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Supporting Online Material
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Materials and Methods
Figs. S1 to S3
References and Notes

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Place Cells and Place Recognition Maintained by Direct Entorhinal-Hippocampal Circuitry

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Place cells in hippocampal area CA1 may receive positional information from the intrahippocampal associative network in area CA3 or directly from the entorhinal cortex. To determine whether direct entorhinal connections support spatial firing and spatial memory, we removed all input from areas CA3 to CA1, thus isolating the CA1 area. Pyramidal cells in the isolated CA1 area developed sharp and stable place fields. Rats with an isolated CA1 area showed normal acquisition of an associative hippocampal-dependent spatial recognition task. Spatial recall was impaired. These results suggest that the hippocampus contains two functionally separable memory circuits: The direct entorhinal-CA1 system is sufficient for recollection-based recognition memory, but recall depends on intact CA3-CA1 connectivity.

The hippocampus and related medial temporal lobe regions play a pivotal role in encoding, consolidation, and retrieval of associations responsible for episodic memory (*1–5*). The hippocampus consists of structurally dissimilar processing modules (subfields) that are interconnected serially as well as directly with the adjacent entorhinal cortex (*6, 7*). This arrangement suggests that individual subfields may subserve discrete computational functions, but evidence linking particular modules to specific memory operations is sparse.

The most conspicuous functional characteristic of pyramidal cells in the hippocampus is their location-specific activity (*2, 8*). Hippocampal "place cells" are influenced by experience and may form a distributed map-like mnemonic representation of the spatial environment that the animal can use for efficient navigation (*2, 9*). But how do distinct hippocampal circuits contribute to such signals, and what is the functional capacity of each part of the place-cell network? One possibility is that place-related firing is a result of intrahippocampal computations. The CA3 area may have the capacity to associate and store patterned information received from the dentate gyrus or directly from the entorhinal cortex (*1, 10–12*). However, place fields have been recorded in area CA1 after selective dentate gyrus lesions (*13*), and place fields were not affected by a moderate reduction of neuronal activity in area CA3 (*14*); these results suggest that positional information from the entorhinal cortex might bypass the dentate gyrus and perhaps also CA3. To determine whether place-related firing in area CA1 is imposed by the associative network in area CA3 or by the direct connections from the entorhinal cortex (*6, 7*), we removed the input from area CA3 so that the remaining CA1 area was innervated only by fibers from the entorhinal cortex.

We first implanted electrodes in four rats in which area CA3 had been removed bilaterally with ibotenic acid (*15*). The CA3 area was removed throughout most of the dorsal hippocampus in all animals (Fig. 1, A to C), whereas area CA1 was largely spared. Neurons appeared normal at the recording position (Fig. 1B). After surgery, the rats were trained to collect scattered food in a square open field, and unit signals were recorded from area CA1. Most of the 42 pyramidal neurons recorded in these animals had distinct and well-defined place fields that were stable for at least 1 hour and thus were similar to those of normal rats (Fig. 1D). These observations suggest that area CA3 may not be necessary for establishing and maintaining place fields in area CA1, and that spatial information from the neocortex may reach the hippocampus primarily through the alternative route: the direct pathway from layer III of the entorhinal cortex.

However, because many functions of hippocampal neurons may be performed with relatively small portions of intact hippocampal tissue (*16*), the place-specific firing in area CA1, as observed in CA3-lesioned rats, could reflect input from remaining CA3 cells at the septal pole or in more temporal parts of the hippocampus. To isolate the direct entorhinal pathway to area CA1 completely, we removed the contralateral hippocampus and made a sequence of continuous miniature razor-blade cuts between areas CA3 and CA1 of the spared hippocampus (Fig. 2). This procedure separated the recording site from area CA3 as well as from subcortical afferents entering through the fimbria-fornix (Fig. 2, M to Q). We recorded place fields from 124 CA1 cells in 11 rats with such lesions. Most units exhibited clear place specificity in the open field after postsurgical training and appeared similar to cells from normal animals (Fig. 2Y). Place fields were stable across recording sessions, even over several days (Fig. 2Y). The discharge was theta-modulated (*17*), indicating that both spatial and temporal firing patterns

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could be imposed through the direct entorhinal input (*18*). The animals were also trained to run on a linear track on which they received reward at the turning points. Disruption of CA3 input did not attenuate the directional modulation that is characteristic of place cells in bidirectional environments (*19*) (Fig. 2Z).

