black-and-white vertical or horizontal stripes in the two-cue phase (see *Materials and Methods*). In the two-cue phase, a similar cue is present in an adjacent ''lure'' quadrant (illustrated as NE) but does not permit the animal to escape from the water. At the beginning of a training trial, an animal is placed in the water, facing the middle of the pool, at the cardinal point opposite and equidistant from the goal and lure cues (illustrated as S). (*B*) In the cued task, the escape platform (dark square) has an equal probability of being in any of the four quadrants but is consistently associated with one of the two striped cues (illustrated as dark gray) and not with the other (illustrated as light gray). These constraints permit eight possible configurations of starting location, goal quadrant, and lure quadrant. Each animal is exposed to each configuration once over each block of 2 days throughout training, in a pseudo-random order. The goal cue (i.e., vertical or horizontal stripes) is kept constant throughout training for each mouse but is counterbalanced within each group. (*C*) In the spatial task, the escape platform is always in the same location (NW in this illustration) but has an equal probability of being associated with each of the two striped cues. These constraints allow four possible configurations for each goal location, as illustrated. Each animal is exposed to each of these configurations once during each training day, in pseudo-random order. Goal location is kept constant for each animal throughout training but is counterbalanced within each group. Importantly, the spatial and cued task have identical sensory, motor, and motivational requirements. It is not possible to distinguish between the two tasks on the basis of a single training trial, but only by observing consistencies across trials. (*D*) Learning is assayed in a probe trial. The configuration of starting location and goal and lure cues is the same as in a training trial, but no escape platform is present; an animal's search strategy is recorded over 60 seconds. Sample search patterns illustrating no bias (*Left*), moderate bias (*Middle*), and extreme bias (*Right*) are shown. Probe trial performance is quantified by comparing occupancy in the goal quadrant with that in the lure