Major Dissociation Between Medial and Lateral Entorhinal Input to Dorsal Hippocampus

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Hippocampal place cells are a model system of how the brain constructs cognitive representations and of how these representations support complex behavior, learning, and memory. There is, however, a lack of detailed knowledge about the properties of hippocampal afferents. We recorded multiple single units from the hippocampus and the medial and lateral entorhinal areas of behaving rats. Although many medial entorhinal neurons had highly specific place fields, lateral entorhinal neurons displayed weak spatial specificity. This finding demonstrates a fundamental dissociation between the information conveyed to the hippocampus by its major input streams, with spatial information represented by the medial and nonspatial information represented by the lateral entorhinal cortex.

The hippocampus and related structures in the medial temporal lobe are crucial components of a brain system that mediates spatial learning, context-dependent learning, and episodic memory (*1*, *2*). These forms of learning have both spatial and nonspatial components, and hippocampal neurons correspondingly have both spatial and nonspatial firing correlates (*3*–*5*). Characterizing the pathways by which spatial and nonspatial information reach the hippocampus is critical for a full understanding of the neural circuitry underlying these forms of memory. The major cortical input to the hippocampus originates in the entorhinal cortex (Fig. 1A), which is divided into two parts on the basis of distinctive cytoarchitecture and connectivity patterns (*6*). The medial entorhinal cortex (MEC) receives its predominant input from the postrhinal cortex (parahippocampal cortex in primates) and forms the medial perforant path into the hippocampus. The lateral entorhinal cortex (LEC) receives its major input from the perirhinal cortex and forms the lateral perforant path (*7*).

The most salient behavioral correlate of hippocampal pyramidal cells in freely moving rats is the spatial location of the rat (*1*, *3*, *8*), and some entorhinal neurons have been shown to display spatial specificity as well (*9*–*12*). Different parts of the MEC and LEC project to different sites along the septal-temporal (dorsal-ventral) axis of the hippocampus (*13*), and accumulating evidence demonstrates functional heterogeneity along this axis (*6*, *14*, *15*). Therefore, it is imperative to precisely localize recording sites in the entorhinal cortex relative to their projections to the hippocampus. Neurons located within the dorsolateral band of the MEC (the band that projects to the dorsal hippocampus) fire in multiple discrete spots in an environment (*16*). These spots are reproducible across repeated sessions, and ensembles of these neurons represent the rat's location with precision. In contrast, cells in the ventromedial band of the MEC (the band that projects to the ventral hippocampus) do not display strong spatial tuning. Here we provide evidence for a major dissociation in spatial tuning along the orthogonal axis of the entorhinal cortex (i.e., between the LEC and the MEC).

Multiple single units were recorded while rats foraged for a food reward in a square chamber with a single white cue card on the west wall (*17*). Figure 1B shows the spatial firing-rate maps of the 10 cells with the highest spatial information scores (*18*) in the CA1 region of the hippocampus, the superficial layers of the MEC, and the superficial layers of the LEC. (The rate maps for all neurons in the sample are shown in fig. S1.) Entorhinal projections to the hippocampus originate in these superficial layers. Most CA1 and many MEC cells displayed very specific place fields, firing robustly and selectively in the square environment. In contrast, only a few cells in the LEC displayed spatial tuning, which was much less specific than that demonstrated by the MEC or CA1. Figure 1C shows the spatial information scores for all neurons from the superficial MEC (n = 52), superficial LEC (n = 68), and CA1 (n = 91). The differences among the three regions were highly significant [CA1: mean = 1.05 ± 0.06 SEM; MEC: mean = 0.61 ± 0.07; LEC: mean = 0.20 ± 0.02; $F(2,208) = 99.2$, $P < 0.0001$]. Post hoc Tukey tests showed that CA1 had higher spatial information scores than did the MEC [$q(208,3) = 6.7$, $P < 0.001$] and LEC [$q(208,3) = 19.8$, $P < 0.001$], and the MEC had higher spatial information scores than did the LEC [$q(208,3) = 11.0$, $P < 0.001$].