The success of the disconnection procedure was verified after recording by injecting a fluorescent retrograde tracer at the recording position. The center of the injection coincided with the recording position (Fig. 2O). This resulted in retrograde labeling of pyramidal neurons in area CA3 in four nonlesioned control animals (Fig. 2, G to K). In contrast, injections into area CA1 in six implanted rats with cuts between areas CA3 and CA1 generally failed to label neurons in area CA3 (Fig. 2, S to W). A few labeled neurons were detected at the septal pole of the hippocampus in two or three of the animals (Fig. 2S), but place fields were neither sharper nor more stable in these animals than in animals without a label in area CA3. Layer III neurons in the entorhinal cortex were labeled in both control rats and lesioned rats (Fig. 2, L and X).

We next investigated whether removal of CA3 input had more subtle effects on place cells in area CA1. Five rats with intact hippocampi were trained in the open field and on the track, and 110 cells recorded from these rats were compared with 166 cells from the lesioned groups (Fig. 3). Repeated measurements from the same rat were pooled. Disruption of CA3 input gave a weak but consistent reduction of the sharpness of place fields from area CA1. This was expressed as an increase in the sparseness of the fields (*20*). The Hodges-Lehmann estimate of median sparseness was 0.46 for lesioned rats and 0.30 for intact rats (Mann-Whitney U test: $Z = 2.9$, $P < 0.005$). The size of the place fields was not significantly altered (28.2% versus 18.9% of the box surface, respectively; $Z = 1.7$). The peak rate was reduced (7.0 Hz versus 10.3 Hz, respectively; $Z = 2.2$, $P < 0.05$). The results were independent of the type of CA3 lesion (Fig. 3, A and B). These effects were small compared to the differences between the firing fields of pyramidal cells and interneurons (Fig. 3, A and B). Place fields were stable across sessions in both lesioned rats and control rats. Removal of CA3 input had no significant effect on how much the peak of the place field moved across a 1- or 24-hour interval (Fig. 3C; $P > 0.05$). Blocking input from area CA3 also failed to change the proportion of directionally modulated place cells on the linear track (Fig. 3D; $P > 0.05$). There was no group difference in average firing rate (1.00 Hz versus 0.91 Hz in legioned and control-operated rats, respectively; $Z = 0.2$, $P > 0.05$).

The direct pathway from the entorhinal cortex thus seems to be sufficient for establishing and maintaining fundamental properties of place cells in area CA1. We next tested whether the reduced circuitry also supported memory.

