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Speed cells in medial entorhinal cortex

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Grid cells in the medial entorhinal cortex (MEC) have spatial firing fields that repeat periodically in a hexagonal pattern. When animals move, activity is translated between grid cells in accordance with the displacement in the environment. For such translation to occur, grid cells must have continuous access to information about instantaneous running speed. However, a powerful entorhinal speed signal has not been identified. Here we show that running speed is represented in the firing rate of a ubiquitous but functionally dedicated population of MEC neurons distinct from other MEC populations, such as grid, head direction and border cells. This entorhinal cell type is characterized by a context-invariant positive, linear response to running speed. Speed cells and grid cells share a similar prospective bias of ~50 to 80 ms. The observations point to speed cells as a key component of the dynamic representation of self-location in the MEC.

0Grid cells are unique in their spatial code[1,2]. Unlike other place-modulated neurons, their population firing pattern not only repeats periodically within a given environment[1], but also seems to apply equally to all explored environments[3], reflecting the uniformity of space despite the unevenness of contextual details. This property makes grid cells ideal candidates for a path integration-based representation of space[1,4-11]. In such a scheme, running speed is integrated across short time windows to obtain the instantaneous displacement of the animal, which in conjunction with head direction input is used to update the representation of the animal´s position. Any path integration mechanism thus requires running speed as a major input. However, while speed has been reported to correlate marginally with entorhinal theta frequency[12] and firing rate of grid cells[13,14], the existence and nature of a reliable and locally

available speed signal has remained unclear. The aim of the present study was to determine whether speed is represented in specific classes of MEC cells.

Experimental control of running speed

1We began the investigation by recording neuronal activity under strict experimental control of the animal's running speed. Rats traversed a 4 m long linear track with their body confined inside a computer-driven bottomless frame that was moved along the track at a pre-set speed ("a Flintstone car"; Fig. 1a). Since the car had no floor, it compelled the animal to engage in natural locomotion at the experimenter-determined speed in order to reach the end of the track, where food reward was delivered. During running, cells were recorded across all layers of MEC (Extended Data Fig.1).

2In initial experiments, we either trained rats to run fast on one half of the track and slow on the other, with a sharp transition in the middle (382 cells, 3 rats), or speed was increased proportionally to the distance from one of the track ends (282 cells, 2 rats). The firing rate of some cells recorded in these protocols followed the speed profile (Fig. 1b), with fast transitions in firing rate at each change in running speed (Fig. 1c).

3In order to disentangle running speed from the position of the animal, the same track segments were traversed at constant running speeds that alternated randomly from run to run between 7, 14, 21 and 28 cm/s. The majority of data in the Flintstone task – 754 cells from 10 rats – were collected with this 4-speed protocol. To identify speed-responsive neurons, we calculated a speed score for each cell, defined as the Pearson product-moment correlation between instantaneous firing rate and running speed, on a scale from -1 to 1. Cells with speed scores higher than the 99^{th} percentile of a shuffled distribution (a value of 0.18) were classified as speed cells. A total of 98 MEC cells (13%) passed this criterion (Fig. 1d-e), significantly more than expected by chance (expected: 7.5 cells, $p=10^{-74}$). As a population, these cells showed significant differences in normalized firing rate between all four blocks of constant running speed (Fig. 1f; Kruskal-Wallis and Tukey-Kramer tests, $p<0.01$; 1 s was excluded around speed jumps; for normalization, see Online Methods). The slope and *y*-intercept of linear regression lines for firing rate as a function of speed were highly dispersed across cells, with frequent cases of non-zero firing at very low speed (Extended Data Fig. 2a; see examples in Fig. 1b,c,e). Negative-linear responses to speed were also observed, although

in only a marginal sub-population of 16 cells (2%) (Extended Data Figs. 2b-d) that was not analyzed further.

Speed cells during free foraging

4Analyses in the Flintstone car do not address directly the question of how much overlap there is between the speed cell population and other known entorhinal cell types, taking into account that limited position sampling prevents measurement of two-dimensional grid periodicity as well as tuning to head direction and geometric borders (Extended Data Fig. 3). Thus, in a second block of experiments, we performed classical free foraging recordings in a 1 m wide square box, where 17 rats covered a wide range of instantaneous speeds, typically from 0 to 50 cm/s. We recorded 2497 MEC cells and obtained for each cell a speed score and a rate-by-speed tuning curve using 2 cm/s bins from 0 to 50 cm/s (as in the Flintstone car but with higher speed resolution). Again, many cells had firing rates that increased linearly with speed (Fig. 2a and Extended Data Figs. 4a and 5). Instantaneous firing rate and running speed exhibited considerable co-variation (Fig. 2b). In behaviorally unfiltered data, as many as 51% of the neurons had a speed score that passed the classification threshold determined by the 99th percentile of a distribution of shuffled data (Fig. 2c). This large proportion (almost 4 times higher than in the Flintstone car) might reflect a difference in the network state between rest and active navigation rather than a genuine correlation between running speed and firing rate, a problem that was not present in the car, where resting periods were left out of the analysis. To overcome this issue, we re-defined the speed score by filtering out static periods (speed < 2 cm/s). With this stricter criterion, used in all subsequent open field analyses, the threshold defined by the 99th percentile of the shuffled distribution (0.18) was passed by 385 neurons, or 15% of all MEC cells (Fig. 2d and Extended Data Fig. 6f) – a percentage almost identical to the estimate from the Flintstone car. Slopes and *y*-intercepts of regression lines for firing rate as a function of speed were as dispersed as in the car (Extended Data Fig. 2a). Cells with similar properties were found in the hippocampus (Fig. 2e and Extended Data Fig. 7b). Out of 964 hippocampal neurons that were active in the open field, 96 cells (10%) passed the threshold determined by the 99th percentile of the shuffled distribution (0.19) (Fig. 2f).

5Once we had established that speed modulation is similar in the open field and the Flintstone car, we could ask whether MEC speed cells form a population of their own. We compared their properties with those of grid, head direction and border cells, classified

respectively by their mean vector length[15], gridness score[13,16], and border score[17,18], with thresholds obtained by shuffling of spike times (Extended Data Figs. 6a). Out of 2497 cells, 518 (21%) were classified as grid cells, 398 (16%) as head direction cells, and 99 (4%) as border cells. All cell categories were represented by a percentage of the population significantly higher than what would be expected by chance, as defined by a binomial distribution (Extended Data Fig. 6c). However, the intersection between speed cells and any of these cell populations was small and, for grid cells and head direction cells, significantly lower than expected by chance. Only 16 speed cells met the criterion for grid cells (expected: 79.9; $p=10^{-17}$), 42 met the head direction criterion (expected: 61.4; p=0.005), and 11 met the criteria for border cells (expected: 15.3; p=0.17) (Extended Data Figs. 4b, 6c-d and 7a). These numbers contrast with the overlap between spatial and directional cells, which was at chance level for grid and head direction cells (88, expected: 82.6, p=0.75) and well above chance for border and head direction cells (40, expected: 15.8, right-tail $p=10^{-6}$). Almost half of the speed cells with a dual classification had low in-field speed scores (speed scores restricted to the data inside the spatial or directional fields), suggesting that, in these cells, the speed modulation was indirect, caused by interactions between speed and other behavioural variables (Extended Data Fig. 6g). Consistent with the functional separation of the speed cells, we found that they had a population distribution of spatial information per spike around one order of magnitude below that of all other MEC cell types, and a distribution of head direction information per spike one order of magnitude below that of head direction cells (Fig. 3a and Extended Data Fig. 6h). Grid, head direction and border cells had similar distributions of speed score (Fig. 3b), typically lower than the criterion for speed cells and not very different from the distribution of shuffled data with a 2 cm/s threshold (Fig. 2d). In sum, because of their distinct firing characteristics and the low levels of overlap with other cell types, MEC speed cells seem to form a population of their own.

6A different pattern was observed in the hippocampus. While the overlap between place cells and speed cells was low (19 cells, expected: 33.4, p=0.004), speed scores calculated separately for the area inside the place field were typically higher than the regular speed scores (Extended Data Figs. 6c,e,g), suggesting a stronger mixture of speed and spatial information in the hippocampus than in the MEC. In agreement with this difference, in-field firing rates in the Flintstone car were not significantly speed-modulated in MEC (Kruskal-Wallis test: $p = 0.12$) whereas a significant but low modulation was present in the hippocampus ($p < 0.05$) (Extended Data Fig. 2e).

7Approximately one-quarter of the speed cells had properties of fast-spiking cells, classified as neurons with a mean firing rate above 10 Hz and a spike width below 0.3 ms[19]. While grid, head direction and border cells had a similar average-firing-rate distribution, typically between 1 and 10 Hz and consistent with mean rates of principal cells[19], speed cells covered a wider range (Fig. 3c). Among 1178 grid, head direction, border and place cells, only 4 (0.3%) passed the criteria for fast spiking cells. In contrast, 95 out of 385 speed cells, or 25%, were classified as fast-spiking in MEC. 27 out of 96 (28%) were classified as fast-spiking in the hippocampus. Speed cells were present in all MEC layers, with a rather homogeneous distribution (minimum 14%; maximum 18%; Fig. 3d).

