Conjunctive Representation of Position, Direction, and Velocity in Entorhinal Cortex

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Grid cells in the medial entorhinal cortex (MEC) are part of an environment-independent spatial coordinate system. To determine how information about location, direction, and distance is integrated in the grid-cell network, we recorded from each principal cell layer of MEC in rats that explored two-dimensional environments. Whereas layer II was predominated by grid cells, grid cells integrated in the grid-cell network, we recorded from each principal cell layer of MEC in rats that coordinate system. To determine how information about location, direction, and distance is during self-motion–based navigation.

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Materials and Methods Figs. S1 to S6 Tables S1 to S3 References
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Grid cells with tesselating firing fields (7) were observed in all principal cell layers (Fig. 1, A and B). To compare their prevalence, we estimated the periodicity of the rate map of each cell by computing a 2D autocorrelation matrix for the rate distribution (Fig. 1C, left), rotating the autocorrelation map in steps of 6°, and calculating the correlation between each rotated map and the original. Grid structure was apparent as a sinusoidal modulation of this correlation, with peaks recurring at multiples of 60° (Fig. 1C, right, and fig. S1) (12). The degree of “gridness” was expressed as the difference between the correlations at the expected peaks (60° and 120°) and the expected troughs (30°, 90°, and 150°) of the function. The proportion of cells with a sinusoidal modulation was layer-dependent (Fig. 1D and table S1). Whereas most well-separated layer II cells had strongly periodic firing fields, only a smaller proportion of the deeper neurons had such characteristics; however, the range of “gridness” among those cells was not different from that of the layer II cells.

To compare the geometric structure of grids in different layers, we defined grid cells as the subset of cells that had higher correlations at 60° and 120° of rotation than at 30°, 90°, and 150° (gridness > 0) (16). All 203 neurons that passed this criterion had stable periodic firing patterns both within and between trials (figs. S1 and S2). Irrespective of layer, the scale of the grid in these cells increased by a factor of 1.5 to 2 from the dorsalmost to the ventralmost recording location, such that cells near the postrhinal border had the densest spacing (~35 to 40 cm) and the smallest firing fields (~500 cm²) (fig. S3). The correlations between distance from the
Postrinal border on the one hand and spacing and field size on the other were significant in all layers (spacing, 0.46 ± 0.94; field size, 0.44 ± 0.94; all, P < 0.005) (17). In all layers, the phase of the grid was distributed, i.e., the vertices of most nearby grid cells were offset relative to each other, but the orientation of the grids was consistent across all simultaneously recorded cells.

Beneath layer II, grid cells were colocalized with head-direction cells whose general properties were similar to those of head-direction cells in other brain areas (18–21) (Fig. 2). In these neurons, firing increased from a low background rate (typically 0.5 Hz) to a high maximum rate (5 to 40 Hz) whenever the rat’s head faced a certain range of directions (Fig. 2D, fig. S4, and table S1). The preferred firing direction varied among cells. The degree of directional tuning was quantified for each cell by comparing the distribution of the rat’s orientation at the time of firing with the distribution of orientations across the entire trial, using Watson’s U² test statistic (20). When these distributions differed significantly and the directional bias was significantly correlated across blocks of the trial, the cell was classified as a head-direction cell. No head-direction cells were found in layer II (Fig. 2E). In contrast, a large proportion of the cells in layers III to VI had directional preferences (273 of 385 cells) (Fig. 2E). The breadth of tuning was not significantly different between layers III, V, and VI (mean U² values, 18.2, 25.2, and 18.3, respectively; mean angular standard deviations, 55.9°, 52.2°, and 58.2°; P > 0.05) (fig. S4). The entire range of head directions was represented in all three layers (Fig. 2F). When several head-direction cells were recorded simultaneously in the same area, their peak firing directions were widely distributed (Fig. 2G and fig. S5). Head-direction cells were usually encountered together with grid cells (fig. S6). Directional tuning curves were always stable across trials (circular correlation, r = 0.84, P < 0.001).
Grid cells and head-direction cells formed overlapping populations. The 2D distribution of gridness and directional tuning was continuous, such that some grid cells were directionally tuned and some head-direction cells had grid correlates (Fig. 3, A to D, and fig. S7). The proportion of grid cells with conjunctive properties was layer-dependent (table S1). The largest proportion was encountered in layers III and V, where 66% and 90% of the grid cells had dual response properties, respectively. In layer VI, the proportion was 28%. No conjunctive cells were observed in layer II. Differences between layers were significant (all four cell layers, \( \chi^2(3) = 28.4, P < 0.001 \); layers III to VI, \( \chi^2(2) = 6.2, P < 0.05 \)).

The degree of directional tuning in cells that met selection criteria for both gridness and directionality was not significantly different from that of pure head-direction cells (mean \( U^2 \) values, 18.0 and 19.4, respectively; \( P > 0.05 \)) (Fig. 3, D and E). Cells with different degrees of gridness and directionality always responded as a coherent ensemble during environmental manipulations such as the rotation of a polarizing cue card (Fig. 3F and fig. S8) (22).