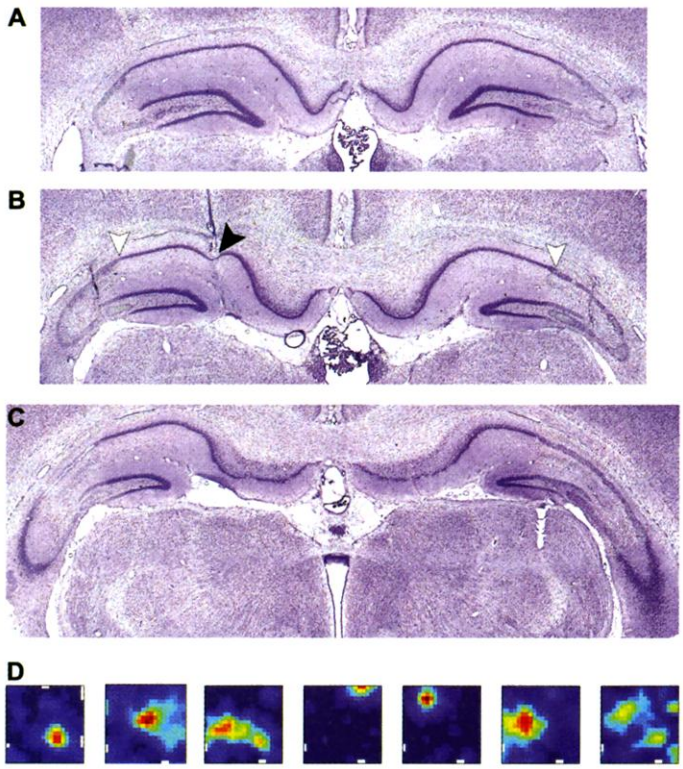

Fig. 1. Place fields in hippocampal area CA1 after removal of dorsal area CA3 by ibotenic acid. (**A** to **C**) Cresyl violet stains of neuronal cell bodies at three coronal levels through the dorsal hippocampus. In (B), the open arrowheads indicate the border between lesioned and intact tissue, and the closed arrowhead indicates the trace of the tetrodes. (**D**) Color-coded firing rate maps for seven well-isolated pyramidal cells recorded from the rat in (A) to (C) during running in a square open field (32 colors, linear scale). Dark red indicates maximum rate (left to right: 9, 11, 3, 12, 6, 11, and 2 Hz); dark blue is close to 0 Hz. Regions not visited by the rat are white.

Spatial recognition and spatial recall were measured after extensive ibotenate-induced CA3 lesions that spared subcortical connections (Fig. 4A) (fig. S1).

Recognition was tested in an annular water maze in which rats found an escape platform at a constant location after one or several laps of swimming. The task had no navigational demands, as the corridor walls guided the rats to the platform. Recognition of the goal location was expressed as slower swimming near the target on probe trials (Fig. 4B) (movie S1). Rats with selective damage to area CA3 showed strong recognition on all probe trials (days 2 to 5; Fig. 4C) and acquired the task as rapidly and accurately as the sham-operated group. Their performance was in sharp contrast to the slow learning of animals with damage to the entire hippocampus (Fig. 4, B and C). There was a significant effect of group on the time spent in a 45° zone around the platform on all retention trials [day 2 onward; $F(2, 24) = 6.4$, $P < 0.01$]. Planned orthogonal comparisons showed that the complete hippocampal lesion group swam less in this zone than did the two other groups [$F(1, 24) = 12.6$, $P < 0.005$]. There was no difference between the sham group and the CA3 group [$F(1, 24) = 1.2$, $P > 0.25$].

Recall was tested in an open Morris water maze. Selective lesions of area CA3 impaired performance (*21, 22*). Rats with CA3 lesions had prolonged escape latencies [$F(1, 14) = 5.8$, $P < 0.05$; days 1 to 3] and showed only weak retention on the probe trials [Fig. 4, D and E; groups × quadrant zones interaction: $F(3, 39) = 3.0$ (day 3) and $F(3, 39) = 3.1$ (day 4), $P < 0.05$].

There are four main implications of the present study. First, direct entorhinal-hippocampal connections have significant capacity for transforming weak location-modulated signals from superficial layers of the entorhinal cortex (*23*) into accurate spatial firing patterns in area CA1, without the participation of the CA3 area (*24*). Second, the fact that hippocampal-dependent spatial recognition memory was spared after isolation of the entorhinal-CA1 circuit implies that place cells in this preparation may still operate as functional ensembles. Because the CA1 area does not seem to have autoassociative properties, these ensemble properties may have been derived from afferent structures such as the entorhinal cortex (*25*). Third, one of the behavioral outcomes of hippocampal associative processing, namely spatial recognition memory, is fully achievable with an isolated entorhinal-CA1 network (*26, 27*). Finally, the isolated entorhinal-CA1 circuit does not support recall of remote locations or trajectories toward these locations, as tested in the open water maze. Such recall depended on an intact CA3 subfield (*28*). Together, the results suggest that the hippocampal formation contains at least two functionally separable circuits with independent mnemonic capacity.