The LEC receives major cortical input from the perirhinal cortex (*6*, *7*). Perirhinal cortex neurons did not display strong spatial selectivity (mean = 0.19 ± 0.02, n = 70) (*19*). The MEC and LEC both receive strong input from the parasubiculum (*6*, *20*). In contrast to the perirhinal cortex, some parasubiculum cells displayed robust spatial tuning (mean = 0.59 ± 0.07, n = 30) (*21*, *22*). The information scores among the five regions (CA1, LEC superficial, MEC superficial, perirhinal, and parasubiculum) were significantly different [$F(4,306) = 75.4$, $P < 0.0001$]. Pairwise comparisons indicated that the parasubiculum was different from both the LEC [$q(306,5) = 8.9$, $P < 0.001$] and the perirhinal cortex [$q(306,5) = 11.2$, $P < 0.001$], but not from the MEC [$q(306,5) = 0.4$, $P > 0.5$, not significant (n.s.)]. Conversely, the perirhinal cortex was different from both the MEC [$q(306,5) = 12.8$, $P < 0.001$] and parasubiculum [$q(306,5) = 11.2$, $P < 0.001$], but not the LEC [$q(306,5) = 2.9$, $P > 0.2$, n.s.] (*23*).

Dorsal hippocampal neurons contain more specific place fields than do ventral hippocampal neurons (*15*). Because different anatomical bands of the MEC and LEC project to the dorsal and ventral hippocampus (Fig. 1A) (*13*), it is important to determine that both the MEC and LEC recording sites were in regions that project to the dorsal hippocampus, in order to make a valid comparison between the two areas. The locations of recording sites of the 12 rats of this study are shown on an unfolded flat-map representation of the dorsolateral projection bands of the MEC and LEC, which project to the dorsal half of the hippocampus (Fig. 2A) (see figs. S2 and S3 for individual maps for each rat and for precise localization of EC recording sites) (*13*) . Figure 2B shows representative histological sections of recordings from CA1 and the MEC, LEC, perirhinal cortex, and parasubiculum. All six MEC rats had tetrodes located in the dorsolateral band (hatched region); a few of the most rostral tetrode tracks encroached on the region that projects to the intermediate hippocampus. In four of the six LEC rats, the tetrodes were in the dorsolateral band, near the rhinal sulcus. In the remaining two rats, the LEC recording sites were in the intermediate band, which projects to the intermediate hippocampus (for one of these rats, no cells recorded in the LEC met inclusion criteria for this study). No strong place fields were recorded in the superficial layers of the LEC in either the intermediate or dorsolateral bands (fig. S4).

Although the quality of spatial information provided by superficial LEC neurons is lower than that provided by superficial MEC

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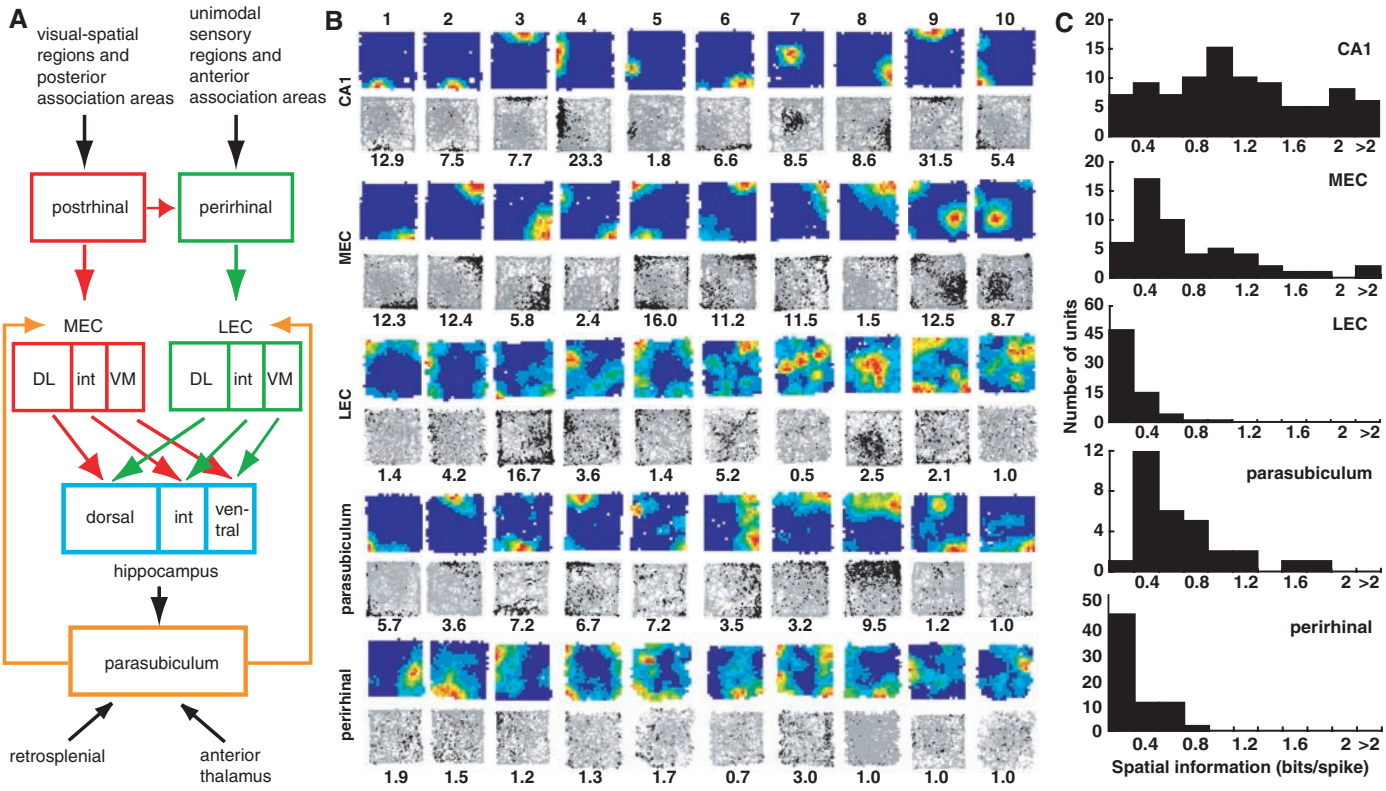

