Supporting Text

Supporting Methods

Subjects. *Experiment 1.* The subjects were 92 male Long–Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed individually and maintained on a 12:12-h light–dark cycle. Food and water were freely available. Rats were assigned to one of five groups, a sham-operated control group (SHAM; $n = 36$) and four groups with bilateral ibotenic acid (IBO) lesions of the hippocampus that varied in septotemporal extent ($n = 56$). Following recovery from surgery, rats were trained on a spatial memory task in the water maze.

Experiment 2. The subjects were 56 male Long–Evans rats weighing 300–350 g at the beginning of the experiment. Rats were housed individually and maintained on a 12:12-hr light–dark cycle. Food and water were freely available. Rats were assigned to one of four groups, a sham-operated control group (SHAM; $n = 30$), two groups (each $n = 8$) with bilateral IBO lesions of the dorsal hippocampus that varied in septotemporal extent (50–75% and 75–100% of total hippocampal volume), and one group ($n = 10$) with bilateral IBO lesions of the ventral hippocampus that involved ≈50% of total hippocampal volume. Following recovery from surgery, rats were trained first on the novel object recognition (NOR) task and then on the spatial memory task in the water maze.

Apparatus. Spatial memory testing was conducted in the Morris water maze (1.8-m diameter) with an "Atlantis platform" (12.7-cm diameter; Spooner *et al.*, ref. 1), which could be raised or lowered remotely during a trial. The platform was located in the center of the northeast quadrant of the pool throughout spatial and visual platform testing. The water was rendered opaque by the addition of powdered milk, and the room was illuminated by four 30-W spotlights pointed at a white ceiling. The water was maintained at room temperature. The testing room contained a number of constant, salient visual cues (posters, objects, and equipment), and an opaque curtain shielded the experimenter from the view of the rat once the trial began. A video camera was mounted on the ceiling directly above the pool and was used in conjunction with a video tracking system (San Diego Instruments, San Diego) to record the swim path of each rat.

NOR was tested in an opaque plastic chamber [35 cm × 41.5 cm × 50 cm (height)]. The stimuli were a plastic animal head and a multicolored jewelry box. Three identical copies of each object were available. A video camera mounted on the wall directly above the chamber was used to record the testing session for offline analysis. Overhead fluorescent lighting illuminated the testing area.

Procedures. *Spatial training.* Each rat received one training session daily for 5 consecutive days. Each daily session began with a single reinforced probe trial, followed by four training trials. For the probe trials, the platform was lowered so that it was inaccessible, and the rat was placed in the water facing the pool wall at one of four start points (north, south, east, or west). The particular start point was counterbalanced across trials for all animals. Upon release into the water, the rat was allowed to swim for 60 sec,

at which point the platform was raised to within 1.5 cm of the water surface. An additional 60 sec was then allowed for the rat to locate the platform and escape from the water. After escaping, the rat remained on the platform for 30 sec before being removed. If the rat failed to escape, it was guided to the platform and remained there for 30 sec.

At the completion of the daily probe trial, four training trials were given with the platform in the raised position (1.5 cm below the water surface), where it remained invisible to the rat but provided a means of escape from the water. The procedure was the same as for the probe trials, except that the rat was allowed 120 sec to find the platform. The intertrial interval for the training trials ranged from 6 to 21 min.

Visual platform testing. Two days after completion of spatial platform training, the rats were tested with the platform in the same location but now 1.5 cm above the water and made salient by the addition of high-contrast colored tape. Rats were given four trials daily for 2 consecutive days. These trials were conducted in the same way as the spatial training trials (intertrial interval = 6–8 min).

NOR. Rats were acclimated to the testing room and chamber 1 day before testing (45 min in the testing room and 5-min exploration of the empty chamber). On the day of testing, a rat was first acclimated again to the testing room for 45 min and then placed in the empty chamber for 1 min. Then the rat was removed, and two identical objects were placed centrally in the chamber (9 cm apart). The rat was then allowed to explore the chamber and the objects for 15 min. After a delay of 3 h, the rat was returned to an empty chamber for 1 min and then reintroduced to the chamber in the presence of two objects: a novel object and a copy of the previously encountered object. The rat was allowed to explore until it accumulated 30 sec of contact time with the objects (nose within 2 cm of object and vibrissae moving). The score was the percent time (compared with the familiar object) that a rat spent exploring the novel object (see ref. 2 for additional details).