quadrant. Probe trial performance can alternatively be assayed using occupancy in a circular zone around goal and lure cues (25-cm diameter; see Fig. S1*A*) or the Gallagher proximity measure (4); all measures showed similar effects in all experiments. (*E*) Timeline of the protocol. Animals are initially shaped to the task; on day 1 they are placed on the platform four times, whereas on days 2 through 5 they are trained to swim to a single gray cue four times. In the two-cue phase they go through four trials per day to escape using either a cued or a spatial strategy; see *Materials and Methods* for details. Probe trials are conducted on the last trial of days 3, 5, and 7.

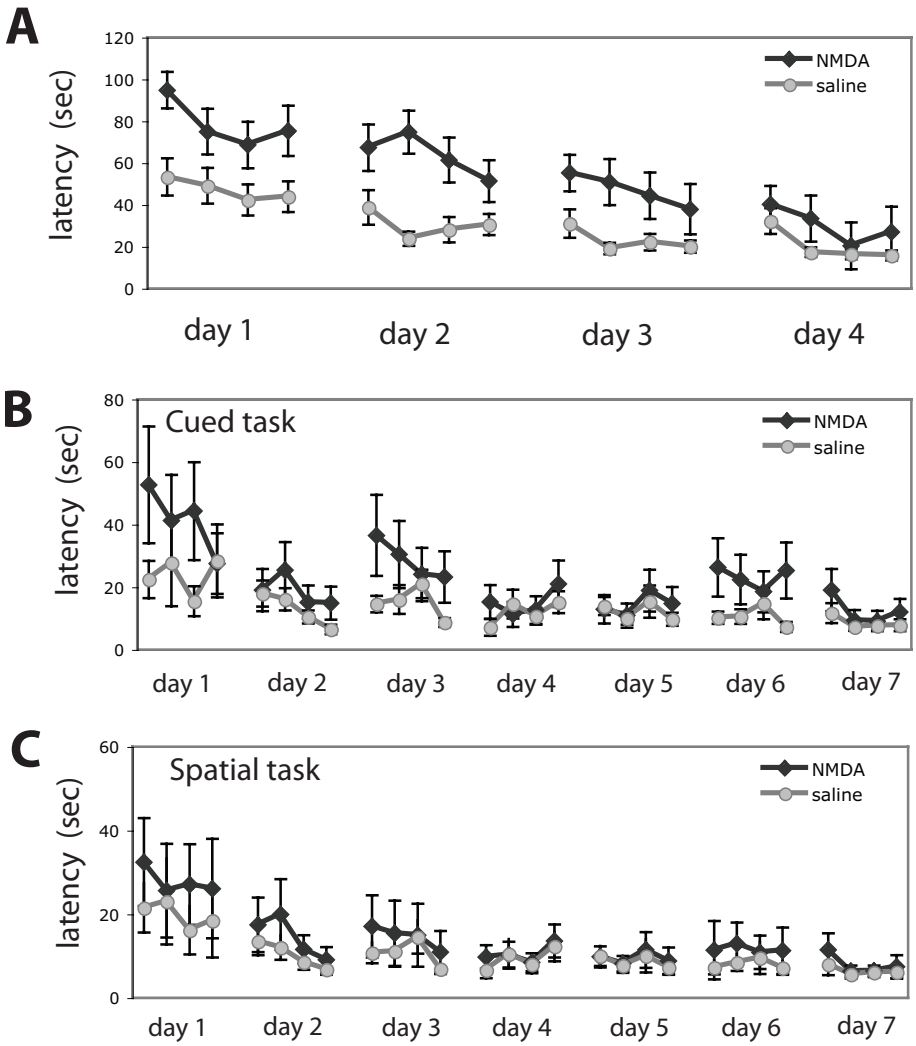

Fig. S2. Effect of dorsal striatal lesion on cued and spatial learning. (*A*) Latencies in one-cue shaping task for the striatal lesion experiment shown in Fig. 1 *A–C* (mean ± SEM). Striatal lesions in this experiment slowed learning in this shaping task. Three-way multivariate ANOVA with lesion as the independent factor and day and trial as nested repeated measures showed a main effect of lesion ($P < 0.001$), day ($P < 0.002$), and trial ($P < 0.01$) and a day-lesion interaction ($P < 0.005$). By the fourth day of one-cue training, the performance of lesioned animals approached that of controls such that the difference between them was only at trend level (one-way ANOVA with lesion as the independent factor and trial as a repeated measure on day 4 only: main effect of trial, $P < 0.01$; main effect of lesion, $P = 0.06$; lesion-trial interaction, $P > 0.2$). The effect of lesion on one-cue latency from the first day of training (i.e., unlikely to be solely a learning effect) raises concerns about the contribution of motor or other deficits to observed effects in the two-cue task; however, as the spatial and cued two-cue tasks have identical sensory, motor, and motivational requirements, normal (or even enhanced) performance in the spatial task provides a better control for these parameters than does performance during one-cue shaping (see Fig. 1*C*). (B) *Latencies during two-cue training in the cued task (mean ± SEM;* n = *8 NMDA,* n = *9 saline solution). ANOVA with task and lesion as between-subjects factors and with day and trial as nested repeated measures