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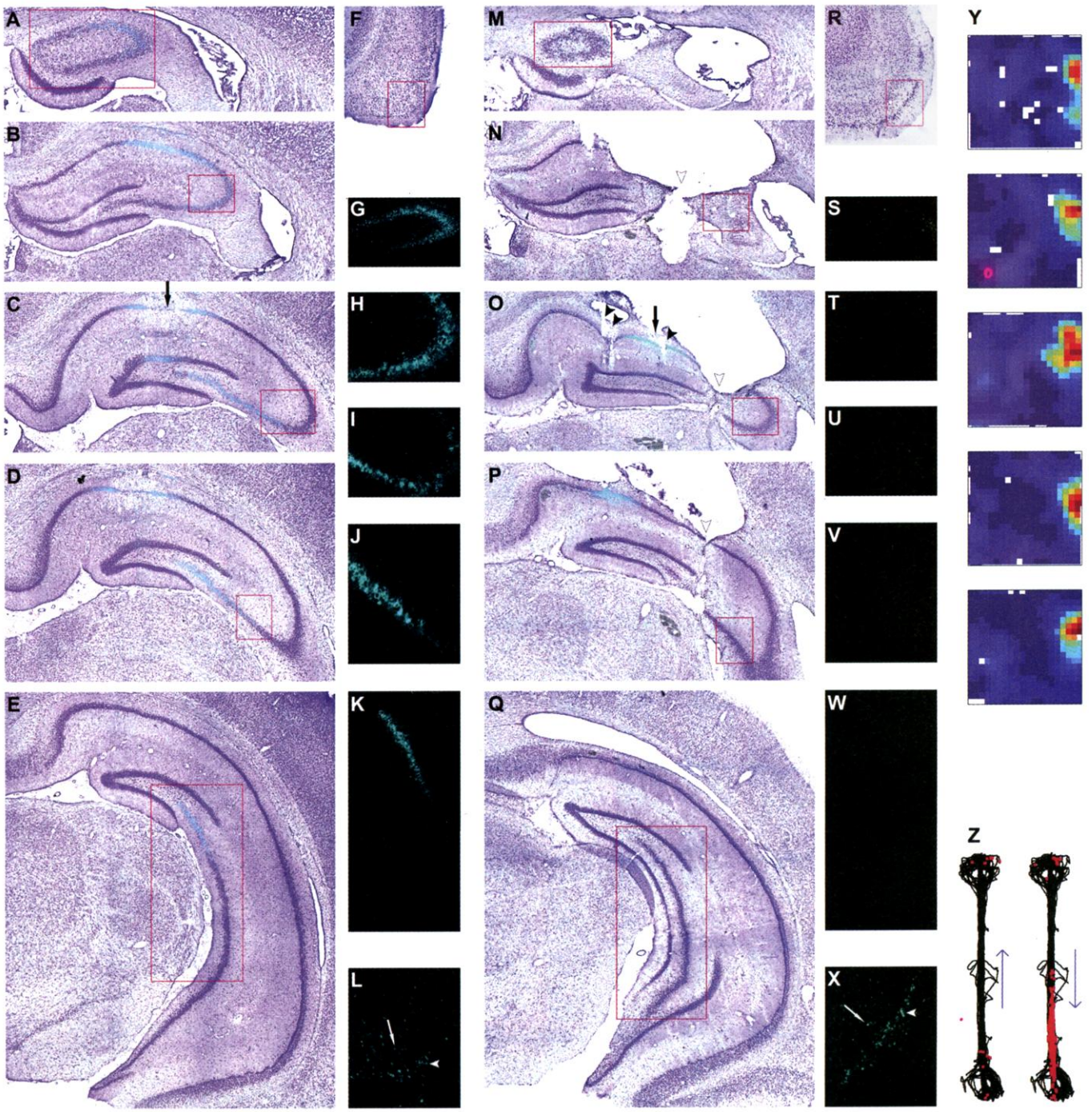