The speed code is context-invariant

8We next asked whether the speed code expressed in the population of MEC speed cells could be used for path integration. For this to happen, not only should it be possible to decode running speed from the activity of speed cells, but the decoding should be context-invariant. We analyzed data from previous experiments[3,13] in which spike activity was recorded from MEC in two rooms (A and B; sequence ABA'; 8 rats). Twenty speed cells were identified. As expected[3], grid cells fired at different locations relative to the box walls in the two rooms (Fig. 4a). Speed cells, in contrast, had invariant speed scores and tuning curves (Figs. 4b-c and Extended Data Figs. 8b-c). In one case, a rat had 4 simultaneously recorded speed cells, a situation fit for decoding. Two simple linear decoders, trained with data from these 4 cells in either A or B, were tested in A'. Reconstructed speed and tracked speed were highly correlated, irrespective of whether room A or room B was used to train the decoder (A: $r = 0.75$, B: $r = 0.74$) (Fig. 4d). In general, the match between reconstructed and actual speed increased with the number of simultaneously recorded cells, reaching an average Pearson correlation of ~0.75 for 6 cells (Fig. 4e; 385 speed cells; all open field sessions where at least one speed cell was recorded). In an experiment where 3 speed cells were recorded with room lights on and off in an on-off-on sequence[1], speed scores and tuning curves were similarly invariant (Fig. 4f-g). Running speed could be reconstructed from preceding trials regardless of whether the decoder was trained with lights on or lights off (Extended Data Fig. 8d), suggesting that optic flow is dispensable for speed cells to be updated. Finally, the speed code was largely invariant also across experimental tasks, as demonstrated when speed cells were recorded in two open field sessions with a Flintstone car session in between. 49 out of 64 cells

(77%) classified as speed cells in the open field were also speed cells in the Flintstone car, with very similar tuning curves and speed scores (Extended Data Fig. 8e). The tuning curves were similar, with no slope adaptation, even in a rat that showed a two-fold difference in average speed between open field and Flintstone car (Fig. 4h-j and Extended Data Fig. 8f). In sum, MEC speed cells express a context-invariant speed code that can be used to decode actual running speed across a variety of experimental manipulations.

The entorhinal speed code is prospective

9We asked if speed cells represent instantaneous speed or have retrospective or prospective components, and if they have, how these components are related to firing locations of spatially modulated cells. Retrospective and prospective firing have been reported for place cells and grid cells under a variety of circumstances[20-26]. If these cells are driven by path integration based on input provided by speed cells, the temporal bias might be derived from the speed signals themselves. To test this hypothesis, we pooled together MEC speed cells from all Flintstone car experiments and calculated correlations between running speed and different temporal shifts of the instantaneous firing rate, in order to find the shift that maximized the correlation. The firing rate of the MEC speed cells correlated better with future speed than simultaneous or past speed (Fig. 5a), both as measured by the maximum of the average correlation curve (54 ms) and by the population statistics of individual correlation maxima (mean: 60 ms, median: 65 ms, Wilcoxon signed rank test: $p<0.01$). A similar prospective bias was found for MEC speed cells in the open field data (average correlation curve maximum: 62 ms, population mean: 82 ms, median: 68 ms, $p<0.01$). This bias was present only in theta-modulated cells (37% of all speed cells), where the speed-related firing ramped up in a characteristic pattern during the course of the theta cycle (Extended Data Fig. 9).

10We next examined the consequences of prospective path integration on the firing locations of simultaneously recorded grid and place cells. The expected amount of anticipation in a prospective path integrator can be estimated directly for episodes of constant running speed (see Online Methods, Eq. 2). If the speed signal anticipates running speed by a fixed time interval τ, its integration will have an anticipation in space proportional to the running speed, with τ as the coefficient of proportionality. Using only constant running episodes from the 4-speeds experiment, we compared the position of the same entorhinal

firing fields (putative grid fields) at 7, 14, 21 and 28 cm/s (Extended Data Fig. 2f). The average field appeared consistently at earlier positions for higher speeds. The relationship between speed and field position was linear, with a coefficient of proportionality similar to the temporal anticipation of MEC speed cells ($\tau = 80\ ms$, r = 0.97) (Fig. 5b). This is compatible with the idea that grid cells are driven by path integration based on input from speed cells. A similar link was not observed in the hippocampus, where speed cells showed a significant retrospective effect (τ between -89 ms and -59 ms) and place cells showed no temporal bias ($\tau = -1\ ms$, r = -0.07) (Fig. 5a-b and Extended Data Fig. 9b).

11The magnitude of the anticipatory shift of the grid fields increased during acceleration episodes. Although we did not find a significant population of cells directly tuned by acceleration (Extended Data Fig. 6b), in MEC the firing rate of the speed cells was positively modulated by acceleration, as expected from their prospective nature (see Online Methods, Eq. 1) (Fig. 5c top; threshold of ±50cm/s^2; Friedman's test for acceleration effects in both tasks: $p<0.01$). This increased firing of speed cells during positive acceleration could make the path integrator run faster and thus generate a larger spatial anticipation than the one observed during constant running speed or negative acceleration. To test this idea, we estimated the impact of positive and negative acceleration, filtered at a threshold of ±50cm/s^2, on the average position of entorhinal spatial firing fields in all Flintstone car experiments. Again, we found an anticipatory shift in field position in MEC cells (Fig. 5d). This shift was also present when the previous analysis was restricted to neurons identified as grid cells during a complementary session in a 2D open field environment (Extended Data Fig. 10d-e). The anticipatory shift increased with the absolute acceleration threshold (Fig. 5e, *Mann-Whitney U-test after Holms-Bonferroni correction: $p<0.01$), an effect that was also found for grid cells in the open field (Fig. 5f, *$p<0.01$). A careful analysis focusing on the choice of an unbiased spatial reference showed that the displacement was asymmetrical so that it was never retrospective, not even for extreme negative acceleration (Extended Data Fig. 10b-e). The shift was significant only in entorhinal layer II, where it was large (Wilcoxon signed-rank test after Holms-Bonferroni correction, $p<0.01$; all spatially modulated cells), and in layer III, where it was small ($p<0.05$) (Flintstone car data, Fig. 5g and Extended Data Fig. 10a). It was strongly modulated by theta activity (Extended Data Figs. 10f-h), in agreement with models suggesting that path integration takes place on a theta cycle basis[7]. In the hippocampus, speed cells showed significant negative modulation by acceleration, compatible with retrospective coding (Eq. 1), but place cells exhibited no significant spatial shift (Figs. 5c-g and Extended

Data Fig. 9b; Friedman's test: $p<0.01$). Taken together, these observations support the idea that entorhinal speed cells contribute to the firing of grid cells via path integration, a process that does not seem to take place in the hippocampus, where speed cells and place cells do not share a common temporal bias.

Discussion

12The main finding of our study is the discovery of a large population of speed cells in the MEC. These cells, which represent a considerable fraction of the MEC neurons – ~15% across all layers – are characterized by a positive-linear response to running speed, and low levels of spatial and directional information. The speed response was independent of visual input, consistent with the idea that the signal is at least partly derived from proprioceptive or motor-efference information in the mesencephalon[27]. Neurons with similar characteristics were found in the hippocampus (~10%), in agreement with prior reports of a speed modulated axon in the hippocampus[28] and one in or around the presubiculum[29]. In the hippocampus, many place cells were also modulated by speed[30,31], in contrast to the speed cell population in MEC, which exhibited little overlap with other cell types. Previous work has demonstrated correlations between running speed and firing rate in grid cells[13,14], but the present data shows that when speed is experimentally disentangled from space, acceleration and behavioral state, the grid cell population exhibits no speed modulation, and only around 1% of all grid cells show a robust speed response. Reconstruction of instantaneous speed would thus be possible only with input from hundreds or thousands of grid or head direction cells. In contrast, speed cells allow for accurate decoding of speed from the activity of only 4-6 specialized cells. With complex functional properties such as prospectiveness and a unique modulation by theta phase, speed cells can hardly be thought of merely as passive integrators of the diffuse speed information contained in the firing of other MEC populations.

13The existence of speed-responsive cells in the entorhinal-hippocampal network has implications for how spatial maps are updated as animals navigate through an environment. Path integration-dependent models of grid cell firing make use of a speed signal, coded either in the frequency of membrane oscillations[9-11,32,33] or in the firing rate of dedicated neurons[4-8]. Irrespective of its nature, the speed signal is needed for dynamic updating of the active grid cell population, in accordance with the animal's movement in space. Two important requirements must be met for a speed signal to enable efficient path integration. The first is a

linear speed-rate relationship, which is required to make the temporal integration of the signal proportional to the displacement of the animal and to allow for a simple combination of multiple inputs without restrictions on variability of slope and *y*-intercept. A non-linear speed input would require sophisticated readout mechanisms in order to avoid instantaneous variations in spacing or phase of the grid map caused by changes in running speed, and in order to allow summation of multiple inputs, each possessing a *y*-intercept that should otherwise be individually subtracted. The present data provide experimental support for the assumption of linearity. The second requirement is contextual invariance. If the speed-rate relationship was context-dependent, the contextual modulation would be inherited by the spatial code and a calibration process would be needed for every novel environment. Our data show that the speed code is invariant across environments, in darkness and in light, with no gain adaptation for different behavioral conditions. The universality of the speed code has a great advantage in that the path integrator needs to be trained only once in the animal's lifetime, allowing it to be used effectively in novel environments and in the absence of strong contextual cues, precisely where it is most needed.

14Finally, the results provide an element that links MEC speed cells with grid cells. While the former code for speed prospectively, with a forward bias of ~50-80 ms, the latter behave as if they were guided by a prospective path integrator. The strong theta modulation of prospectiveness in speed and grid cells suggest that the integration occurs on a theta cycle basis. The anticipation of preferred firing position in the grid cells is enhanced during acceleration periods but persists when the animals run at constant speed. The temporal bias is consistent with previous reports of alternating modes of prospective and retrospective firing in grid cells[25] but the present observations suggest that, if low speed rather than average maps are used as a spatial reference, the modes reflect positive and negative acceleration, respectively, and are generated by the prospective firing pattern of local speed cells. Positive acceleration at the beginning of a movement may put the position estimated by the grid network ahead of the actual one, while negative acceleration at the end of a movement might compensate, bringing estimated and actual positions back together when the animal stops. Following this logic, the overall effect should be purely prospective, as is apparent when precautions are taken to ensure that the reference is not itself biased towards prospection. In contrast to the observations in MEC, no direct link could be established between speed cells and place cells in the hippocampus. While hippocampal speed cells encode speed retrospectively, place cells exhibit no systematic shift in firing location in response to speed or

acceleration. Place cells may under some circumstances inherit prospective firing from grid cells[24] but the present data suggest that, in general, temporal biases in the hippocampus follow a logic of their own, independent of path-integration processes that take place in the MEC[21,22,26,34].

15 The unique association of the predictive code with layer II grid cells is among the major functional differences described so far in the MEC circuit, and might so provide a key to understanding the computational steps underlying the dynamic representation of space. How the prospective speed signal is generated, why it is translated primarily to grid cells of layer II, how theta oscillations contribute to this process, and how the prospective firing of layer II is integrated with non-prospective activity in other parts of the network are questions that remain to be addressed.