Fig. 1. Spatial firing properties in CA1 and parahippocampal areas. (**A**) Simplified connectivity between hippocampus and parahippocampal areas related to this study (*6*, *7*). Not all feedforward, feedback, or cross-stream pathways are represented. DL, dorsolateral band; int, intermediate band; VM, ventromedial band. (**B**) Firing-rate maps for the 10 cells showing the highest spatial information scores (ranked left to right) in each area. Red represents maximal firing, whereas blue represents no firing. The grayscale image below each rate map shows the trajectories of the rat in the 10-min session (gray lines) and the location of the rat when each spike was fired (black dots). Numbers represent the maximum firing rate for each cell. (**C**) Histograms of spatial information scores for each area. The spatial information score represents the amount of information (in bits per spike) about the rat's location that is conveyed by the firing of the cell (*18*). Note the differences in the *y*-axis scales.

neurons, some of the LEC rate maps displayed a modest amount of spatial selectivity (e.g., cells 4 and 8 of Fig. 1B). Putative spatial selectivity can arise as an artifact of inhomogeneous sampling of the environment, or of random bursts of activity, or from responsiveness to an aspect of the environment that is correlated with location (e.g., responsiveness to a localized odor). One test of true spatial selectivity is if the spatial firing patterns of a cell are replicated across repeated sessions. We systematically recorded successive sessions separated by 5 to 20 min in two rats (LEC rat 115 and MEC rat 123), and the firing-rate maps of superficial MEC cells were more correlated between the two sessions than the rate maps of superficial LEC cells [mean correlation for MEC = 0.63 ± 0.07, $n = 21$; for LEC = 0.30 ± 0.06, $n = 24$; $t(43) = 4.2$, $P < 0.0001$]. Because the paper covering the floor of the recording chamber was replaced between sessions, the reproducible firing patterns in MEC were not the result of floor-based odor cues. A similar analysis was performed for all cells in the sample by correlating the spatial firing patterns for the first half of a session with the spatial firing patterns for the second half. This analysis confirmed that CA1 and MEC spatial firing patterns were much more consistent within a session than were LEC patterns (fig. S5).