Which object was novel (plastic animal head or jewelry box), and the left/right position of the novel object were counterbalanced within each group. The experimenter was blind to the group membership of the rats during testing and offline data analysis. After completion of the NOR task, the rats were trained on the water maze.

Surgery and Histology. Anesthesia was maintained throughout surgery with isoflurane gas (0.8–2.0% isoflurane delivered in O_2 at 1 liter/min). The rat was placed in a stereotaxic instrument (Kopf Instruments, Tujunga, CA), and the incisor bar was adjusted until bregma was level with lambda. For the four lesion groups, bilateral excitotoxic hippocampal lesions were produced by local microinjections of ibotenic acid (IBO, Biosearch). IBO was dissolved in 0.1 M PBS to provide a solution with a concentration of 10 mg/ml, pH 7.4. IBO was injected at a rate of 0.1 μl/min with a 10-μl Hamilton syringe mounted on a stereotaxic frame and held with a microinjector (model 5000, Kopf Instruments). The syringe needle was lowered to the target coordinate and left in place for 1 min before beginning the injection. Following the injection, the syringe needle was left in place for a further 2 min to reduce the spread of IBO up the needle tract.

In experiment 1, four sets of surgical coordinates (modified from Jarrard, ref. 3) were used to create lesions of the dorsal hippocampus and to damage a range of hippocampal tissue, beginning in the dorsal portion. The first injection of IBO was always in the anterior-most aspect of the dorsal hippocampus (bregma –2.4), and additional injections moved progressively caudal and ventral. Lesions of different extent were made by injecting a total of either 0.165 μl of IBO into 6 sites within each hippocampus, 0.255 μl of IBO into 9 sites within each hippocampus, 0.34 μl of IBO into 12 sites within each hippocampus, or a total of 0.51 μl of IBO into 18 sites within each hippocampus (see Clark *et al.*, ref. 2). In experiment 2, to produce dorsal hippocampal lesions that damage 50–75% and 75–100% of total hippocampal volume, either a total of 0.34 μl of IBO was injected into 12 sites within each hippocampus, or a total of 0.51 μl of IBO was injected into 18 sites within each hippocampus. A third set of lesion coordinates was used to produce ventral hippocampal lesions that damaged ≈50% of total hippocampal volume. Ventral lesions were made by injecting a total of 0.355 μl of IBO into 13 sites within each hippocampus.

The procedure for the SHAM group was the same as for the lesion groups, with the exception that the dura was not punctured, the syringe needle was not lowered into the cortex, and no IBO was injected. Once awake and responsive, each rat was returned to its home cage in the colony room for a 14-day recovery period. In experiment 1, water-maze training began 5-7 weeks following surgery and after other behavioral testing (not reported here).

At completion of testing, the rats were administered an overdose of sodium pentobarbital and perfused transcardially with buffered 0.9% NaCl solution followed by 10% formaldehyde solution (in 0.1 M phosphate buffer, pH 7.4). The brains were then removed and cryoprotected in 20% glycerol/10% formaldehyde. Coronal sections (50 μm) were cut with a freezing Microtome beginning at the level of the anterior commissure and continuing caudally through the length of the hippocampus. Every fifth section was mounted and stained with thionin to assess the extent of the lesions.

To calculate the extent of damage, the damaged region (defined as tissue that was either missing or necrotic) was drawn onto the appropriate coronal section (4) by using an MZ6-series microscope (Leica, Deerfield, IL) and a computer-assisted drawing program. A software tool was then used to calculate the area of damage at each coronal level, and the sum of the damaged sections was calculated as a percent of normal area (as derived from the representative atlas sections). The total damage was the average of the damage on the left and right sides. Hippocampal damage was calculated from 11 coronal sections (bregma –2.88 to –6.8 mm in half-millimeter intervals), damage to the subiculum was calculated from five coronal sections (bregma –4.80 to –6.80 mm in half-millimeter intervals).