Methods summary

Materials and Methods are described in the online version of the paper. References unique to this section appear only in the online paper.

Supplementary Information accompanies the paper on www.nature.com/nature**.**

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Competing interests statement

The authors declare that they have no competing financial interests.

Keywords

Entorhinal cortex, hippocampus, grid cells, head direction cells, speed, velocity, space, theta rhythm.

Figure 1 | Speed-responsive MEC cells in a linear task.

a, Flintstone car. **b**, Mean firing rate (green) and running speed (grey) as a function of position for 3 representative speed cells in MEC. Left and middle: linear speed protocol; right: step protocol. Pearson correlations between instantaneous running speed and firing rate are indicated. **c**, Representative speed cells during decelerating and accelerating events of the step protocol (left and right subpanels, respectively). Top: firing rate (red, left axis) and running speed (grey, right axis) as a function of time relative to the event onset. Bottom: spike raster plots. **d**, Distribution of speed scores for all cells in the 4-speed experiment (purple curve) and for 100 shuffles of the spike timestamps of each cell (grey bars; counts normalized by number of shuffles). Dashed line shows the 99^{th} percentile of the shuffled distribution (0.18). **e**, Tuning curves of 5 representative speed cells in **d**. **f**, Normalized average firing rates (Online Methods) for all 98 speed cells in all 4 speed groups (means ± s.e.m.).

Figure 2 | Speed cells in an open field.

a, Firing rate as a function of position, head direction (hd), and running speed in 3 representative MEC speed cells during free foraging in an open field. Each row shows one cell. Left: colour-coded spatial rate maps. Scale bar to the right. Center: firing rate (colour) as a function of head direction (*x* axis) and running speed (*y* axis). Right: Firing rate as a function of running speed. **b**, Traces showing *z*-scores for firing rate (colour) and speed (grey) for 7 representative entorhinal speed cells during 2 minutes of free foraging (different sessions). Maximum values of firing rate and speed are indicated (left and right, respectively). **c**, Distribution of speed scores (Pearson correlation between instantaneous firing rate and running speed) for all MEC cells in the open field (purple curve) and for 100 shuffles of the spike timestamps of each cell

(grey bars; count normalized by 100). Dashed line, 99th percentile of the shuffled distribution (value at the top). **d**, As **c**, but including only running periods (speed > 2 cm/s). **e**, Speed cells in the hippocampus (as in **a**). **f**, Distribution of hippocampal speed scores, excluding static periods as in **d**.

Figure 3 | Entorhinal speed cells form a population of their own.

a, Logarithmic-scale scatter plot showing head-direction and spatial information of 1206 neurons (dots) classified by statistical criteria as speed, grid, head-direction, or border cells. Cell identity is colour-coded. Black dots represent 62 speed cells (out of 385) that satisfied criteria for at least one additional cell type. Curves show normalized counts of spatial (top) and head-directional (right) information for each cell category. **b**, Distribution of speed scores across cells of all categories. **c**, Distributions of firing rate, averaged over non-silent periods (firing rate > 1 Hz). **d**, Distribution of functional cell types across entorhinal layers (only proportions higher than 1%). Colour code at the top left.

Figure 4 | Invariance of the entorhinal speed code.

a, Correlation of grid maps on two trials in the same room (A vs. A') or in different rooms (A vs. B) in a representative rat. **b-c**, Tunning curves (**b**) and speed scores (**c**) of 4 simultaneously recorded speed cells (unique colors) on successive trials in rooms A and B. Note room-independent speed-rate relationships. **d**, Two linear decoders, trained with the activity of these 4 speed cells in either A or B, were used to decode running speed in A'. Reconstructions from A and B are very similar (Pearson correlation: 0.99) and match actual speed in A' (reconstruction quality, or Pearson correlation with running speed, A: 0.75, B: 0.74). **e**, Reconstruction quality

as a function of the number of simultaneously recorded speed cells (all trials in open field). The training data set comprised the initial 70% of the session and the decoders were tested on the remaining 30%. **f-g**, tuning curves (**f**) and speed scores (**g**) of 3 simultaneously recorded speed cells during 2 regular sessions with a trial in darkness in between. **h**, Tuning curves in open field (o.f.) and Flintstone car for speed cells from rat 14566, trained to run twice as fast in the car as in the open field (Extended Data Fig. 8f). Note gain invariance. **i**, Tuning curves had similar variability across open field sessions and between open field and car (Mann-Whitney U-test p: 0.34; all speed cells from rat 14566). **j**, Speed score, *y*-intercept and slope for second open-field trial (grey) or car trial (colour) against first open-field trial (same cells as **i**).

Figure 5 | Prospective coding of entorhinal speed cells and grid cells.

a, Correlation between running speed and temporal shifts of instantaneous firing rate for speed cells in MEC (top) and hippocampus (bottom). Left: car; right: open field. Green bars show normalized counts of temporal shifts that maximize correlation. Average correlation curves are shown in purple. Note prospective bias in MEC speed cells and retrospective bias in hippocampal cells. **b**, Relation between speed and spatial anticipation (peak field position relative to that of 7 cm/s group). Data from 4-speed car experiment. Note linear relation in grid cells (MEC) but not place cells (hippocampus). **c**, Normalized activity of speed cells (mean ± s.e.m.) as a function of speed during intervals of positive (red) and negative (black) acceleration (threshold:±50 cm/s^2). **d**, Average fields of putative grid cells (top) and place cells (bottom) in the car after filtering for extreme acceleration (as in **c**) or without filtering (grey). Δ is spatial anticipation during positive compared to negative acceleration. **e**,

Spatial shifts Δ corresponding to different acceleration thresholds (mean ± s.e.m.) for grid cells (green) and place cells (grey) (*p<0.01). **f**, Spatial shifts as in **e**, but for open field sessions (see Online Methods) (*p<0.01). **g**, Spatial shifts Δ for spatially modulated cells in the Flintstone car classified by recording location and MEC layer (mean ± s.e.m.; *p<0.01, #p<0.05).

Online methods:

Subjects

26 male Long Evans rats (ages 3-6 months; 350-500 g at implantation and testing) were housed individually in transparent Plexiglass cages (54 × 44 × 35 cm). 8 of these rats were taken from a previous study of grid-cell activity during hippocampal remapping[3,13] and 1 was from a study of grid cells in darkness[1]. Speed cells were not reported in those studies. All rats were maintained on a 12-h light/ 12-h dark schedule and tested in the dark phase. After surgery, the rats were placed on a food deprivation schedule that initially kept them at ~ 90% of their free-feeding body weight but was progressively loosened depending on task performance.

Electrode implantation and surgery

Tetrodes were constructed from four twisted 17 μm polyimide-coated platinum-iridium (90%–10%) wires (California Fine Wire, CA) and mounted in groups of 4 into microdrives with a single turning screw and no separation between tetrodes. The electrode tips were plated with platinum to reduce electrode impedances to between 150–300 kΩ at 1 kHz.

Anesthesia was induced by placing the animal in a closed glass box filled with isoflurane vapor. Following this, the animals were rapidly moved into the stereotaxic frame, which had a mask connected to an isoflurane pump. Air flow was kept at 1 liter/minute with 0.5-3.5% isoflurane as determined by physiological monitoring. Local anesthetic (Xylocain) was applied on the skin before making the incision. Holes were drilled on the dorsal skull anterior to the transverse sinus to reach the entorhinal cortex, and posterior to bregma to reach the

hippocampus. Rats were implanted with two microdrives aiming at entorhinal cortex alone bilaterally and a third microdrive aimed at the right hippocampus. The coordinates for entorhinal implants were: 4.5-4.8 mm medio-lateral relative to lambda, 0.2-0.7 mm anterior to the border of the sinus depending on the target layer, and 1.5-1.8 mm dorso-ventral relative to the surface of the brain. The inclination of the entorhinal tetrodes was 8°, with the tips pointing in the anterior direction. Out of 13 drives that were used to record from MEC layer II, 3 tracks were observed to reach the very dorsal tip of the layer, at the transition to parasubiculum. After corroborating that the data from these drives was equivalent to the rest of MEC layer II in every analyzed aspect (cell type proportions, theta modulation, prospectiveness of speed and grid cells), we pooled the data from all 13 drives together. The coordinates for hippocampal implants were: 2.7 mm medio-lateral, -3.3mm antero-posterior relative to bregma, and 1.5 mm dorso-ventral relative to the brain surface. These tetrodes were implanted vertically. Jeweller's screws and dental cement were used to secure the drive to the skull. Two screws per microdrive were additionally connected to the system ground. Tetrodes in MEC were implanted by a similar approach in the remapping and darkness study[1,3,13].

Data collection

For data collection, the rat was connected to the recording equipment (Axona Ltd., Herts, U.K.) via AC-coupled unity-gain operational amplifiers close to its head, using a counterbalanced cable that allowed the animal to move freely within the available space. Tetrodes were lowered in steps of 50 μm every day in search of new cells. All data from the same day were pooled together for cell classification, so that each cell recorded at a given depth was counted only once. In separate analyses, cells were not included if a cell had been recorded on the same tetrode at a distance of less than 200 μm. Cell counts with these separate analyses were similar to those performed on the total data set (Extended Data Fig. 6f). Recorded signals were amplified 10 000 to 25 000 times and band-pass filtered between 0.8 and 6.7 kHz. Triggered spikes were stored to disk at 48 kHz (50 samples per waveform, 8 bits/sample) with a 32 bit time stamp (clock rate at 96 kHz). EEG was recorded single-ended from one electrode per drive. The EEG was amplified 5000-10 000 times, lowpass-filtered at 500 Hz, sampled at 4800 Hz, and stored with the unit data. A tracker system (Axona Ltd.) was used to record the position of a pair of LEDs attached to the head stage at a rate of 50 samples per second, allowing to track for position and head direction. The *x* and *y* components of the velocity and acceleration vectors were computed from the tracked positions using a Kalman filter and smoother (RTS).

