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Principles governing the integration of landmark and self-motion cues in entorhinal cortical codes for navigation

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Abstract

To guide navigation, the nervous system integrates multisensory self-motion and landmark information. We examined how these inputs generate the representation of self-location by recording entorhinal grid, border and speed cells in mice navigating virtual environments. Manipulating the gain between the animal's locomotion and the visual scene revealed that border cells responded to landmark cues while grid and speed cells responded to combinations of locomotion, optic flow, and landmark cues in a context-dependent manner, with optic flow becoming more influential when it was faster than expected. A network model explained these results, providing principled regimes under which grid cells remain coherent with or break away from the landmark reference frame. Moreover, during path integration-based navigation, mice estimated their position following the principles predicted by our recordings. Together, these results provide a quantitative framework for understanding how landmark and self-motion cues combine during navigation to generate spatial representations and guide behavior.

Main Text

To accurately navigate, the brain must combine information regarding self-motion and the sensory perception of landmarks to form an estimate of spatial position. The neural substrates thought to support such position coding include functionally-defined cell types that reside in medial entorhinal cortex (MEC)¹. Grid cells represent positional information by firing in multiple place-specific locations, which form a regular array of firing activity that covers the environment². MEC head direction cells fire when an animal faces a particular direction, updating their representation based on self-motion while remaining anchored to visual cues^{3,4}. Border cells increase their firing rate near environmental boundaries, even when these boundaries are represented by visual cues alone^{5,6}. Finally, speed cells change their firing rate with the running speed of the animal^{7,8}. Thus, as a population, MEC neurons have the capacity to generate an internal map of space, with their

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codes likely emerging from interactions between self-motion cues, such as locomotion and optic flow, and sensory cues regarding environmental landmarks.

However, the principles by which MEC cells integrate self-motion versus landmark cues to generate their functional response properties remain incompletely understood. While several works indicate that grid cell firing patterns rely on the integration of self-motion cues^{2,9–11}, increasing evidence suggests that the grid pattern emerges from a complex interaction of self-motion and sensory landmark features. For example, grid cells deform in response to geometric changes in the environment, distort in polarized environments, depend on input regarding boundaries to maintain an error free map of space and, in mice, rapidly destabilize after the removal of visual landmarks^{2,12–19}. Yet, many of these studies involved the complete removal of self-motion or landmark cues. Fewer studies have examined situations in which self-motion and landmark cues systematically disagree, which could elucidate the principles governing their interaction.

The principles underlying how self-motion and landmark cues integrate to generate speed cell firing patterns remain equally unknown. In MEC, speed cells retain their general coding features in complete darkness, but firing rates and the slopes of linear fits between firing rate and running speed decrease¹⁶, suggesting that visual inputs calibrate their response features. Visual inputs could provide a measure of self-motion in the form of optic flow²⁰, which must be combined with other multisensory signals to generate a unified self-motion percept²¹. However, the influence of optic flow on MEC speed cells has not been directly measured. In addition, previous works often ascribe the neural basis of path integration-based navigation to MEC functionally-defined cell types^{1,2,9}, but the degree to which behaviorally-measured path integration position estimates and MEC neural codes follow the same cue combination principles remains unclear^{13–17}.

Here, we examine the principles by which functionally-defined MEC cell classes integrate self-motion (through locomotion and optic flow cues) with visual landmark cues (Fig. 1), as well as what relationship these computations might have to behavioral position estimates. To do this, we analyzed the neural activity and navigational behavior of mice while they explored virtual reality (VR) environments, which enable precise control over the animal's sensory experience^{22,23}. By combining these experimental approaches with an attractor-based network model, we propose a framework for understanding how optic flow, locomotion and landmark cues interact to generate competing drives on MEC firing patterns and position estimates during path integration-based navigation.

Results

Cell classification and virtual reality setup

We recorded MEC neural activity in 21 mice as they navigated mono-directional VR linear tracks for water reward (Fig. 1b-d and Supplementary Fig. 1). Well-trained mice signaled their engagement with the task by slowing down in anticipation of the reward (Supplementary Fig. 2). Spatially-responsive cells were classified based on their spatial tuning in an open field (grid cell $n = 151/1136$, border $n = 160/1136$, Online Methods). In VR, MEC neurons recorded in the open field were identified by matching waveforms

(781/1136 cells; 96/151 grid cells, 97/160 border cells; Fig. 1b-c and Supplementary Fig. 3) and many had clear, spatially stable firing fields at one or more locations on the track (mean half-and-half stability $\pm$ standard error of the mean [SEM]; grid = 0.40 ± 0.02 , border = 0.54 ± 0.02 ; Fig. 1e-f and Supplementary Fig. 4). Grid cell stability in VR was similar to grid cell stability on a real linear track in a separate dataset²⁴ (Supplementary Fig. 4g,h), indicating that the grid network was engaged during navigation in the virtual environment.

In VR, border cells were more spatially stable than grid cells (Wilcoxon rank-sum $p = 4.4 \times 10^{-5}$) and, on average, the firing rate of stable border cells peaked near prominent visual landmarks in the environment (repeated measures ANOVA $p = 0.016$; Fig. 1e, Online Methods). In contrast, the firing rate of stable grid cells did not peak near landmarks but was uniformly distributed with respect to the track (repeated measures ANOVA $p = 0.18$; Fig. 1e, Online Methods). This suggests that grid and border cells are driven by different sets of cues in VR, with border cell firing likely determined by the location of visual landmarks. We next investigated this further using manipulations of the virtual environment.