Supporting Results

Experiment 1. *Neurohistological findings.* On the basis of the size of the dorsal hippocampal lesion, rats were assigned to one of four lesion groups without knowledge of

their behavioral data; 5–30% (n = 5), 30–50% (n = 24), 50–75% (n = 11), or 75–100% (n = 16) of total hippocampal volume. All rats sustained bilateral damage to all of the cell fields of the hippocampus (CA cell fields, dentate gyrus). The extent of damage to the left and right hippocampus was similar for all groups (P values >0.1). In cases where the lesion was not complete at a particular level of the dorsal hippocampus, the sparing was typically restricted to the most medial aspect of the dentate gyrus or CA1 cell field. In all rats there was minor damage to the cortex and to the fimbria overlying the dorsal hippocampus, which was associated with the placement of the Hamilton syringe during surgery. There was no evidence of damage to the amygdala or perirhinal cortex (see Fig. 6).

Dorsal 5–30%. Five rats had lesions that involved 5.5–28.4% of total hippocampal volume, with an average lesion size of 20.4%. One rat also had minor damage to the dorsal subiculum (<1%).

Dorsal 30–50%. Twenty-four rats had lesions that involved 30.2–49% of total hippocampal volume, with an average lesion size of 39.7%. In nine rats, there was minor damage to the subiculum (<5%).

Dorsal 50–75%. Eleven rats had lesions that involved 50.6–70.3% of total hippocampal volume, with an average lesion size of 57.3%. Nine rats also had minor damage to the subiculum (mean = 3.2%, range = 0.1–12.4%).

Dorsal 75–100%. Sixteen rats had lesions that involved 76.2–97.3% of total hippocampal volume, with an average lesion size of 85.5%. In all cases, the lesion extended into the subiculum (mean = 28.4%, range = 3.1–65.9%). In 11 of the rats in this group, there was also very minor bilateral damage to the entorhinal cortex (see Fig. 8).

Experiment 2. *Neurohistological findings.* All rats sustained bilateral damage to all cell fields of the hippocampus. The extent of damage to the left and right hippocampus was similar for all groups (P values >0.4). There was minor damage to the cortex and to the fimbria overlying the dorsal hippocampus that was associated with the placement of the Hamilton syringe during surgery. There was no damage to either the amygdala or the perirhinal cortex.

Dorsal hippocampal lesions. In cases where the lesion was not complete at a particular level of the dorsal hippocampus, the sparing was restricted to the most medial aspect of the dentate gyrus or dorsal CA1 cell field.

Dorsal 50–75%. Eight rats had lesions that involved 51.8–70.3% of total hippocampal volume with an average lesion size of 57.2%. Additionally, all rats sustained damage to the subiculum (mean = 10.6%, range = 1.5–21%). One rat had minor unilateral encroachment of the lesion into the right entorhinal cortex (see Fig. 8).

Dorsal 75–100%. Eight rats had lesions that involved 76.6–85.7% of total hippocampal volume, with an average lesion size of 82.9%. All rats also sustained damage to the

subiculum (mean = 15.1%, range = 0.7–33.9%). Two rats had minor bilateral encroachment into the entorhinal cortex, and an additional rat had minor unilateral damage to the right entorhinal cortex (see Fig. 8).

Ventral hippocampal lesions. Ventral hippocampal lesions ranged in size from 43.9% to 53.5% of total hippocampal volume, with an average lesion size of 49.5%. In cases where the lesion was not complete at a particular level, the sparing was generally restricted to the medial dorsal hippocampus, or a portion of the ventral lateralmost CA1/2 cell field. All rats also sustained damage to the subiculum (mean = 18.3%, range = 6.7–31.7%). One rat also had minor unilateral damage to the left entorhinal cortex, and an additional rat had minor bilateral damage to the entorhinal cortex. Last, all had minor damage to nuclei of the dorsolateral and ventrolateral geniculate, as well as the optic tract, which was associated with the placement of the Hamilton syringe (see Figs. 7 and 8).