revealed a significant main effect of day* (P < *0.001), significant effects of lesion and of task (both* P < *0.01), a trend toward an effect of trial* (P = *0.07), and a significant day-lesion interaction* (P < *0.01). In the cued task, latencies improved over days (ANOVA with lesion as the independent factor and day and trial as nested repeated measures; main effect of day,* P < *0.05) and were significantly slower in the lesioned group* (P = *0.05).* (C) *Latencies during two-cue training in the spatial task (mean ± SEM;* n = *8 NMDA,* n = *9 saline solution). Latencies improved during training (ANOVA, main effect of day,* P < *0.001) and were slightly elevated on day 1 in the lesioned group, leading to a main effect of lesion* (P < *0.04) and a day-lesion interaction* (P < *0.01).*

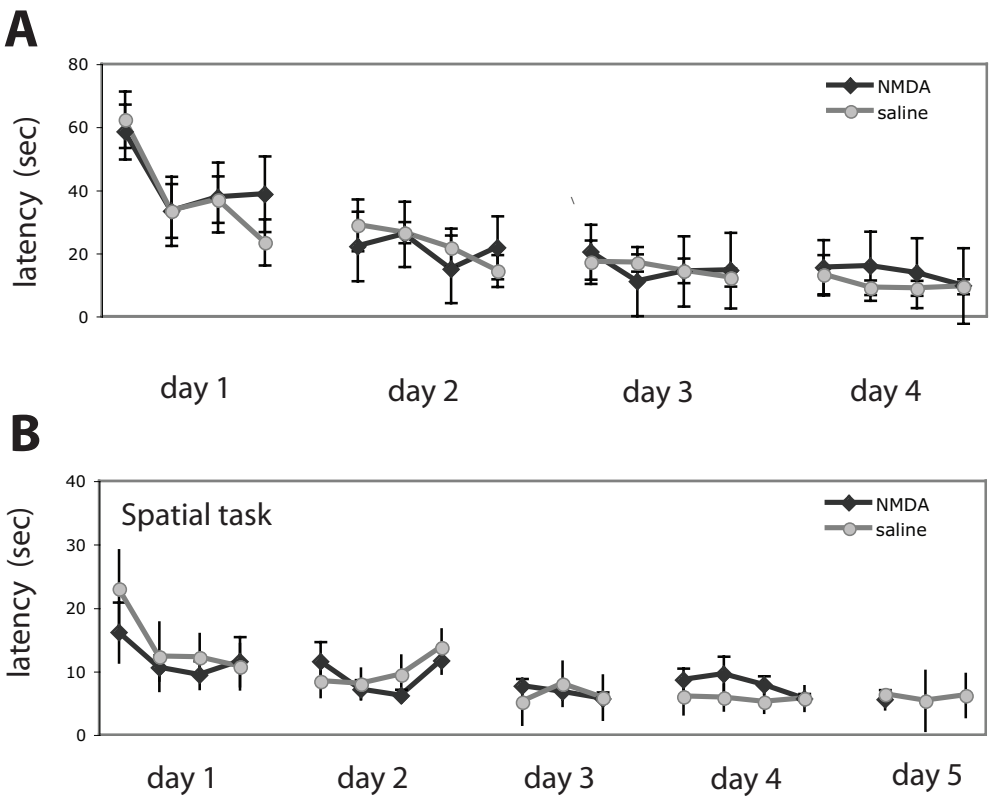

Fig. S3. Replication of effects of dorsal striatal lesions on spatial learning. (*A*) In this replication experiment (Fig. 1 *D* and *E*), animals with dorsal striatal lesions showed equivalent latencies in the one-cue shaping task to control animals. ANOVA with day and trial as nested repeated measures showed a main effect of day and trial ($P < 0.001$) and a day–trial interaction ($P < 0.01$) but no effect of lesion or interactions (all $P > 0.1$). (*B*) Striatal lesions had no effect on latencies during learning of the two-cue spatial task. ANOVA with day and trial as nested repeated measures showed a main effect of day ($P < 0.01$) but no significant effect of lesion and no significant interactions (all $P > 0.1$). The fact that enhanced spatial learning was again seen in a cohort of animals in which latencies during training were unchanged rules out the possibility that the enhancement of spatial learning in the first experiment (Fig. 1 *A–C;* Fig. S2) was related to the longer latencies during training.

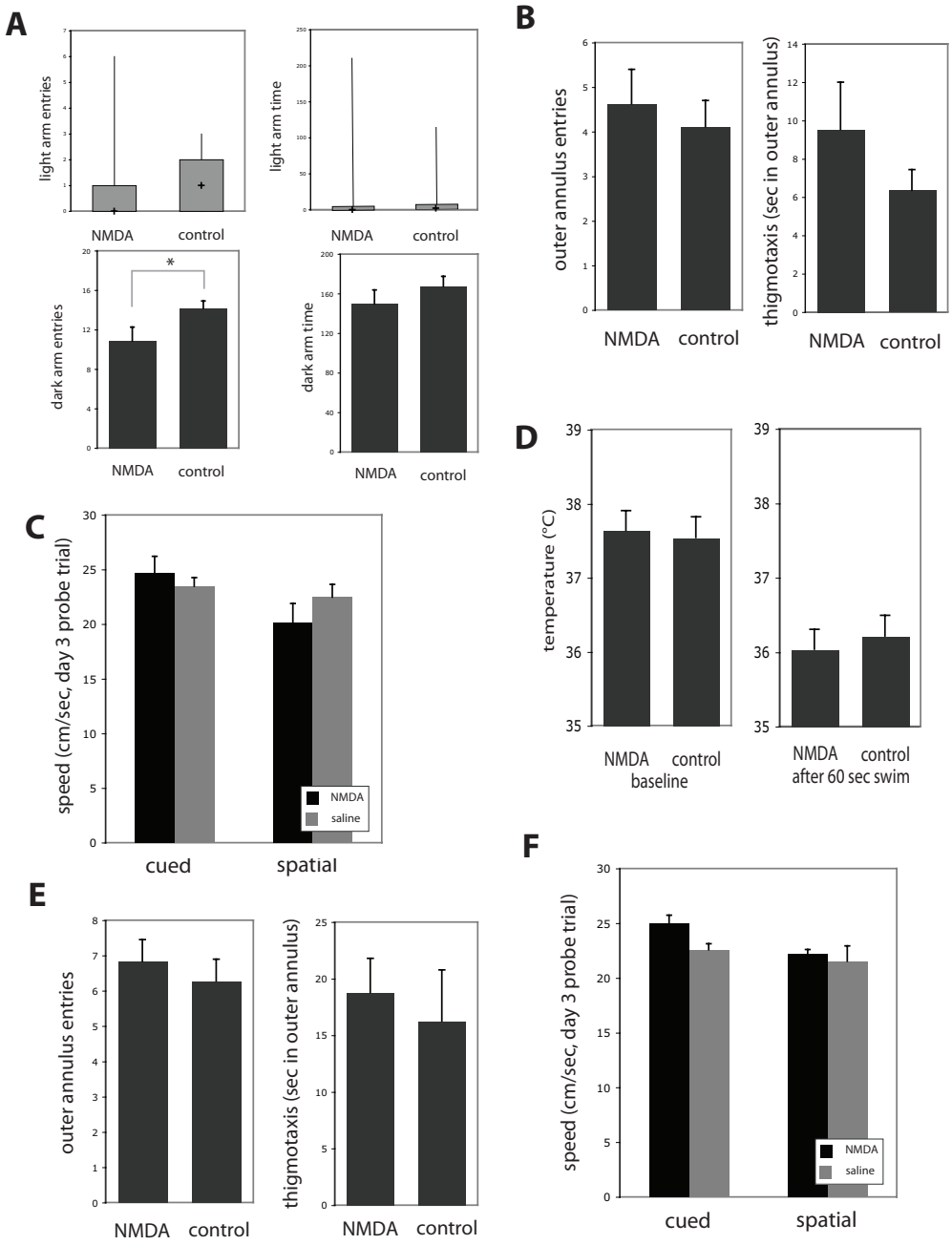

Fig. S4. Striatal lesions do not alter anxiety or swim speed or disrupt thermoregulation. (*A*) It has been argued that increasing anxiety can increase striatum-dependent navigation strategies at the cost of spatial learning strategies; our results therefore might be explained by decreased anxiety in striatum-lesioned animals. To evaluate this possibility, animals with large dorsal striatal