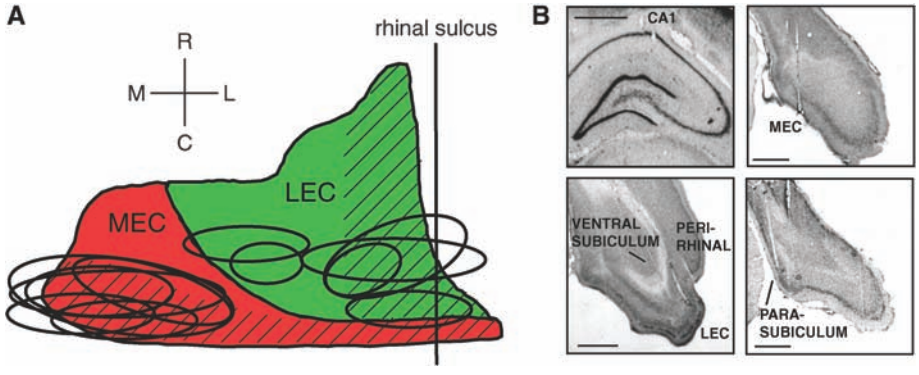

Fig. 2. Recording sites in the MEC and LEC. (**A**) Averaged flat-map reconstruction of the entorhinal cortex (*13*). The dorsolateral projection band, which sends projections to the dorsal half of the hippocampus, is shown hatched. Ovals denote the spread of tetrode penetrations for each rat. R, rostral; M, medial; C, caudal; L, lateral. (**B**) Representative histological sections showing tetrode tracks from CA1 and the LEC, perirhinal cortex, MEC, and parasubiculum. Scale bars, 1 mm.

The similarity of spatial information between the parasubiculum and the MEC raises questions about whether the spatial selectivity in the MEC is generated in the MEC from nonspatial postrhinal inputs (Fig. 1A) (*16*, *24*) or derived from the parasubiculum. In addition to its inputs from the subiculum, the parasubiculum receives input from the retrosplenial cortex and from the anterior thalamus (*6*, *25*), two regions that contain cells that represent head direction and movement through space (*26*, *27*). The parasubiculum may thus be part of a network that creates spatial representations on the basis of self-motion cues through interactions with the retrosplenial cortex and anterior thalamus, and then sends this

representation to the MEC. Regardless of the site of origin of the spatial signal, our results demonstrate a fundamental distinction between the functional correlates of the two major streams of input into the hippocampus from the neocortex. Superficial layers in the MEC contain exquisitely tuned "place cells," which may arise from a grid-like representation of space (fig. S6) (*16*). In contrast, there were few robust place cells in the superficial layers of the LEC under the present conditions of unstructured foraging in an environment with few spatial landmarks. Perhaps cells from the dorsolateral band of the LEC display spatial firing under other conditions (e.g., in a visually complex environment or in a more structured behavioral task). Alternatively, because CA1 neurons respond to individual items or discrete stimuli in conjunction with spatial location (*3*–*5*), it is possible that the LEC stream carries this nonspatial information from the perirhinal cortex into the hippocampus, where it is combined with spatial information from the MEC stream to create conjunctive object-place (or event-place) representations in the hippocampus proper (*28*, *29*). Consistent with this notion of parallel input streams, perirhinal cortex lesions disrupt exploratory behavior based on novel configurations of objects, whereas postrhinal cortex lesions disrupt exploratory behavior based on novel configurations of an object and a spatial context (*30*). This process may be a rodent analog of a dissociation in humans between item and source memory localized to the perirhinal and parahippocampal cortices, respectively (*31*).