Experiment 1: Latency. Repeated-measures ANOVA revealed that during the course of training, the SHAM and dorsal hippocampal lesion groups (5–30%, 30–50%, 50–75% and 75–100%) reduced the time needed to escape to the platform [$F(4, 348) = 75.9$, $P < 0.0001$]. There also was a main effect of group [$F(4, 87) = 14.7$, $P < 0.0001$] and a Group × Session interaction ([$F(16, 348) = 4.0$, $P < 0.0001$], indicating that the groups differed in their mean latencies to find the platform and in the rate of learning across the training sessions.

Planned *t* tests revealed that rats with dorsal hippocampal lesions that damaged only 5–30% of total hippocampal volume performed similarly to the SHAM group [Fig. 1*A*; sessions 1–5: SHAM group, 16.1 ± 1.0 sec; 5–30% group, 13.2 ± 1.7 sec; $t(39) = 1.0$, $P > 0.1$]. In contrast, the three groups with large dorsal hippocampal lesions (30–50%, 50–75%, 75–100%) were slower than either the SHAM group or the 5–30% group to find the hidden platform (Fig. 1*A*; sessions 1–5: 30–50% group, 28.2 ± 2.5 sec; 50–75% group, 35.4 ± 4.0 sec; 75–100% group, 31.9 ± 2.7; all P values <0.05). Last, the three groups with large dorsal lesions performed similarly (Fig. 1*A*; sessions 1–5, all P values >0.1).

The latency to find the visible platform ranged from 6.1 to 10.5 sec (Fig. 1*A*). The only difference among groups was that the 30–50% dorsal hippocampal group (mean latency = 10.5 ± 1.2 sec) was measurably slower to find the visual platform than the SHAM group [mean latency = 7.8 ± 0.5 sec; $t(58) = 2.3$, $P < 0.05$].

Experiment 2: Latency. Repeated-measures ANOVA revealed that during the course of training all groups (SHAM, dorsal 50–75%, dorsal 75–100%, and ventral 50%) reduced the time needed to escape to the platform [$F(4, 208) = 97.8$, $P < 0.0001$]. There also was a main effect of Group [$F(3, 52) = 8.2$, $P < 0.0001$] and a Group × Session interaction [$F(12, 208) = 2.7$, $P < 0.01$]. Planned *t* tests revealed that rats with dorsal hippocampal lesions that damaged either 50–75% or 75–100% of hippocampal volume were slower than the SHAM group to find the hidden platform (sessions 1–5: SHAM, 19.2 ± 1.5 sec; dorsal 50–75%, 31.0 ± 4.3 sec; dorsal 75–100%, 34.2 ± 3.7 sec; all P values <0.01). Further, the dorsal 75–100% group also was slower than the ventral 50% group to find the hidden platform [ventral 50% group, 22.7 ± 1.9 sec; $t(16) = 2.9$, $P< 0.01$], and the

dorsal 50–75% group was marginally slower than the ventral 50% group $t(16) = 1.9$, $P = 0.07$]. There was no difference between the SHAM and ventral 50% groups $t(38) = 1.2$, $P > 0.2$].

The average latency to find the visual platform ranged from 8.4 to 6.1 sec. Although the difference between group latencies was small, the dorsal 75–100% group (mean latency 9.4 ± 0.8 sec) was slower than the ventral 50% group to find the visual platform [mean latency, 6.1 ± 0.4 sec; $t(14) = 3.83$, $P < 0.01$], and the dorsal 50–75% was marginally slower than the ventral 50% group [dorsal 50–75%, 7.4 ± 0.5 sec, $t(16) = 2.0$, $P = 0.06$]. Finally, the dorsal 75–100% group was slower to find the platform than the dorsal 50–75% group [$t(14) = 2.2$, $P < 0.05$].

Experiment 2: NOR. We evaluated the total amount of time spent exploring the objects during the 15-min familiarization period. The four groups averaged 106 sec of contact time with the objects (SHAM, 87.3 ± 4.5 sec; dorsal 50–75%, 70.8 ± 11.5 sec; dorsal 75–100%, 136.5 ± 21.7 sec; ventral 50%, 129.6 ± 9.7 sec). The ventral 50% and dorsal 75–100% hippocampal groups explored the objects more than the other two groups (P values <0.05).

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