Cells with conjunctive grid and head-direction properties may update the representation of spatial location by integrating position and direction information as the animal moves around; however, translocation of the position vector between grid cells with shifted firing vertices may require additional information about the animal’s instantaneous speed of movement (23–27). We thus asked whether speed was expressed in the firing rates of any of the cells that we recorded (Fig. 4). Because all position points were used in this analysis, including those outside the firing fields, the correlation between speed and rate was generally low (Fig. 4A). Yet, regression analyses showed a consistent positive speed-rate relation in nearly all grid cells (141/150 cells, \( P < 0.001 \)), head-direction cells (153/220 cells, \( P < 0.001 \)) and grid × head-direction cells (45/53 cells, \( P < 0.001 \)), suggesting that a substantial proportion of the network expressed information about how fast the animal was moving (Fig. 4B).

The slope of the regression line was steeper for conjunctive cells and grid cells than for head-direction cells (\( F(2,422) = 5.5, P < 0.005 \); conjunctive versus head-direction, \( P = 0.009 \); grid versus head-direction, \( P = 0.01 \); conjunctive versus grid, \( P = 0.60 \); Tukey HSD test). The y-intercept of the regression line was positive in 422 out of 423 cells (Fig. 4C). Average rates at the lowest velocity (0 to 3 cm/s) were 2.26 ± 0.03 Hz (grid cells), 2.50 ± 0.02 Hz (head-direction cells) and 2.39 ± 0.05 Hz (grid × head-direction cells). Thus, grid structure and directional tuning can be maintained during brief stops along the rat’s trajectory. Few stops were longer than 5 s.

These results imply that, despite the differential hippocampal and neocortical connections of superficial and deep layers of the MEC (10, 28), all layers together operate as an integrated unit, with considerable interaction between grid cells, present in all principal cell
layers, and head-direction cells, present in layers III to VI. Principal neurons from layer II to layer V have apical dendrites that extend up to the pial surface (14, 29). Layer V cells have extensive axonal connections to the superficial layers (14, 15), and local axons of layer II and III cells may contact the dendrites of deeper cells (30). This implies that visuospatial and movement-related signals from the post-rhinal and retrosplenial cortices (10, 28) and directional signals from the dorsal presubiculum (18–20, 31–34) may activate the entire MEC circuit even when the axonal input is specific to one or a few layers.

The results show that the spatial map in MEC comprises both grid cells and head-direction cells. These cell types form a continuous population, with grid cells expressing variable degrees of directional modulation and head-direction cells expressing variable degrees of grid structure. Conjunctive representations among input variables appear in many theoretical models for neural systems that perform coordinate transformations, and similar conjunctions of head direction and location have been observed in some cells in the dorsal presubiculum (35). Analogous conjunctive cells may be involved in the computation of head-centered coordinates from retinal-location and eye-position data (“gain fields”) in the posterior parietal cortex (36, 37) and in the continuous updating of head direction cells on the basis of conjunctions between current head direction and head angular velocity (38, 39). Our results suggest that, as the animal moves, the position vector may be updated through integration of position, direction, and speed signals in the grid-cell network (23–27). Conjunctive cells are likely to play a critical role in this process. The conjunctive cells are located predominantly in layers III and V, where principal neurons have extensive axonal projections to the grid-cell population in layer II (14, 15). The ability of superficial cells to read out signals carried by these axons may be critical for translating activity over the population of grid cells, i.e., between cells with a different spatial phase, in a manner consistent with the rat’s motion. The direction of translation may be determined by the head-direction input; the distance may be controlled by the speed modulation. In essence, this mechanism would perform path integration in the MEC network.

The integration of directional and positional information is strongly facilitated by the intermingled localization of the grid cells and the head-direction cells and the distributed representation of spatial phase (7) and head orientation in these cell types. Within a diameter of a few hundred micrometers or less, the complete range of positions and distances appears to be represented. It remains to be determined whether the integration of position, direction, and velocity is confined to the local circuitry, as in the columns of the isocortex (40), or extends across the entire entorhinal sheet of grid cells, spanning all grid spacings.
The activity of the network was not interrupted by brief stops on the rat’s trajectory. The firing rate of grid cells increased with velocity. (Head-direction cells, and grid cells had positive slope values, i.e., the firing rate is maximal at the vertices of the grid and minimal at the center of each triangle. Rotations of the firing rate map (or its 2D autocorrelation function) come into correspondence with the unrotated map every 60°.


12. The firing field of a grid cell has the form of a regular grid whose unit cell is a pair of inverted equilateral triangles or a rhombus with interior angles of 60° and 120°. Firing rate is maximal at the vertices of the grid and minimal at the center of each triangle. Rotations of the firing rate map (or its 2D autocorrelation function) come into correspondence with the unrotated map every 60°.


16. Materials and methods are available as supporting material on *Science* Online.

17. If this linear trend persists throughout the dorsal-to-ventral extent of MEC, it predicts grids with spacings of about 2 m in the most ventral portions of MEC.


22. A similar coupling has been reported for thalamic head-direction cells and hippocampal place cells (21).


41. We thank S. Molden and A. Treves for discussion and correspondence with the unrotated map every 60°.

Supporting Online Material

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Materials and Methods

Figs. S1 to S9

Table S1