Head oscillations, amplified by the distance of the LEDs from the head of the animal, could generate a spurious correlation between tracked acceleration and position. Anticipated spatial firing (Fig. 5d-f) could reflect such spurious correlations. In the following, we present several factors suggesting that the correlation is rather generated by genuine prospective coding: a) The prospective nature of speed and spatial cells is not present in the hippocampus, but all hippocampal data were recorded simultaneously with data from MEC, sharing a common tracking signal. Moreover, in grid cells the spatial shifts were layer specific, and in speed cells prospective firing was only present in theta-modulated cells (Extended Data Fig. 9a). b) Under extreme conditions, firing fields shifted ~10 cm. In the worst scenario, with head oscillations of 90º, this corresponds to a distance between LEDs and head of more than 7 cm. However, LEDs were placed at most 2-3 cm away from each other, given the small size of Axona microdrives. A more realistic oscillation of 30º would require a distance of 19 cm to generate a similar field shift. c) The acceleration-related grid field shift was asymmetric, showing anticipation only during epochs with positive acceleration, with no effect during negative acceleration (Extended Data Fig. 10b-e). d) The shift was also strongly modulated by theta phase, while acceleration and theta phase were not correlated (Extended Data Fig. 10h, bottom). e) A qualitatively similar anticipatory shift was observed in the absence of acceleration (Fig. 5b).

Spike sorting and cell classification Spike sorting was performed offline using graphical cluster-cutting software (tint; Axona Ltd.). Clustering was performed manually in two-dimensional projections of the multidimensional parameter space (consisting of waveform amplitudes), using autocorrelation and cross-correlation functions as additional separation tools and separation criteria. In general, the stability of the tetrodes allowed for all sessions in a day to be merged for clustering purposes.

Flintstone car and open field Every day the rats were first trained in an open field (1 m × 1 m × 50 cm box) and then in a Flintstone car on a 4 m long linear track, with possible repetition of both types of trials. There was a total of 2010 recording sessions (total for all 26 rats). In the open field, the animal was trained to collect chocolate crumbs thrown randomly into the box, one at a time, in trials that lasted at least 20 min and as long as the animal would exhibit active foraging behavior. Flintstone car trials varied depending on the protocol. With very few exceptions, a given rat was always trained with the same protocol. In general terms, sessions consisted of 10 to 25 runs on the linear track lasting at most 25 min. Naïve rats generally explored the possibility of jumping over the limits of the car. This behavior was

discouraged by placing the animals back in the correct position inside the car. Escape attempts typically stopped after 1 or 2 runs, when rats discovered that a chocolate crumb was placed at each end of the track to motivate running. On rare occasions, for training purposes, additional ground chocolate was distributed randomly along the track. This made the rat focus on the track and prevented it from taking alternative strategies such as jumping over the car. Between runs the rat rested on the end of the track for a random interval between 10 and 20 s. A 6 s lasting beep of increasing pitch indicated the beginning of the next run. Between trials, the rat rested on a towel in a large flower pot on a pedestal.

The Flintstone car had a minimalistic design to prevent the rat from using it as a sitting platform or spatial reference frame. It was 28 cm long and 17 cm wide, with two ball-bearing wheels at each end. The car was supported by Plexiglas rails running slightly below the track along the sides (Fig. 1a). These lateral rails could barely be seen by the rat, giving support to the car without the need of lateral walls. At each end of the car was a wide mesh fence measuring 17 cm × 16 cm to prevent the rat from moving ahead or behind the car while not obscuring their vision or sensation of velocity. A 25W battery-powered motor (Japan Servo) under the track was attached to two sets of guide lines, each one pulling from one end of the car. A motorcycle battery was used as an isolated power source to avoid 50 Hz AC noise. Braided fishing line (>20lb) was used as the guidance line. While the car was constructed in a minimalistic fashion, curtains were placed at both sides of the track and filled with a variety of salient visual cues, so as to make the laboratory the most salient spatial reference frame. Different scripts within the DacqUSB acquisition software (Axona Ltd) were used to control the motor that moved the Flintstone car. The digital output of the recording system was transformed into analog by means of a custom-built digital-to-analog converter and fed as a control signal to the motor. To park the car consistently in the same position at the beginning of each run, two mechanical sensors were placed at the extremes of the track, and their output was fed to the digital input of the recording system.

Linear protocol. In order to secure that similar track segments were covered across a wide range of speeds, a linear relationship between speed and position was established by setting the car speed to vary exponentially with time.

2-speed step protocol. The track was divided into 2 equal halves and different speeds were chosen for each half. The transition between them was sudden and occurred always in the same place.

4-speed step protocol. This protocol was designed to obtain multiple transitions between 4 different speed groups: 7, 14, 21 and 28 cm/s. Every run was divided into 6 segments (S1-6), 3 of them corresponding to the outbound run and the other 3 to the inbound run. S1: the speed was set at 7 cm/s for a fixed amount of time so as to cover roughly the first third of the track. S2: the speed was chosen randomly between the 4 options. The point for the next transition was also chosen randomly and varied from run to run within a range of ~75 cm. S3: the speed was chosen randomly again and kept until the end of the track was reached. S4: as in S2, the speed and the transition point toward S5 were chosen randomly. S5: the speed was chosen randomly and kept until a fixed position (the same as for the transition between S1 and S2). S6: the speed was set to 28 cm/s until the end of the track. The protocol has elements of complexity that exceed the aims of this paper. For analyses of behavior at different speeds, only segments S2 to S5, where space and speed were randomly related, were taken into account. Periods 1 s around each transition were excluded.

The linear protocol and the 2-speed step protocol were used only for visualization of speed-rate relationships, considering that space and speed were correlated in these two protocols. Further analyses were performed with the 4-speed protocol, in which the two variables could be disentangled.

Remapping experiment. Recordings were obtained from 8 rats while they foraged freely in enclosures in rooms A and B, following the order A-B-A'. In each recording session, the protocol was similar to the open field protocol described above. Enclosures could be either 1 m wide square boxes or circular boxes 90 cm or 180 cm in diameter[3,13]. Each trial in one enclosure lasted 10 min.

Darkness experiment. Recordings were obtained from a rat foraging freely in a circular box 1 m in diameter with the lights of the room turned either on or off, following the order on-off-on[1] (10 min each). Switching off lights resulted in complete darkness.

Rate maps and speed tuning

Rate maps that showed firing rate as a function of location, head direction, or speed were constructed with similar procedures. Histograms of spike count on one hand and time spent in the location on the other were built for each cell, using equally spaced bins (bin size: 2.5 cm for spatial maps, 6° for head direction maps and 2 cm/s for speed maps). Each bin of the rate map was obtained as the ratio between the spike count and the time spent, smoothed by a

Gaussian filter (standard deviation: 4 cm for spatial maps, 6° for HD maps and 3 cm/s for speed maps). In speed maps, where coverage is very inhomogeneous, only bins accounting for at least 0.5% of the data were included as valid. In composite rate maps for speed vs. head direction, this threshold was divided by the number of head direction bins. Instantaneous firing rate was obtained by dividing the whole session into 20 ms bins, coinciding with the frames of the tracking camera. A temporal histogram of spiking was then obtained, smoothed with a 250 ms wide gaussian filter. Spatial and head directional information measures[35] were based on these maps.

Speed tuning was determined by calculating a speed score for each cell. The speed score was defined as the Pearson product-moment correlation between the cell's instantaneous firing rate and running speed, on a scale from -1 to 1. The variability of the speed tuning curve when comparing two sessions A and B (Fig. 4i) was calculated as the average across bins of the absolute normalized change in firing rate, $|(A-B)/(A+B)|$.

Shuffling

Chance-level statistics was constructed for a given variable W through a shuffling procedure. At each repetition, the entire sequence of spikes fired by the cell was time-shifted along the animal's path by a random interval between 30 s and the total trial length minus 30 s, with the end of the trial wrapped to the beginning. The shuffled instance of the variable W was then calculated using the shifted spikes, and the collection of 100 repetitions for each cell composed the chance-level statistics. For cell-type classifications, all shuffled data of the corresponding score was pooled together and the 99th percentile of the distribution was used as a classification criterion.

In order to distinguish speed-correlated effects from changes in behavioral state (foraging vs. sitting still), we dismissed all data produced at a running speed lower than 2 cm/s in the calculation of the observed and shuffled speed scores.

Normalization of speed cell activity

Because of the variability in baseline and slope, a simple or normalized average of speed cell activity would not properly capture the population behavior. To obtain a better normalization

method, we applied to any firing rate measure f of a speed cell expressed in Hz the linear transformation:

$$f_n = \frac{(f - A)}{B * 50cm/s},$$

where A [Hz] and B [cm^{-1}] are the y-intercept and slope of the cell's speed tuning. This linear transformation aims to achieve for every cell a normalized dimensionless activity of 0 when the rat is still and 1 when it runs at 50 cm/s, allowing for proper population averaging.

Unbiased analysis of cells modulated by running speed

The modulation depth of a cell was defined as the difference between the maximum and minimum firing rates in its speed tuning curve. A cell was classified as modulated by speed if its modulation depth was significantly higher than the 99th percentile of a distribution of modulation depths obtained from shuffling 1000 times the cell's spike timestamps. It is worth noting that the nature of the modulation depth does not allow for the mixture of information coming from different cells, so that every individual cell had its own threshold. This selection method has no bias toward linear coding of speed, but for all types of data a majority of significantly modulated cells exhibited a positive, linear code, as measured by the linearity index (regression of the tuning curve) (Extended Data Fig. 2b).

Measures used for cell type classification

Gridness score[13,15,16]:

The gridness score for each cell was determined from a series of expanding circular samples of the autocorrelogram, each centered on the central peak but with the central peak excluded. The radius of the central peak was defined as either the first local minimum in a curve showing correlation as a function of average distance from the centre, or as the first incidence where the correlation was under 0.2, whichever occurred first. The radius of the successive circular samples was increased in steps of 1 bin (2.5 cm) from a minimum of 10 cm more than the radius of the central peak, to a maximum of 90 cm. For each sample, we calculated the Pearson correlation of the ring with its rotation in α degrees first for angles of 60° and 120° and then for angles of 30°, 90° and 150°. We then defined the minimum difference between any of the elements in the first group (60° and 120°) and any of the elements in the second

(30°, 90° and 150°). The cell's gridness score was defined as the highest minimum difference between group-1 and group-2 rotations in the entire set of successive circular samples.