lesions (see Fig. 1*A*) were tested in the elevated plus maze, a standard test of anxiety. Lesioned and control animals showed no difference in entries into or time spent in the light arms ($n = 16$ lesioned, $n = 18$ control; minimum–first quartile–median–third quartile–maximum; Mann-Whitney *U* test: all $P > 0.05$) or in time spent in the dark arms (mean ± SEM; *t* test, $P > 0.2$), but they did enter the dark arms slightly less frequently than controls (*t* test, $P < 0.05$). This pattern of results is consistent with a slightly reduced overall level of exploratory activity but not with altered anxiety. (*B*) Thigmotaxis, or tendency to ''hug'' the wall during exploration in the water maze, is thought to reflect anxiety level during the trial. Thigmotaxis was measured during probe trials; data are shown from the day-3 probe trial (see Fig. 2). There was no difference between lesioned animals ($n = 16$; mean ± SEM) and controls ($n = 18$) in either entries into or time spent in a 5-cm annulus around the edge of the pool (*t* test, all $P > 0.2$). There was likewise no main effect of task or task–lesion interaction in 2×2 ANOVA when animals were split by task (all main effects and interactions, $P > 0.2$). (*C*) Striatal lesions do not alter swim speed. Swim speed was measured on all probe trial days; data are shown from day 3. There was no significant effect of lesion on swim speed in either the cued task (mean ± SEM; *t* test, $P > 0.2$ for both tasks). (*D*) Striatal lesions do not disrupt thermoregulation. Temperature was measured using s.c. capsules in NMDA lesioned and control animals ($n = 8$ each group) before (*Left*; mean ± SEM) and immediately after (*Right*) a 1-minute swim. No significant differences between groups were seen in either group (*t* test, all $P > 0.2$). (*E*) The effect of dorsal striatal lesions on anxiety, as measured by thigmotaxis, was also examined in the replication experiment (Fig. 1*E*); data from the day-3 probe trial are shown. No significant differences between groups were found (mean ± SEM; all $P > 0.2$). (*F*) These lesions likewise had no effect on swim speed in either the cued or spatial task; data from the day-3 probe trial are shown. No significant differences between groups were found (mean ± SEM; *t* test, all $P > 0.1$).