References and Notes

1. J. O'Keefe, L. Nadel, *The Hippocampus as a Cognitive Map* (Clarendon Press, Oxford, UK, 1978).
2. H. Eichenbaum, *Neuron* **44**, 109 (2004).
3. J. O'Keefe, *Exp. Neurol.* **51**, 78 (1976).
4. E. R. Wood, P. A. Dudchenko, H. Eichenbaum, *Nature* **397**, 613 (1999).
5. M. A. Moita, S. Rosis, Y. Zhou, J. E. LeDoux, H. T. Blair, *Neuron* **37**, 485 (2003).
6. M. P. Witter, D. G. Amaral, in *The Rat Nervous System*, G. Paxinos, Ed. (Elsevier, New York, ed. 3, 2004), pp. 635–704.
7. R. D. Burwell, *Ann. N.Y. Acad. Sci.* **911**, 25 (2000).
8. R. Muller, *Neuron* **17**, 813 (1996).
9. C. A. Barnes, B. L. McNaughton, S. J. Mizumori, B. W. Leonard, L. H. Lin, *Prog. Brain Res.* **83**, 287 (1990).
10. S. J. Mizumori, K. E. Ward, A. M. Lavoie, *Brain Res.* **570**, 188 (1992).
11. G. J. Quirk, R. U. Muller, J. L. Kubie, J. B. Ranck Jr., *J. Neurosci.* **12**, 1945 (1992).
12. L. M. Frank, E. N. Brown, M. Wilson, *Neuron* **27**, 169 (2000).
13. C. L. Dolorfo, D. G. Amaral, *J. Comp. Neurol.* **398**, 25 (1998).
14. M.-B. Moser, E. I. Moser, *Hippocampus* **8**, 608 (1998).
15. M. W. Jung, S. I. Wiener, B. L. McNaughton, *J. Neurosci.* **14**, 7347 (1994).
16. M. Fyhn, S. Molden, M. P. Witter, E. I. Moser, M. B. Moser, *Science* **305**, 1258 (2004).
17. Materials and methods are available as supporting material on *Science* Online.
18. W. E. Skaggs, B. L. McNaughton, M. A. Wilson, C. A. Barnes, *Hippocampus* **6**, 149 (1996).
19. R. D. Burwell, M. L. Shapiro, M. T. O'Malley, H. Eichenbaum, *Neuroreport* **9**, 3013 (1998).
20. M. Caballero-Bleda, M. P. Witter, *J. Comp. Neurol.* **328**, 115 (1993).
21. J. S. Taube, *Hippocampus* **5**, 569 (1995).
22. Projections to the MEC and LEC originate in different regions of the parasubiculum (*20*). We did not sample the different regions adequately to determine whether there was a difference in spatial tuning between areas that project to the MEC or LEC, or to determine whether there were differences in spatial tuning between the dorsal and ventral parasubiculum.
23. Because there were no differences in the spatial information scores between the deep and superficial layers of either the parasubiculum or perirhinal cortex, recordings from these layers were combined for each area. Comparing the information scores of CA1, the superficial MEC, and the superficial LEC, with only the superficial cells of the parasubiculum or perirhinal cortex, yielded no change in the results.
24. R. D. Burwell, D. M. Hafeman, *Neuroscience* **119**, 577 (2003).
25. T. van Groen, J. M. Wyss, *Brain Res.* **518**, 227 (1990).
26. J. S. Taube, *J. Neurosci.* **15**, 70 (1995).
27. L. L. Chen, L. Lin, E. J. Green, C. A. Barnes, B. L. McNaughton, *Exp. Brain Res.* **101**, 8 (1994).
28. W. A. Suzuki, E. K. Miller, R. Desimone, *J. Neurophysiol.* **78**, 1062 (1997).
29. D. Gaffan, *Exp. Brain Res.* **123**, 201 (1998).
30. G. Norman, M. J. Eacott, *Behav. Neurosci.* **119**, 557 (2005).
31. L. Davachi, J. P. Mitchell, A. D. Wagner, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 2157 (2003).

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Supporting Online Material

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Materials and Methods
Figs. S1 to S6
References

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Early Asymmetry of Gene Transcription in Embryonic Human Left and Right Cerebral Cortex

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The human left and right cerebral hemispheres are anatomically and functionally asymmetric. To test whether human cortical asymmetry has a molecular basis, we studied gene expression levels between the left and right embryonic hemispheres using serial analysis of gene expression (SAGE). We identified and verified 27 differentially expressed genes, which suggests that human cortical asymmetry is accompanied by early, marked transcriptional asymmetries. *LMO4* is consistently more highly expressed in the right perisylvian human cerebral cortex than in the left and is essential for cortical development in mice, suggesting that human left-right specialization reflects asymmetric cortical development at early stages.

One of the most remarkable aspects of the human cerebral cortex is that the two hemispheres are specialized for distinct cognitive and behavioral functions. Whereas the right cerebral cortex regulates movement of the left side of the body and vice versa, ~90% of the human population is naturally more skilled with the right hand than with the left (*1*). This motor asymmetry is strongly correlated with language dominance: Language function is predominantly localized to a distributed network in the left perisylvian cortex in 97% of right-handers and ~60% of left-handers (*2*, *3*). Functional asymmetries exist in mathematical ability and in spatial and facial recognition as well. These functional asymmetries have been related to anatomical asymmetries of the cortex that are somewhat more subtle (*2*, *4*). For example, the posterior end of the sylvian fissure is higher in the right hemisphere than in the left (*5*). The planum temporale, a region in the posterior portion of the superior temporal sulcus in which Wernike's area resides, is larger in the left hemisphere than in the right in more than 65% of examined adult and 56 to 79% of examined fetus and infant brains, so the anatomical asymmetries are less marked than

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