Mean vector length (head direction score)[36]:

Given the head direction tuning map of a cell, if the bin i with orientation θ_i expressed in radians is associated with a firing rate λ_i, the mean vector length was computed as

$$m.v.l. = \frac{1}{N}\left|\sum_{i=1}^{N} \lambda_i e^{i\theta_i}\right|,$$

where the sum was performed over all N directional bins and the modulus of the resulting complex number was obtained.

Information per spike[35]:

Given a spatial or head direction map with mean firing rate λ and a value λ_i for each of its N bins, information rate was computed as

$$Information = \sum_{i=1}^{N} p_i \frac{\lambda_i}{\lambda}\ log_2\left(\frac{\lambda_i}{\lambda}\right),$$

where p_i is the occupancy probability of bin *i*.

Border score[17,18]:

The border score was computed as the difference between the maximal length of a wall touching on any single firing field of the cell and the average distance of the field from the nearest wall, divided by the sum of those values. The range of border scores was thus from -1 to 1. Firing fields were defined as collections of neighboring pixels with firing rates higher than 20% of the cell's peak firing rate and a size of at least 200 cm^2.

Theta index[37]:

For a given cell, the normalized temporal autocorrelogram was obtained using bins of 5 ms. The theta index was defined as the difference between the trough (50-70 ms) and the peak (100-140 ms).

Estimation of the significance of overlaps between cell populations

The observed population overlaps were compared with the ones that would result from an independent random assignment of categories. The probability of a neuron to be randomly assigned to category A was set as $p_A=N_A/N$, where N is the total number of neurons and N_A the total number of neurons belonging to A. In this way, in a population of N neurons the expectation value of the size of the subpopulation randomly assigned to category A is $p_A*N=N_A$, identical to the observed group size. Since the assignments are random and independent, the probability of a neuron to be assigned simultaneously to categories A and B is $p_{AB}=p_A*p_B$, and the expectation value for the overlap between both populations is $p_{AB}*N$. When speed was one of the categories, the observed overlap N_{AB} was consistently found to be lower than $p_{AB}*N$ (Extended Data Fig. 6c). To estimate the significance of this difference, N_{AB} was compared with the full probability distribution. In a Bernoulli process, the probability of succeeding k times when tossing N times a coin, each time with probability of success p_{AB}, is given by the binomial distribution,

$$P(k) = \binom{N}{k} {p_{AB}}^k (1-p_{AB})^{N-k},$$

and the left tail p-value associated to N_{AB} is

$$p = \sum_{k=0}^{N_{AB}} P(k).$$

Decoding of running speed from speed cell activity

A simple linear decoder was implemented[38]. A linear relationship between firing rate and speed averaged over 1 s bins is expressed as

$$S_{tr} = R_{tr} f,$$

where S_{tr} is a column vector with the speed bins used for training, R_{tr} is a matrix containing, as columns, the corresponding bins of firing rate for each neuron and an additional column of ones to account for y-intercepts, and f is the linear filter, also a column vector, with length equal to the number of neurons plus one. Training the filter is equivalent to inverting this equation,

$$f = ({S_{tr}}^T S_{tr})^{-1} {S_{tr}}^T R_{tr}.$$

Once f is obtained, the reconstructed speed for a different set of firing rates R_{test} of the same neurons is obtained as

$$S_{rec} = R_{test} f,$$

and the reconstruction quality is defined as the Pearson correlation between S_{rec} and the actual speed S_{test}.

Fields on the linear track

Grid and place fields on the linear track were individualized from 1D spatial maps, treating outbound and inbound runs separately. Fields were identified as isolated local maxima in the rate map above 2Hz, decaying at both sides to either half of their amplitude or below 2 Hz before a new local maximum appeared. A Gaussian fit around the peak of the field was used to estimate amplitude, center and standard deviation of the field. The *z*-score for any position on the track was defined as its distance to the closest field center divided by the standard deviation of the field. The sign of the *z*-score was adjusted for inbound and outbound runs such that the running direction always went from negative to positive values.

For a quantification of spatial shifts in real space (Fig. 5b) the position relative to the field center rather than the *z*-score was used. The two measures are different only in the normalization by field standard deviation. Gaussian fits were used to estimate the field centers subject to different running speeds.

Quantifying the temporal anticipation of the grid field

We define two different sets of kinetic variables. The position, speed and acceleration of the rat are represented by $[x(t), v(t), a(t)]$. The same quantities calculated by a prospective path integrator (which we assume to be free of errors) are represented instead by $[\tilde{x}(t), \tilde{v}(t), \tilde{a}(t)]$. For simplicity and without loss of generality, we assume all these quantities to be zero at time $t = 0$.

If prospective speed cells anticipate the running speed by τ, we can write

$$\tilde{v}(t) = v(t+\tau) = v(t) + \tau a(t) + \frac{\tau^2}{2}\frac{\partial a(t)}{\partial t} + \cdots, \quad (1)$$

where we have used the Taylor series expansion of $v(t+\tau)$ around t. The position of the animal at any time t can be expressed as:

$$x(t) = \int_0^t v(u)du.$$

However, a prospective path integrator that used $\tilde{v}(t)$ as a speed signal would calculate the position at time t as

$$\tilde{x}(t) = \int_0^t \tilde{v}(u)du = x(t) + \tau v(t) + \frac{\tau^2}{2}a(t) + \cdots,$$

where we have used (1).

In the 4 speeds experiment, we can choose to work with segments of constant running speed, where the acceleration and all other derivatives of the speed are close to zero. Thus, a grid field will suffer a spatial anticipation following

$$\tilde{x}(t) \approx x(t) + \tau v(t). \quad (2)$$

Intuitively, if the anticipation of the grid field is of a temporal nature, it will be seen in space as proportional to the running speed, with τ as the coefficient of proportionality.

Acceleration-related field shift on the linear track

On the linear track, outbound and inbound runs were treated as different sessions. In order to pool together in the analysis fields with different width, the *z*-score rather than the position on the track was used as the spatial variable, defining the running direction always from negative to positive values. For every identified field, positive and negative acceleration maps were constructed by filtering only segments of the trajectory where acceleration passed a pre-set positive or negative threshold, e.g. ±50 cm/s^2. The variable Δ was defined as the spatial shift of the positive map that maximized its correlation with the negative map. A field was considered for further analysis only when the correlation between positive and negative maps at its maximizing shift Δ was above 0.9.

This measure did not allow for the dissection of the prospective and retrospective components of the shift, assuming alternating modes[25]. To make this distinction, the average firing field was used as a reference. We used only experiments where this reference could be assumed to be nearly unbiased, i.e. the 4-speed protocol, where most of the time was spent at the lowest speed (7 cm/s) and acceleration was close to zero. In this case, Δ was defined as the spatial shift of the positive or negative map that maximized its correlation with the reference field. Positive (negative) values of Δ along the running direction characterized prospective (retrospective) coding.

Open field acceleration

While in 1D the sign of acceleration is always well defined, in 2D this happens only when the acceleration vector points approximately in the same or in the opposite direction of the velocity vector. These vectors, obtained from their *x* and *y* components of the position, were decomposed into magnitude (a and v) and direction (a_d and v_d). All open field analyses considering the sign of acceleration include only segments of the trajectory where the absolute value of $\cos(a_d - v_d)$ is greater than 0.8. This excludes deviations greater than 37° approximately. The effective acceleration was thus defined through its magnitude a and the sign given by the sign of $\cos(a_d - v_d)$.

Field shift in the open field

Since in open field experiments spatial fields are never traversed twice in the same way, a map-based method was developed to estimate field shift caused by acceleration. The running direction of the rat was used to divide the session where a place cell or grid cell was recorded into four groups: North, East, West and South. Different groups were treated as if they were independent sessions. For every acceleration threshold a_t, two spatial maps were constructed, selecting only time stamps where acceleration was well defined and either positive ($a>a_t$) or negative ($a<-a_t$). The first of these maps was then displaced to both sides in the running direction (N, E, W or S) in order to determine the displacement Δ that maximized the correlation between both maps. Only maps with a maximum firing rate above 10 Hz and with a maximum correlation between maps above 0.5 were included in the analysis. All cells in all 4 running directions were pooled together for the population analysis.

Theta rhythm

A band-pass filter with cutoff frequencies of 6 Hz and 12 Hz was applied to the raw EEG data in order to obtain the theta component of the local field potential. A Hilbert transform was used to decompose the resulting oscillation into amplitude and phase. The phase was then unwrapped into a mostly monotonically increasing signal by adding 2π at every phase reset. The phase at which spike and position timestamps occurred was obtained from the unwrapped phase by interpolation followed by a modulo 2π operation. These values were used to construct histograms of phase precession in the space vs theta phase domain. The theta index[37] was used to assess the theta modulation of individual neurons.

Clustering of theta-phase related behavior

The theta cycle was binned (16 bins) and the average normalized firing rate of each speed cell for each bin was obtained. This data was used as an input into a *k*-means clustering algorithm (MATLAB) with the number of clusters *k* varying between 4 and 10. In every case we used the best result out of 10 replicates, defined as the one with the lowest within-cluster sum of point-to-centroid distance, which ensured a stable solution. Four qualitatively different behaviors were consistently found. For all values of *k* greater than 4, the "ramping" cluster split into sub-clusters of ramping activity with different grades of steepness. After, merging these sub-clusters into one, solutions with different values of *k* were very similar to each other. The results in Extended Data Fig. 9 use k=7.

Histology

Electrodes were not moved after the final recording session. Anesthesia was induced by placing the animal in a closed glass box filled with isoflurane vapor. The rats then received an overdose of Equithesin and were perfused intracardially with saline and 4% formaldehyde. The brains were extracted and stored in formaldehyde, and frozen sagittal sections (30 µm) were cut. All sections were mounted on glass slides and stained with cresyl violet. With the use of a light microscope, equipped with a digital camera, the positions of the recording electrodes were registered in relation to relevant borders between subfields. Final positions of the recording electrodes were indicated on photomicrographs obtained in AxioVision. The exact position of the electrodes at recording was extrapolated using the read-out of the tetrode turning protocol.