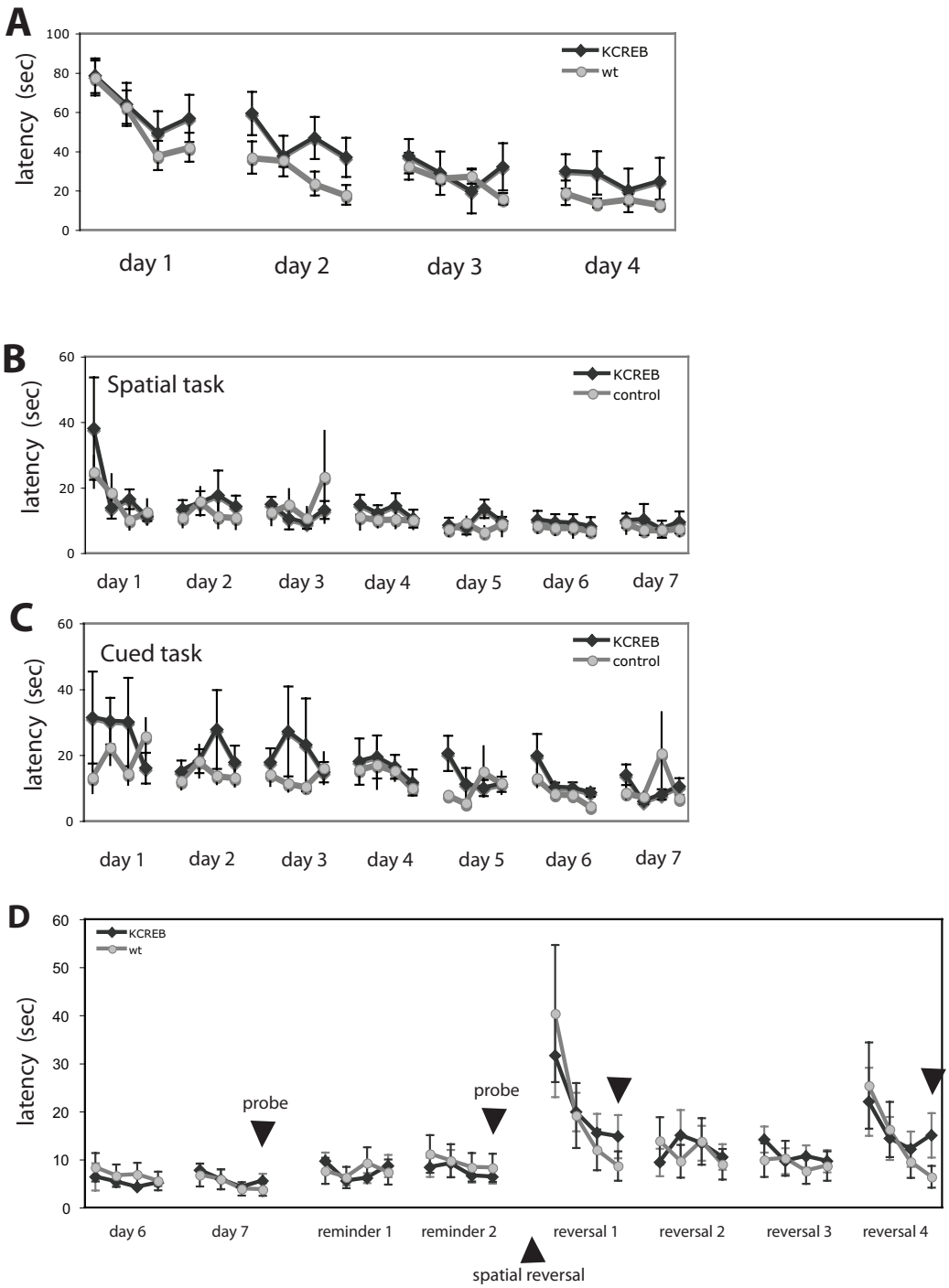

Fig. S5. Cued and spatial learning in str-KCREB transgenic mice. (*A*) Disruption of striatal CREB function through transgenic expression of a dominant-interfering transgene did not affect learning in the one-cue shaping task. ANOVA with genotype as the independent factor and day and trial as nested repeated measures revealed main effects of day and trial (both $P < 0.001$) and a day–trial interaction ($P < 0.005$), but no main effect of genotype or interactions. (*B*) In the two-cue task there was a main effect of day (ANOVA with nested repeated measures; $P < 0.001$) and task ($P = 0.001$), but no main effect of genotype or interactions. Analyzing only animals trained in the spatial task (one-way ANOVA with nested repeated measures) again showed a main effect of day ($P < 0.001$), a trend-level effect of trial ($P = 0.08$), and a day–trial interaction ($P < 0.005$), but no significant main effect of genotype or interactions. (*C*) Latencies in the cued task showed a main effect of day (one-way ANOVA with nested repeated measures, $P < 0.001$), but no main effect of genotype or interactions. (*D*) Latencies in the reversal experiment. Both genotypes, trained in the spatial task, showed equivalently low latencies to escape during the last two trials of the initial spatial training (re-plotted from *B*) and on the two reminder training days. Latencies increased dramatically after spatial reversal, with no statistically significant difference between groups, and quickly declined with ongoing training.