Fig. 2. Place fields in area CA1 after disruption of fibers from areas CA3 to CA1. (**A** to **X**) Cresyl violet and fluorescence images from an unlesioned control rat (A to L) and a rat with area CA1 isolated from area CA3 by a series of longitudinally oriented razor-blade cuts at the border between these subfields (M to X). Both rats received an injection of the retrograde tracer aminostilbamidine in the dorsal CA1 area [arrows in (C) and (O)]. Fluorescent images (green) are superimposed on the images of the cresyl violet sections [(A to F) and (M to R)]. In (M) to (Q), open arrowheads mark the surgical cut, and closed arrowheads indicate the location of three tetrodes. Panels (G) to (L) and (S) to (X) show magnified fluorescence images [(G) to (K) and (S) to (W) correspond to the red boxes in (A) to (E) and (M) to (Q), respectively; (L) and (X) correspond to the red boxes in (F) and (R), respectively]. Arrows in (L) and (X) indicate examples of layer III cells, and arrowheads show layer II cells. (**Y**) Color-coded firing rate map for a cell that was recorded for five consecutive days (top to bottom) in the lesioned rat in panels (M) to (X) (scale as in Fig. 1D; maximum firing rates: 8, 12, 12, 17, and 11 Hz, respectively). (**Z**) Directionally selective firing on a linear track in a different cell from the same rat. Red dots indicate where individual spikes were recorded on the rat's path (black). Spikes are shown separately for movement in each of the two directions (arrows). The cell had a distinct place field in one direction (peak rate, 22 Hz) but not in the other (peak rate, 0.8 Hz).

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24. Place fields were also observed in mice with selective *N*-methyl-D-aspartate receptor deletion in area CA3 (*29*). However, place selectivity disintegrated in these animals when landmarks were partially eliminated, which suggests that the CA3 area may be necessary for pattern completion under increased memory demands.
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27. Recognition of the goal location not only required a familiarity judgment but also involved an associative operation, that is, recollection of the fact that the place might contain an escape platform (*5*).
28. Area CA3 may also be necessary for nonspatial tasks that require association of discontiguous events (*30*).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/296/5576/2243/DC1
Materials and Methods
Fig. S1
Movie S1

20 February 2002; accepted 10 May 2002

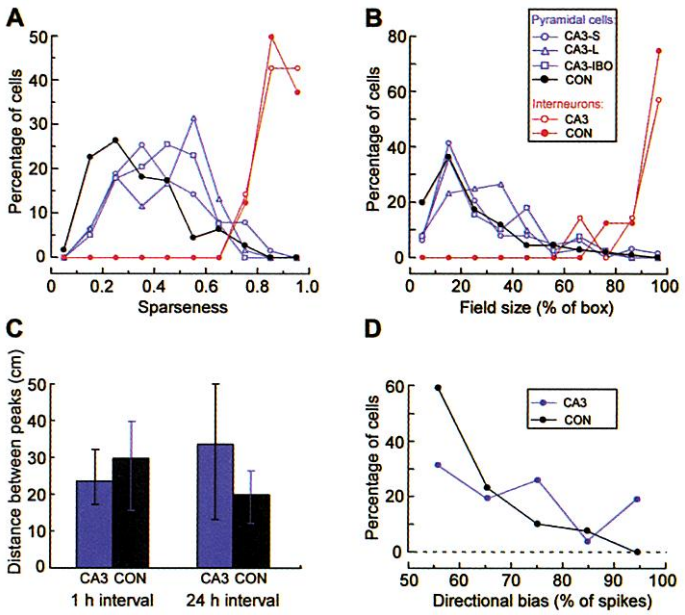

Fig. 3. Quantitative description of place fields in area CA1 after blocking input from area CA3. (**A**) Distribution of place cells in categories of increasing sparseness (bins of 0.1; 238 active pyramidal cells) in control rats (CON; black) and rats with lesions (blue). Input from area CA3 was removed either by ibotenic acid (CA3-IBO), small cuts (three cuts; CA3-S), or large cuts (four or five cuts; CA3-L). Interneurons (red) are shown for comparison. (**B**) Distribution of place field size (percentage of box surface) in the same experimental groups (bins of 10%; $n = 238$). (**C**) Stability of place fields in the box across a 1-hour interval ($n = 97$) or a 24-hour interval ($n = 34$). Error bars indicate SEM. (**D**) Distribution of directional specificity of place fields on the linear track (bins of 10%; $n = 110$).