Legend for Extended Data

Extended Data Figure 1 | Nissl-stained sagittal brain sections showing representative recording locations in MEC and hippocampus.

Red dots indicate final location of tetrodes. Rat number, hemisphere (R=right, L=left) and entorhinal layers or hippocampal regions where cells were recorded are indicated.

Extended Data Figure 2 | Linear relationship between speed and firing rate in speed cells but not spatially modulated cells of the MEC or the hippocampus.

a, Scatterplot showing slope and *y*-intercept of regression lines for each entorhinal speed cell recorded in the Flintstone car (blue circles) and in the open field (grey circles). Note wide range of slopes and *y*-intercepts. **b**, Identification of speed-modulated cells using analyses that do not assume linearity (see Online Methods). The linearity of these cells is represented by the regression of the tuning curves (red), which clusters mostly around 1 (speed cells) and marginally around -1 (anti-speed cells), in contrast with the distribution of linearity indexes of the shuffled population (grey, 100 shuffling steps, count normalized by the number of steps). This holds across experimental protocols and brain regions, as indicated. **c**, Spatial maps and average speed along the track of 4 representative anti-speed cells in the Flintstone car under linear or 2-speed step protocols, plotted as in Fig. 1b. **d**, Firing rate as a function of position, head direction (hd), and running speed for 6 representative anti-speed cells recorded in MEC during free running in a square open field. Each row shows one cell. Left: colour-coded spatial rate maps. Scale bar to the right. Center: Firing rate as a function of head direction (*x* axis) and running speed (*y* axis). Firing rates in left and center diagrams share the same colour code. Right: Firing rate as a function of running speed. **e**, Speed modulation of firing fields in MEC (top) and hippocampus (bottom). Left: average normalized firing profile of fields in each of the 4-speed groups in the Flintstone car. Right: for each field, the area under the curve 1 standard deviation around the average field center is computed to obtain mean firing rate across firing fields for each speed group (mean ± s.e.m.). In MEC statistical tests showed no significant effect of speed on the average normalized firing rate (Kruskal-Wallis test, $p = 0.12$). In the hippocampus, in contrast, the same tests showed a significant trend in the modulation by speed, due exclusively to the difference between 7 and 28 cm/s (Kruskal-Wallis and Tukey-Kramer tests, $p < 0.05$). Note that similar tests on entorhinal speed cells (Fig. 1f) showed significant differences between all groups ($p<0.01$). **f**, Average firing fields, as in **e**, but using position relative to field center instead of field z-score as the spatial variable (running is always from negative to positive values). This allows direct measurement of firing position as a function of running speed, connecting Eq. 2 with Fig. 5b. Gaussian fits are used to determine firing position, defined as the field center for each speed category.

Extended Data Figure 3 | The Flintstone car does not affect firing properties of grid and place cells on the linear track.

As opposed to recording from a passive rat sitting on a classical car[39], the Flintstone task does not alter the spatial and temporal firing properties of grid cells (top 4 cells) or place cells (bottom 4 cells). Every cell was recorded under 3 conditions: experimenter-determined running in the Flintstone car ('car'), free foraging on the same linear track but with the Flintstone car removed ('free'), and open field. Each block of panels shows data for one cell. Left side of each panel: from top to bottom, the animal's trajectory (black curve) and spike positions (coloured dots) for free sessions and car sessions; corresponding colour-coded rate maps, with red indicating peak rate and dark blue silence; and overall firing rate across the *x* dimension of the track for free (grey) and car (colour) conditions. Note the similarity between spatial maps recorded in the car and the free condition. Right side of each panel: from top to bottom, colour-coded open field rate map and temporal cross-correlograms of spiking in free and car conditions. Note the similarity of the two cross-correlograms.

Extended Data Figure 4 | MEC speed cells are generally not modulated by space or direction.

Examples of MEC speed cells. Three sets of data are shown for each cell. Left: colour-coded spatial rate maps. Scale bar to the right of the first map. Center: Firing rate as a function of head direction (*x* axis) and running speed (*y* axis). Same colour code as for the rate map. Maximum firing rate is indicated in the upper left corner. Right: Firing rate as a function of running speed. **a**, 12 representative MEC speed cells (from a total sample of 385 speed cells in 17 animals), which in general are poorly modulated by space or direction. **b**, Examples of speed cells that passed one additional cell-type criterion (from left to right: border, grid, head direction).

Extended Data Figure 5 | MEC speed cells in a single animal.

All MEC speed cells recorded in one animal (rat 14740). For each cell we show four plots. From left to right: spatial rate maps, head direction vs speed rate maps, spatial autocorrelograms used to calculate the gridness score, and speed tuning curves (right). Symbols for rate map, head direction vs. speed map, and tuning curve as in Extended Data Fig. 4. The spatial autocorrelogram is colour-coded from $r = -1$ (blue) to $r = +1$ (red).

Extended Data Figure 6 | Speed cells form a separate cell class.

a, Observed data (purple) and 100 step-shuffled distributions (grey; count normalized by 100) of different variables used to classify cell types. The dashed lines represent the 99th percentile threshold of the shuffled distribution, with the exception of the distributions of border score and spatial information used for border cell classification, where a dual 95th percentile criterion was used. Threshold values are indicated in boxes. **b**, A similar comparison with shuffled data shows no signs of 'acceleration cells' in MEC. The acceleration score was defined as the correlation between instantaneous firing rate and acceleration. Left: cells recorded in the open field had a distribution of acceleration score (purple) very similar to that of the shuffled population (grey bars). The number of cells exceeding the 99th percentile of the shuffled distribution (0.11) were 21 more than the

average chance level (observed: 46 out of 2497, expected: 25, p-value: 10^{-4}). This might be explained by the fact that out of these 46 cells, 20 were speed cells, which are as a population modulated by acceleration due to their prospective nature (Fig. 5c and Eq. 1). Center: The partial correlation between firing rate on one side and speed and acceleration on the other was computed for these speed cells with high acceleration modulation. In all cases, the partial correlation with speed was higher than the partial correlation with acceleration, with more than a two-fold difference on average. Right: potential modulation by acceleration was also studied by restricting the calculation of the acceleration score to fragments of 2 s around the onsets for the highest speed change in the 4-speeds experiment (from 7 to 28 cm/s), where potential 'acceleration cells' should exhibit a peak in their firing rate. Cells recorded in this experiment had a distribution of acceleration scores (purple) very similar to that of the shuffled distribution (grey), and only 8 out of 997 cells had a score above the 99th percentile of the shuffled distribution (0.45; expected: 10, p-value: 0.78). **c**, Tables showing the significance of population sizes and population overlaps using classification thresholds based on the 99th (top) and the 95th (bottom) percentile of the shuffled distribution. G, grid cells; HD, head direction cells; S, speed cells; B, border cells; P, place cells; +, conjunctive cells satisfying criteria for more than one cell class. Expected chance levels are obtained from Bernoulli distributions. For single categories, the right tail p-value is indicated. For overlap between categories, the left-tail p-value is indicated, while in the case of the overlap between head direction and border cells, which clearly exceeds chance levels (~40% of border cells are also head direction cells), the right tail p-value is added in parentheses. The mixture in the coding of speed and other behavioral variables was always smaller than the mixture between spatial and directional coding. For hippocampal data, the statistics includes only cells that were active in the open field (not including sleep sessions). Note that all cell categories are defined by comparison with a shuffled distribution, i.e. not by applying arbitrary thresholds. This procedure does not always define populations of significant magnitude (see **b**) and exhibits consistent results for the overlap of populations at the 99th and 95th percentile level. **d**, Scatter plots showing distributions of scores and cell-type classifications. Each dot represents a cell, with the same colour-code as used in Fig. 3a. *X* and *Y* axes show scores used for cell-type classification (gridness score, speed score, mean vector length head direction score, border score, or spatial information). Dashed lines represent the classification threshold for each score. **e**, Scatter plot as in **d** showing overlap between the speed-cell and the place-cell populations in the hippocampus. In this case, speed score and spatial information were used for classification. **f**, Recording across multiple days can generate an unwanted bias in the estimation of population sizes, since a single cell could be counted many times. To avoid this bias, we reduced our original dataset by discarding a cell if another cell had been recorded at a distance of less than 200 μm on the same tetrode on an earlier day. In this reduced population of 608 cells, 18% were speed cells, confirming that the results in Figs. 2 and 3 are free of this kind of bias. **g**, The speed scores of cells in MEC (left and center) and hippocampus (right) were plotted against the in-field speed score of the cells, calculated only with data from the spatial bins with a firing rate above the median. This quantity is a correction for spatially and directionally modulated cells, but has no meaning for other cells. Left: out of 16 grid cells that passed the speed cell criterion, 11 (69%) had in-field speed scores clearly below threshold, while the remaining population had similar regular and in-field scores (Mann-Whitney U-test, p = 0.31). Similarly, out of 11 border cells, 5 (45%) had very low in-field scores, and the remaining had similar regular and in-field scores (p = 0.82). Center: a similar approach was implemented using head-direction bins instead of spatial bins. Out of 42 MEC head direction cells with high speed score, 17 (40%) had in-field scores below threshold, while the remaining

population had similar regular and in-field scores ($p = 0.57$). Right: different conclusions were obtained in the analysis of hippocampal place cells. Out of 19 place cells with high speed score, 6 (32%) had low in-field scores. The remaining population had in-field scores significantly higher than the corresponding regular speed scores (Mann-Whitney U-test, $p < 0.02$). In addition, 33 other place cells with low regular speed score had in-field speed scores higher than threshold, suggesting a stronger mixture between speed and spatial coding in the hippocampus. **h**, Population distribution (mean ± s.e.m.) of various quantities for all MEC cell types (S=speed, G=grid, HD= head direction, B=border).

Extended Data Figure 7 | Representative examples of conjunctive grid and head direction cells in MEC and speed cells in the hippocampus.

Three sets of data are shown for each cell. Left: colour-coded spatial rate maps. Scale bar to the right of the first map. Center: Firing rate as a function of head direction (*x* axis) and running speed (*y* axis). Same colour code as for the rate maps. Right: Firing rate as a function of running speed. **a**, MEC conjunctive cells do not exhibit strong modulation by speed. **b**, Hippocampal speed cells have characteristics that are similar to entorhinal ones.