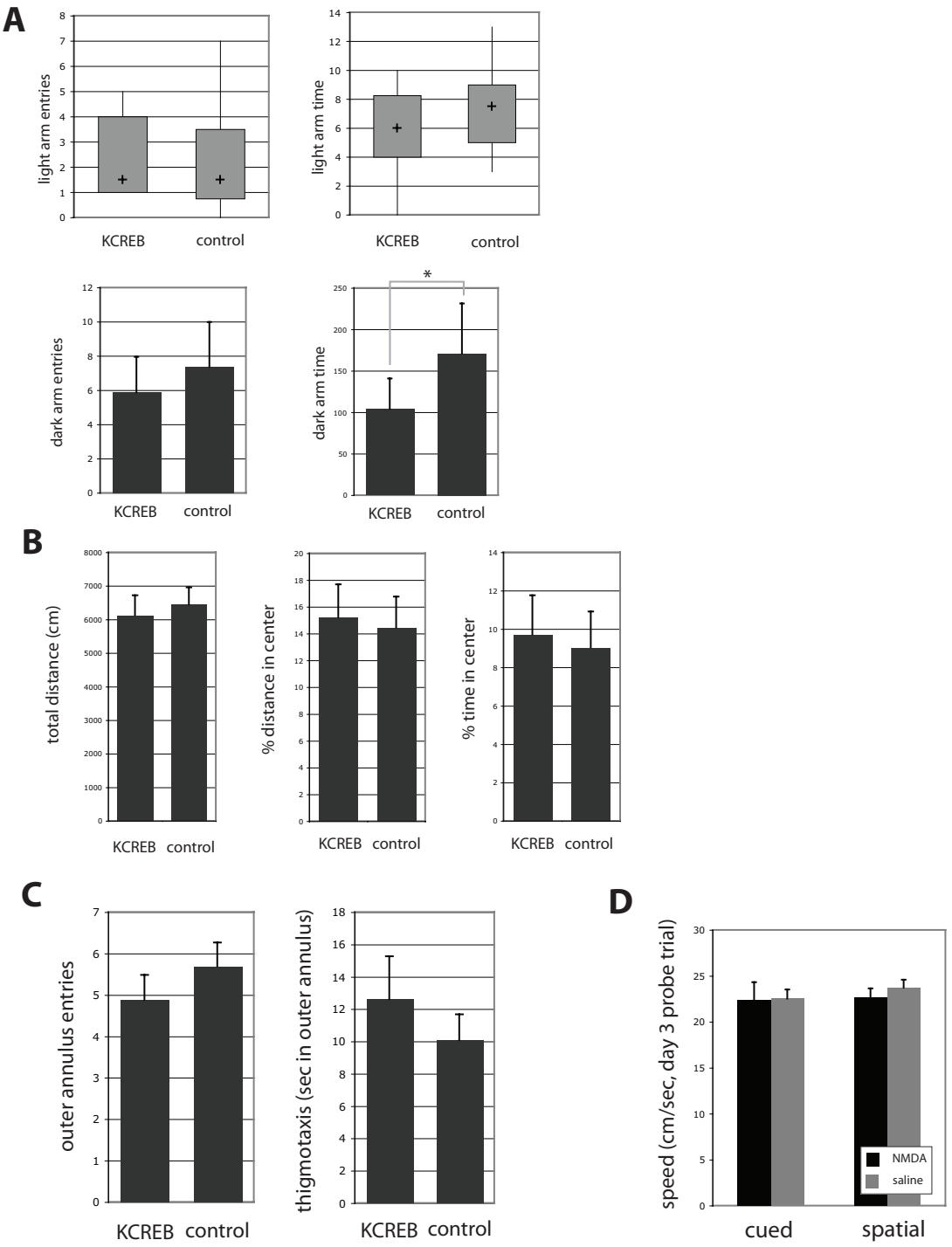

Fig. S6. Tests of anxiety and activity in KCREB transgenic mice. We tested KCREB mice and litter-mate controls for anxiety to exclude the possibility that decreased anxiety may contribute to the observed enhancement in spatial learning and impairment in cued learning (Fig. 6). (*A*) In the elevated plus maze, there was no difference between transgenic mice ($n = 8$) and controls ($n = 8$) in entries into or time spent in the open arms (minimum–first quartile–median–third quartile–maximum; Mann-Whitney test, $P > 0.2$), or in the entries into the closed arms (mean ± SEM; *t* test, $P > 0.2$). The KCREB mice did have a greater tendency to stay in the middle of the plus maze, resulting in a significantly lower dark arm dwell time (*t* test, $P < 0.05$); however, this pattern of results is not suggestive of altered anxiety. (*B*) In the open field, KCREB and control mice showed similar overall activity, as has been reported previously (16), and similar time spent in the center—a measure of anxiety—as measured either by percent path length or percent time ($n = 8, 8$; mean ± SEM; *t* test, all $P > 0.5$). (*C*) Thigmotaxis was similar between KCREB mice and controls, as measured either by entries into or time spent in the outer annulus. Data shown are from the day-3 probe trial ($n = 16, 16$; mean ± SEM; *t* test, all $P > 0.2$). There were likewise no significant effects in 2×2 ANOVA when animals were further subdivided based on the task (i.e., spatial or cued). (*D*) KCREB and control animals swam at similar speeds in both tasks; data from the day-3 probe trial are shown. No significant differences between groups were found (mean ± SEM; *t* test, all $P > 0.1$).