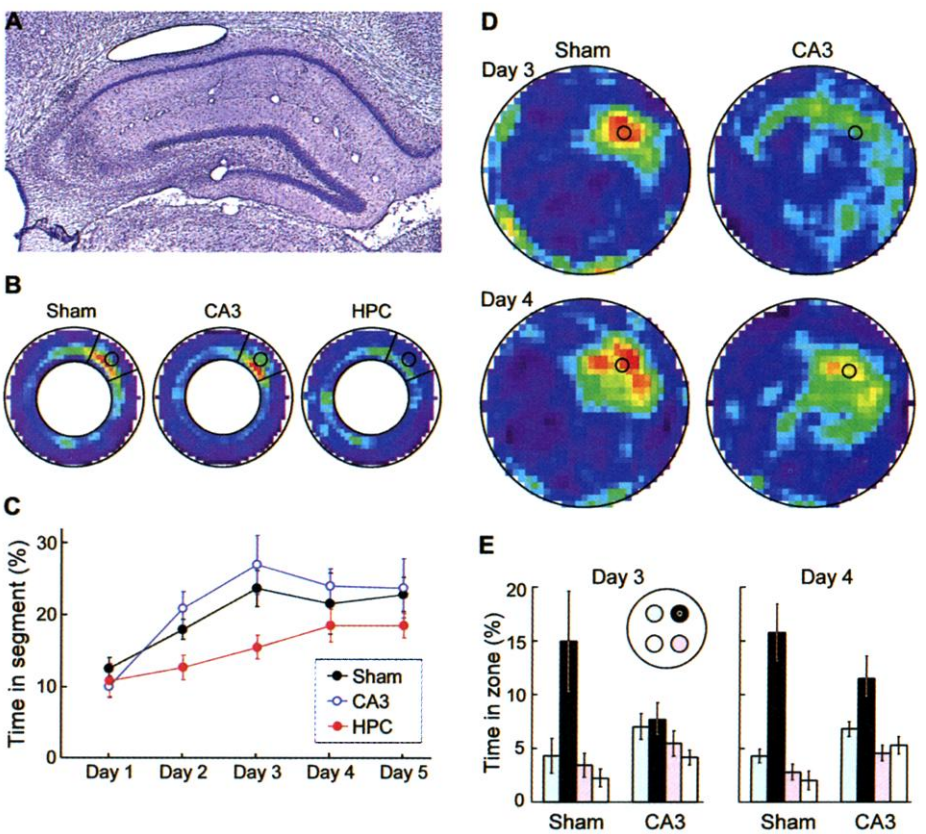

Fig. 4. The direct CA1–entorhinal cortex circuit supports spatial recognition memory but not navigation memory. (**A**) Cresyl violet stain of neuronal cell bodies in the dorsal hippocampus of a rat with a representative lesion in area CA3. These lesions were substantially larger than those in Fig. 1 (see fig. S1). (**B**) Color-coded pixel maps showing time distribution on an overnight retention trial in the annular water maze (day 3; group averages). Red corresponds to mean pixel times of 0.5 s; purple indicates times close to 0 s. Each diagram highlights the location of the platform (circle) and a 45° segment around the platform (radiating lines). HPC, hippocampus. (**C**) Time spent inside the 45° target segment on daily probe trials in the annular corridor (means ± SEM). (**D**) Color-coded pixel maps showing navigation on two probe trials in an open water maze after sham surgery or CA3 lesions. Red corresponds to pixel times of 0.25 s (group means); purple indicates times close to 0 s. The platform location is highlighted (circle). (**E**) Time spent in circular zones (inset) on each probe trial in (D) (one zone per quadrant; zone radius, 20 cm; platform in the center of the black zone). Error bars indicate SEM.