Extended Data Figure 8 | The speed code is context-invariant.

a, Colour-coded rate maps showing realignment in a grid cell recorded in rooms A and B. The sequence of recording was ABA'. In MEC, change of room causes change in grid phase and grid orientation; in the hippocampus, this is accompanied by global remapping[3]. **b**, Speed score, tuning curve y-intercept and slope in room A vs. room B for 20 speed cells recorded in the room-change experiment in **a** (8 rats). Each dot corresponds to one cell. Values distributed around the diagonals, suggesting similar values in A and B. **c**, Percentage change for the same quantities between trials A and B and between A and A' (mean ± s.e.m). In each case, the difference between the two distributions was non-significant (Wilcoxon signed rank test, speed score: $p = 0.9$, y-intercept: $p=0.54$, slope: $p=0.49$). **d**, Reconstructed speed (purple and black) compared to actual speed in darkness (grey). Speed was decoded from the activity of 3 speed cells, with decoders trained either in the lights-on condition (black) or the lights-off condition (purple). Pearson correlation between reconstructions: 0.97. Correlation between decoded speed and actual speed (reconstruction quality): 0.45 with the 'light on' decoder and 0.48 with the 'lights off' decoder. **e**, All speed cells that were recorded in the open field both before and after trials in the Flintstone car were selected from recordings in the MEC (top) and the hippocampus (bottom). Speed score (left), speed tuning curve y-intercept (center) and slope (right) were compared within sessions. Grey dots show the comparison between pre-open field and post-open field recordings (*X* axis and *Y* axis, respectively). Colored circles indicate the comparison between pre-open field (*X* axis) and Flintstone car (*Y* axis). In case the speed score in the Flintstone car was below threshold, an open circle was used instead of the filled colored circle (15 out of 64 in MEC (23%) and 8 out of 16 in the Hippocampus (50%)). The results indicate that, although in both areas many speed cells maintain their firing properties even across extremely different contextual and behavioral situations, MEC speed cells seem to exhibit a more universal code than hippocampal speed cells. **f**, Overall distribution of running speed in the open field (o.f.; grey) and in the

Flintstone car experiments (linear speed profile; red) for rat 14566. This was the rat with the largest difference in speed across behaviors (open field: 10±8 cm/s, Flintstone car: 20±13 cm/s, mean±s.d.) Yet the difference did not generate adaptation in the slope of the speed-rate tuning curve (Figs. 4f-h).

Extended Data Figure 9 | Theta modulation of MEC speed cells.

a, Plots of temporal bias, as in Fig. 5a, for MEC speed cells with weak (left) and strong (right) theta modulation (all cells from open field). Only the latter were prospective (discriminating threshold θ_{index} = 0.2; see Extended Data Fig. 8f). Among cells classified as fast spiking, 30% in MEC and 19% in the hippocampus were speed cells. The proportions of theta-modulated fast-spiking cells are 32% for MEC and 13% for the hippocampus. **b**, Temporal bias of speed cells classified according to location, task and theta modulation. Different measures are used: the maximum of the average correlation curve (peak of mean) and the mean (mean of peaks) and median (median of peaks) of the distribution of maxima of individual correlation curves. The anticipation of the speed cell response to the movements of the animal cannot be related to the learned prediction of the Flintstone car protocol, since in all cases the leads are similar to those found in spontaneous open field behavior. Similar and even larger leads in neural activity over body kinematics have been described in the motor cortex of monkeys[40] as well as rats[41]. Since the motor networks are supposed to be one of the sources of speed information feeding the hippocampal navigation systems, with prominent direct connections from secondary motor cortex to MEC[42], we cannot discard the hypothesis that the lead is simply inherited from this source. Alternatively, other simple network mechanisms such as anticipated synchronization could generate this effect locally without the involvement of predictions or learning in a cognitive sense. **c**, MEC speed cells ordered according to increasing theta modulation index. Colour-coded firing rate profile across the theta cycle is plotted, with each line representing a different cell. Firing rate is normalized for visualization purposes. Red arrow-heads indicate the threshold (θ_{index} = 0.2) used in **a** and **b**. The plot reveals that theta modulated cells have a characteristic behavior, exhibiting a ramp of activity that develops roughly along the first two thirds of the cycle and falls to near-zero during the last third. **d**, Representative examples of the activity of ramping (strongly modulated) and flat (weakly modulated) speed cells at different speeds (colour-coded). Top 4 cells are ramping. Rat number is indicated in the top-left corner. Note that ramps corresponding to different speeds do not run in parallel. Instead, the ramp slope increases with speed. One possible explanation for this is that the ramp represents the integration of speed (distance traveled) from the beginning of the theta cycle rather than speed itself. Note also that the ramp/silent division of the theta cycle roughly coincides with the reset/look ahead division arising from the analysis of grid cell activity (Extended Data Fig. 10f,h). **e**, Normalized firing rate profile (mean ± s.d.) for 4 clusters resulting from applying a *k*-means algorithm to the data in **c**. The number of clusters *k* was set to 7, and all clusters exhibiting a ramping behavior were merged together (similar results were obtained by applying the same procedure with *k*=4 … 10). Note that most speed cells fall into the ramping (#1) or flat (#2) clusters. The sum of counts is 321, lower than the total cell number of 385, because for some cells a simultaneous EEG recording was not available. **f**, Average dynamics along the theta cycle of the normalized firing rate of speed cells belonging to each of the 4 clusters for different running speeds (colour-coded as in **c**). **g**, First 2 principal components of the data. Note that the first principal component represents the ramping pattern. **h**, Scatter representation of the data in **a** across the principal components in **e**. Colours indicate clusters as in **e**. **i-j**, Distribution of clusters (**i**) and

theta indexes (**j**) for different MEC layers. **k**, Plots obtained from the 25 most ramping (left) or flat (right) MEC speed cells (all trials). Each block shows the distribution of correlations between running speed and different temporal shifts of the instantaneous firing rate (left), together with a profile of normalized activity across the theta cycle for positive and negative acceleration with a threshold of ±50 cm/s^2 (right, top) and the difference between the two curves (right, bottom; mean ± s.e.m.). Only ramping cells express pronounced prospective behavior, as seen both by a positive temporal shift (ramping: 206 ± 22 ms, $p < 0.01$; flat: -23 ± 19 ms, $p = 0.31$; Wilcoxon signed rank tests) and by a marked difference between positive and negative acceleration curves along the ramp of activity. Friedman's tests show a significantly higher firing rate for positive acceleration in ramping cells and for negative acceleration in flat cells ($p < 0.01$).

Extended Data Figure 10 | Grid cells in MEC layer II express strong prospective theta-modulated spatial coding.

a, Average fields of spatially modulated MEC and CA cells in the Flintstone car, filtering for only positive acceleration (>50 cm/s^2; red) or only negative acceleration (<-50 cm/s^2; black). Recording layer (II, III or V) in MEC or subfield in the hippocampus (CA1, CA3) is indicated in each case, and the average unfiltered field is shown in grey. Space is represented by the *z*-score of the field and running direction is always defined from left to right. Note that fields were significantly shifted only in MEC layers II (strongly) and III (weakly), i.e. not in MEC layer V or in the hippocampus (Fig. 5g). **b**, To rule out the possibility of a retrospective effect during negative acceleration, we restricted the analysis to the 4-speed experiment. Since rats spent most of the time running at very low speed and nearly zero acceleration, the temporal bias of the average field is reduced to a minimum, and it can be used as a reliable reference. The plot shows shifted fields for different positive and negative acceleration thresholds using only data from the 4-speed experiment. Acceleration threshold is colour-coded (scale bars to the right). Note that negative acceleration, regardless of its magnitude, has a very small effect on the field position, keeping the field close to the reference average field in all cases. In contrast, positive acceleration produces a prospective advance of the field that increases with acceleration threshold. **c**, Position of the average fields peaks in **b** as a function of positive or negative acceleration threshold. Note the increase in prospective shift with increasing positive acceleration threshold. In contrast, negative acceleration produces no effect apart from a small retrospective offset. Such an offset is expected as a consequence of prospection during positive acceleration, since the average field at the lowest speed, used as a reference, should have a small, yet non-zero, prospective bias. **d**, Shifted fields as in **b**, but using only cells that could be classified as grid cells based on rotational symmetry in a complementary open field recording (using the 99th percentile of a shuffled distribution as the classification criterion). The acceleration threshold was ±50 cm/s^2. **e**, Shifts that maximized the correlation between positive or negative acceleration-related fields and the reference average field shown in **d** (mean ± s.e.m; *Wilcoxon signed-rank test after Holms-Bonferroni correction: $p<0.01$). **f**, Phase map of the pool of all putative grid cells, indicating "look ahead" and "reset" stages over two theta cycles (see **h**). In the "look ahead" stage, the grid network engages in forward sweeps, related to phase precession proper[24]. In the "reset" stage the spatial representation suffers a sudden jump back, opposite to the running direction, and the correlation between grid cell firing phase and position is very poor. **g**, Similar phase maps filtering for only positive (>50 cm/s^2; top) or only negative acceleration (<-50 cm/s^2; black). **h**, Top: average firing rate along two

theta cycles. The local minima, indicated with dashed lines, were used to define the frontiers between the "look ahead" and "reset" stages[7,35,43,44]. During the "look ahead" stage, phase precession proper takes place, while during the "reset" stage, the spatial code jumps back and remains relatively static as theta phase increases (see **f**). Center: in three consecutive rows, the average dynamics of Δ along two theta cycles for different acceleration thresholds (colour-coded; from top to bottom: MEC layers II, III and V). **N**ote that the prospective shift of grid fields increases during the "reset" stage and decreases during the "look ahead" stage. This speaks strongly against the idea that the prospective effect is a by-product of forward sweeps of different magnitude, and in favor of transient and local distortions in the representation of location. Bottom: Acceleration is not strongly modulated by theta phase, as observed when computing the overall average (grey) and the average restricted to positive (red) or negative (black) acceleration. **i**, Frequency distribution of ratio between intrinsic firing frequency and LFP theta frequency in grid cells and speed cells. In grid cells (green), the mean intrinsic firing frequency is 3% higher than the theta frequency obtained from the LFP power spectrum (Mann-Whitney U-test: $p = 10^{-21}$). This difference is due to phase precession. In contrast, in speed cells (grey), the mean intrinsic firing frequency is only 0.6% higher than the LFP theta frequency ($p = 0.043$), suggesting that a similar mechanism is not present in this population.