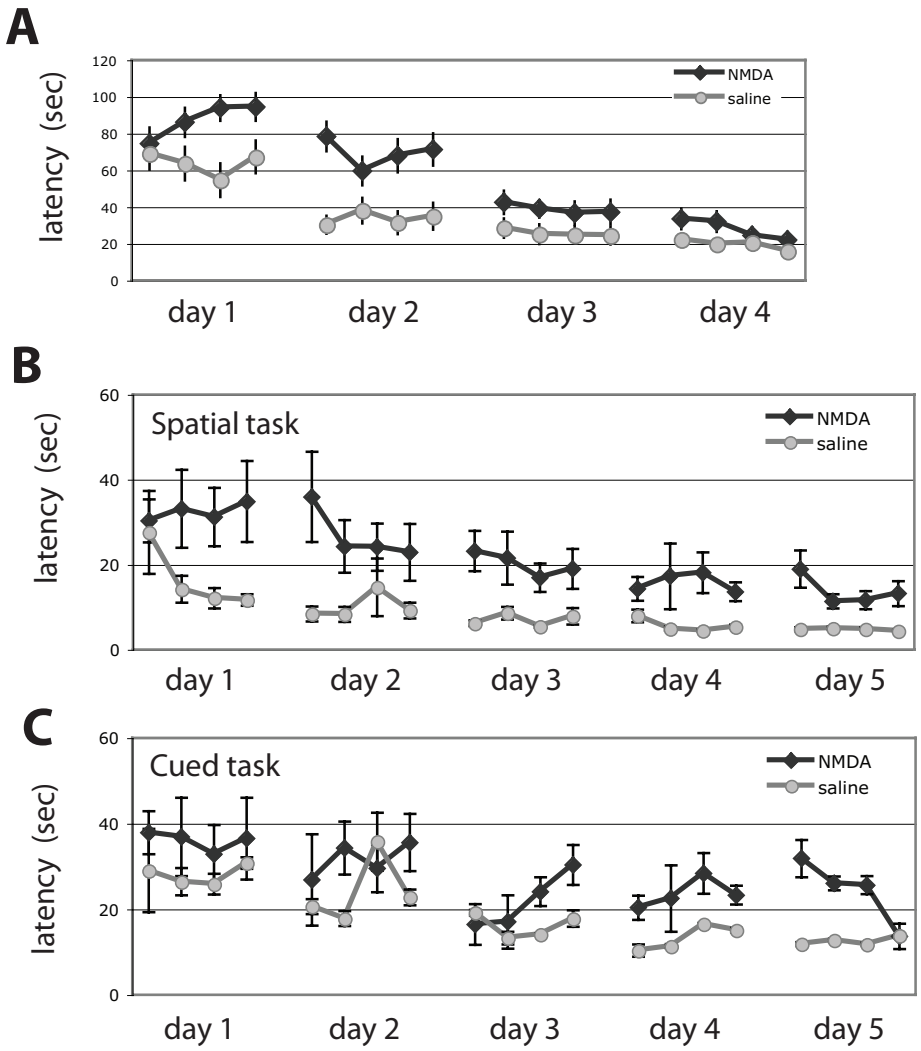

Fig. S7. Cued and spatial learning after hippocampal lesions. (*A*) Lesioned mice showed longer latencies early in the one-cue shaping task, but achieved equivalent escape performance by the end of the fourth day. ANOVA (lesion as the independent factor; day and trial as nested repeated measures) revealed a main effect of day ($P < 0.001$) and of lesion ($P < 0.05$). However, as in the first experiment with large striatal lesions (Fig. 2; Fig. S4), this difference in one-cue latencies is unlikely to explain the subsequently observed deficit in spatial learning (Fig. 3), because latencies were equivalent at the end of one-cue training and because performance was actually better in the probe trial in the two-cue cued task in the lesioned animals than in controls (Fig. 3); cued and spatial tasks share all sensory, motor, and motivational characteristics. (*B*) Latencies in the two-cue phase of the spatial task. ANOVA with nested repeated measures for day and trial showed main effects of day and lesion (both $P < 0.001$). Lesioned animals showed higher latencies throughout, consistent with a deficit in spatial learning (although latencies are also affected by nonspecific factors). (*C*) Latencies in the two-cue phase of the cued task. ANOVA showed a main effect of day ($P < 0.001$) and of lesion ($P < 0.05$).

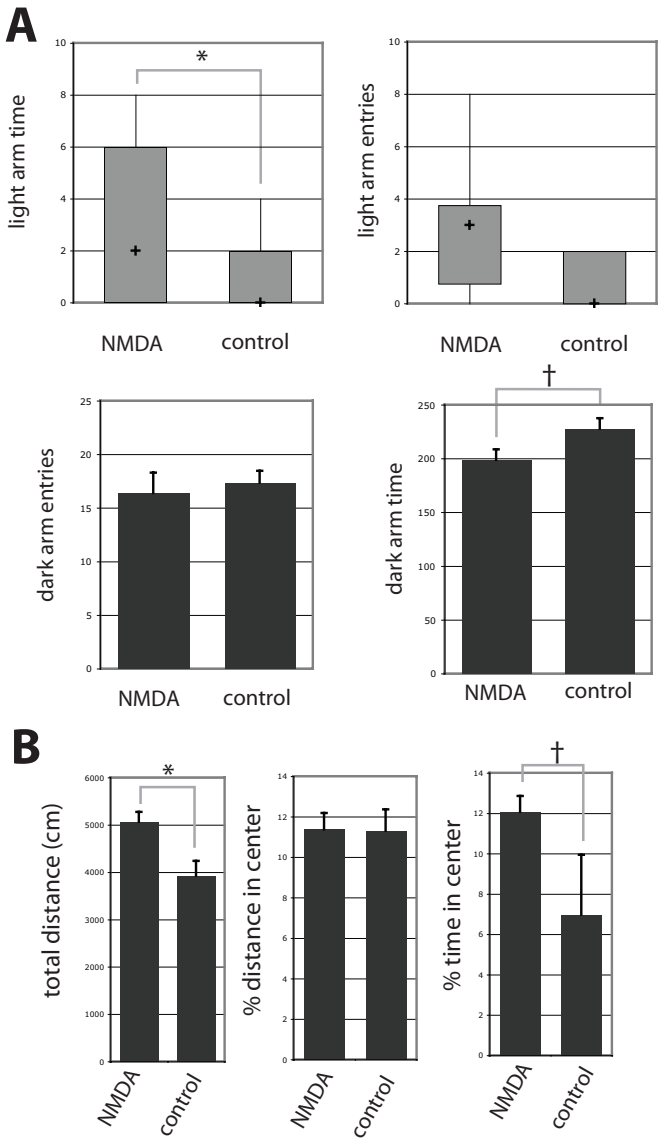

Fig. S8. Hippocampus-lesioned mice show a trend toward reduced anxiety. As noted earlier, increased anxiety has been shown to potentiate striatum-dependent learning in some contexts; increased anxiety in our hippocampus-lesioned animals could therefore partially explain our results (Fig. 3). To evaluate this possibility, we analyzed anxiety in hippocampus-lesioned animals (Fig. 3) in the elevated plus maze. (*A*) Lesioned animals spent more time in the open arms ($n = 16$ lesioned, $n = 17$ control; minimum–first quartile–median–third quartile–maximum; Mann-Whitney U test, $P < 0.05$) and significantly less time in the closed arms (t test, $P < 0.05$); this pattern is consistent with reduced anxiety. This renders it unlikely that the lesions explain the impairment of spatial learning and enhancement of cued learning that we observe. There was no significant difference in the two groups' entries into open and closed arms. (*B*) These animals were also analyzed in the open field to assay basal locomotor activity and anxiety. Over a 10-min session, lesioned mice exhibited significantly more exploratory activity than controls ($n = 23$ NMDA, $n = 22$ controls; t test, $P < 0.005$). There was a trend toward lesioned animals spending a greater percentage of their time in the center, which would be indicative of reduced anxiety, but it did not reach significance (t test, $P > 0.1$); there was no difference in the percentage path length spent in the center by the two groups (t test, $P > 0.2$). (*C*) Finally, hippocampal lesions produced no significant effect on swim speed in either the cued or the spatial task; data from the day-3 probe trial are shown. No significant differences between groups were found (mean ± SEM; t test, all $P > 0.1$).