References

1 Hafting, T., Fyhn, M., Molden, S., Moser, M. B. & Moser, E. I. Microstructure of a spatial map in the entorhinal cortex. *Nature* **436**, 801-806, doi:10.1038/nature03721 (2005).

2 Moser, E. I. *et al.* Grid cells and cortical representation. *Nature reviews. Neuroscience* **15**, 466-481, doi:10.1038/nrn3766 (2014).

3 Fyhn, M., Hafting, T., Treves, A., Moser, M. B. & Moser, E. I. Hippocampal remapping and grid realignment in entorhinal cortex. *Nature* **446**, 190-194, doi:10.1038/nature05601 (2007).

4 McNaughton, B. L., Battaglia, F. P., Jensen, O., Moser, E. I. & Moser, M. B. Path integration and the neural basis of the 'cognitive map'. *Nature reviews. Neuroscience* **7**, 663-678, doi:10.1038/nrn1932 (2006).

5 Fuhs, M. C. & Touretzky, D. S. A spin glass model of path integration in rat medial entorhinal cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 4266-4276, doi:10.1523/JNEUROSCI.4353-05.2006 (2006).

6 Burak, Y. & Fiete, I. R. Accurate path integration in continuous attractor network models of grid cells. *PLoS Comput Biol* **5**, e1000291, doi:10.1371/journal.pcbi.1000291 (2009).

7 Navratilova, Z., Giocomo, L. M., Fellous, J. M., Hasselmo, M. E. & McNaughton, B. L. Phase precession and variable spatial scaling in a periodic attractor map model of medial entorhinal grid cells with realistic after-spike dynamics. *Hippocampus* **22**, 772-789, doi:10.1002/hipo.20939 (2012).

8 Couey, J. J. *et al.* Recurrent inhibitory circuitry as a mechanism for grid formation. *Nature neuroscience* **16**, 318-324, doi:10.1038/nn.3310 (2013).

9 Burgess, N., Barry, C. & O'Keefe, J. An oscillatory interference model of grid cell firing. *Hippocampus* **17**, 801-812, doi:10.1002/hipo.20327 (2007).

10 Hasselmo, M. E. & Brandon, M. P. A model combining oscillations and attractor dynamics for generation of grid cell firing. *Front Neural Circuits* **6**, 30, doi:10.3389/fncir.2012.00030 (2012).

11 Bush, D. & Burgess, N. A hybrid oscillatory interference/continuous attractor network model of grid cell firing. *J Neurosci* **34**, 5065-5079, doi:10.1523/JNEUROSCI.4017-13.2014 (2014).

12 Jeewajee, A., Barry, C., O'Keefe, J. & Burgess, N. Grid cells and theta as oscillatory interference: electrophysiological data from freely moving rats. *Hippocampus* **18**, 1175-1185, doi:10.1002/hipo.20510 (2008).
13 Sargolini, F. *et al.* Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science* **312**, 758-762, doi:10.1126/science.1125572 (2006).
14 Wills, T. J., Barry, C. & Cacucci, F. The abrupt development of adult-like grid cell firing in the medial entorhinal cortex. *Front Neural Circuits* **6**, 21, doi:10.3389/fncir.2012.00021 (2012).
15 Langston, R. F. *et al.* Development of the spatial representation system in the rat. *Science* **328**, 1576-1580, doi:10.1126/science.1188210 (2010).
16 Stensola, H. *et al.* The entorhinal grid map is discretized. *Nature* **492**, 72-78, doi:10.1038/nature11649 (2012).
17 Solstad, T., Boccara, C. N., Kropff, E., Moser, M. B. & Moser, E. I. Representation of geometric borders in the entorhinal cortex. *Science* **322**, 1865-1868, doi:10.1126/science.1166466 (2008).
18 Bjerknes, T. L., Moser, E. I. & Moser, M. B. Representation of geometric borders in the developing rat. *Neuron* **82**, 71-78, doi:10.1016/j.neuron.2014.02.014 (2014).
19 Buetfering, C., Allen, K. & Monyer, H. Parvalbumin interneurons provide grid cell-driven recurrent inhibition in the medial entorhinal cortex. *Nature neuroscience* **17**, 710-718, doi:10.1038/nn.3696 (2014).
20 Muller, R. U. & Kubie, J. L. The firing of hippocampal place cells predicts the future position of freely moving rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **9**, 4101-4110 (1989).
21 Frank, L. M., Brown, E. N. & Wilson, M. Trajectory encoding in the hippocampus and entorhinal cortex. *Neuron* **27**, 169-178 (2000).
22 Wood, E. R., Dudchenko, P. A., Robitsek, R. J. & Eichenbaum, H. Hippocampal neurons encode information about different types of memory episodes occurring in the same location. *Neuron* **27**, 623-633 (2000).
23 Ferbinteanu, J. & Shapiro, M. L. Prospective and retrospective memory coding in the hippocampus. *Neuron* **40**, 1227-1239 (2003).
24 Gupta, A. S., van der Meer, M. A., Touretzky, D. S. & Redish, A. D. Segmentation of spatial experience by hippocampal theta sequences. *Nature neuroscience* **15**, 1032-1039, doi:10.1038/nn.3138 (2012).
25 De Almeida, L., Idiart, M., Villavicencio, A. & Lisman, J. Alternating predictive and short-term memory modes of entorhinal grid cells. *Hippocampus* **22**, 1647-1651, doi:10.1002/hipo.22030 (2012).
26 Bieri, K. W., Bobbitt, K. N. & Colgin, L. L. Slow and fast gamma rhythms coordinate different spatial coding modes in hippocampal place cells. *Neuron* **82**, 670-681, doi:10.1016/j.neuron.2014.03.013 (2014).
27 Lee, A. M. *et al.* Identification of a brainstem circuit regulating visual cortical state in parallel with locomotion. *Neuron* **83**, 455-466, doi:10.1016/j.neuron.2014.06.031 (2014).
28 O'Keefe, J., Burgess, N., Donnett, J. G., Jeffery, K. J. & Maguire, E. A. Place cells, navigational accuracy, and the human hippocampus. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **353**, 1333-1340, doi:10.1098/rstb.1998.0287 (1998).
29 Lever, C. *et al.* Spatial coding in the hippocampal formation: input, information type, plasticity and behaviour. (2003).
30 McNaughton, B. L., Barnes, C. A. & O'Keefe, J. The contributions of position, direction, and velocity to single unit activity in the hippocampus of freely-moving rats. *Exp Brain Res* **52**, 41-49 (1983).
31 Czurko, A., Hirase, H., Csicsvari, J. & Buzsaki, G. Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. *Eur J Neurosci* **11**, 344-352 (1999).

32 Hasselmo, M. E., Giocomo, L. M. & Zilli, E. A. Grid cell firing may arise from interference of theta frequency membrane potential oscillations in single neurons. *Hippocampus* **17**, 1252-1271, doi:10.1002/hipo.20374 (2007).
33 Blair, H. T., Welday, A. C. & Zhang, K. Scale-invariant memory representations emerge from moire interference between grid fields that produce theta oscillations: a computational model. *J Neurosci* **27**, 3211-3229, doi:10.1523/JNEUROSCI.4724-06.2007 (2007).
34 Battaglia, F. P., Sutherland, G. R. & McNaughton, B. L. Local sensory cues and place cell directionality: additional evidence of prospective coding in the hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 4541-4550, doi:10.1523/JNEUROSCI.4896-03.2004 (2004).

References for Methods

35 Skaggs, W. E., McNaughton, B. L., Wilson, M. A. & Barnes, C. A. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. *Hippocampus* **6**, 149-172, doi:10.1002/(SICI)1098-1063(1996)6:2<149::AID-HIPO6>3.0.CO;2-K (1996).
36 Boccara, C. N. *et al.* Grid cells in pre- and parasubiculum. *Nature neuroscience* **13**, 987-994, doi:10.1038/nn.2602 (2010).
37 Cacucci, F., Lever, C., Wills, T. J., Burgess, N. & O'Keefe, J. Theta-modulated place-by-direction cells in the hippocampal formation in the rat. *J Neurosci* **24**, 8265-8277, doi:10.1523/JNEUROSCI.2635-04.2004 (2004).
38 Serruya, M. D., Hatsopoulos, N. G., Paninski, L., Fellows, M. R. & Donoghue, J. P. Instant neural control of a movement signal. *Nature* **416**, 141-142, doi:10.1038/416141a (2002).

References for Extended Data Figure Legends

39 Terrazas, A. *et al.* Self-motion and the hippocampal spatial metric. *J Neurosci* **25**, 8085-8096, doi:10.1523/JNEUROSCI.0693-05.2005 (2005).
40 Paninski, L., Fellows, M. R., Hatsopoulos, N. G. & Donoghue, J. P. Spatiotemporal tuning of motor cortical neurons for hand position and velocity. *Journal of neurophysiology* **91**, 515-532, doi:10.1152/jn.00587.2002 (2004).
41 Chapin, J. K., Moxon, K. A., Markowitz, R. S. & Nicolelis, M. A. Real-time control of a robot arm using simultaneously recorded neurons in the motor cortex. *Nature neuroscience* **2**, 664-670, doi:10.1038/10223 (1999).
42 Burwell, R. D. & Amaral, D. G. Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *The Journal of comparative neurology* **398**, 179-205 (1998).
43 O'Keefe, J. & Recce, M. L. Phase relationship between hippocampal place units and the EEG theta rhythm. *Hippocampus* **3**, 317-330, doi:10.1002/hipo.450030307 (1993).
44 Hafting, T., Fyhn, M., Bonnevie, T., Moser, M. B. & Moser, E. I. Hippocampus-independent phase precession in entorhinal grid cells. *Nature* **453**, 1248-1252, doi:10.1038/nature06957 (2008).