Gain manipulations separate visual and motor contributions to MEC neural codes

To ascertain the relative contribution of locomotion versus visual cues (optic flow and visual landmarks) to the firing patterns of MEC neurons, we put locomotion and visual cues into conflict by altering the gain of the transformation between the rotation of the ball and translation of the VR track (Fig. 2)²³. Manipulations followed an A-B-A' design, with gain in B either decreasing (0.5x; Fig. 2a) or increasing (1.5x; Fig. 2a) the translation of the visual scene. To avoid plasticity in the representation of the virtual environment, we restricted the number of gain manipulation trials for any given session ($n = 5$ for gain decrease; $n = 10$ for gain increase). Despite altered gain in B, mice continued to slow down at the reward location, indicating a landmark-based navigational strategy in this task (Supplementary Fig. 2). Across both gain change conditions, we recorded 80 grid cells (gain decrease $n = 65$, gain increase $n = 56$) and 68 border cells (gain decrease $n = 44$, gain increase $n = 48$) (Fig. 2d and Supplementary Fig. 5). Grid and border cell firing fields were analyzed with respect to virtual position on the track, rather than actual distance traveled on the ball. To quantify the response of grid and border cells to altered gain, we then considered three possible response types: that the spatial firing pattern degenerated, coherently remapped or did not change between baseline and gain trials (Fig. 2b-c).

Most grid and border cells retain stable firing patterns during gain manipulations

We first examined whether the spatial firing patterns of grid or border cells degenerated during gain manipulation trials. We quantified degeneration by computing the spatial stability between matched numbers of trials from the A and B periods (Online Methods). As we recorded a subset of MEC cells over multiple blocks of gain manipulation trials, we computed spatial stability values within each trial block separately. Grid and border cell stability did not significantly decrease from the A to B period, except for grid gain increase sessions, in which stability decreased but was comparable to B period values observed during all other gain sessions (stability A vs B $\pm$ SEM, Wilcoxon tests: grid gain decrease A = 0.32 ± 0.03 , B = 0.32 ± 0.02 , $n = 98$ gain manipulations from 65 cells, $p = 0.83$; border gain decrease A = 0.39 ± 0.04 , B = 0.38 ± 0.04 , $n = 48$ gain manipulations from 44 cells, $p =$

0.89; grid gain increase $A = 0.43 \pm 0.03$, $B = 0.31 \pm 0.03$, $n = 88$ manipulations from 56 cells, $p = 0.00047$; border gain increase $A = 0.41 \pm 0.01$, $B = 0.40 \pm 0.01$, $n = 53$ gain manipulations from 48 cells, $p = 0.88$; Fig. 3a). Stability remained significantly above zero for grid and border cells across both gain changes (all $p < 1e-10$), and firing rates did not change (median (Hz) $\pm$ SEM, Wilcoxon tests: grid gain decrease, $A = 2.70 \pm 0.90$, $B = 2.54 \pm 0.73$, $n = 33$, $p = 0.95$; border gain decrease, $A = 2.22 \pm 0.54$, $B = 2.35 \pm 0.46$, $n = 28$, $p = 0.49$; grid gain increase, $A = 1.50 \pm 0.60$, $B = 1.76 \pm 0.72$, $n = 37$, $p = 0.36$; border gain increase, $A = 2.60 \pm 0.65$, $B = 2.20 \pm 0.65$, $n = 30$, $p = 0.70$; Supplementary Fig. 6a). Thus, while some degeneration was present in a subset of cells, most cells retained a high level of stability during gain changes that was comparable to baseline.

To quantify structured changes in firing rate maps during gain manipulations, we next identified cells that remained stable during gain manipulations (stability > 0.2 in both the A and B period; upper right quadrant of Fig. 3a). A majority of grid and border cells had stable responses to gain manipulations (# cells with 1 stable trial block/# cells: grid gain decrease, 33/65; gain increase, 37/56; border gain decrease, 28/44; gain increase, 30/48). This thresholding analysis identified similar numbers of stable cells when applied to adjacent blocks of trials within the A period, confirming that degeneration during gain changes was comparable to baseline instability (Supplementary Fig. 7a). For subsequent analyses of structured changes in firing patterns, only stable trial blocks were used.

Gain changes cause coherent remapping in grid cells but little change in border cells

Next, we analyzed whether stable grid and border cells changed their firing patterns during gain manipulations by computing lagged cross-correlations between the A and B period rate maps (Fig. 3b). Rate maps were computed with respect to virtual position on the track, rather than distance run on the ball. This spatial cross-correlation measure quantitatively discriminates between the possibilities shown in Fig. 2b-c. For example, a large peak in the cross-correlation at zero spatial lag indicates firing patterns do not change between baseline and a gain manipulation. Such a result would be expected if grid or border cell responses were primarily determined by visual cues, thereby locking their firing patterns to position on the virtual track, regardless of any change in gain. Alternatively, a large peak at a nonzero spatial lag indicates a form of coherent remapping in which firing patterns systematically shift in space due to a gain change. And finally, the lack of a large peak indicates a more complex remapping with significant changes in firing patterns between baseline and a gain manipulation. For cells with multiple stable gain manipulation responses, correlations were averaged across manipulations.

For adjacent blocks of 5 trials within baseline, grid and border cells had high cross-correlations centered around zero spatial lag, confirming that these cells had spatially stable and coherent patterns in the baseline (A) condition (correlation value $\pm$ SEM: grid gain decrease (33) = 0.54 ± 0.03 , border gain decrease (28) = 0.60 ± 0.03 , grid gain increase (37) = 0.56 ± 0.02 , border gain increase (30) = 0.61 ± 0.03 ; Fig. 3b). Grid cells remapped during both gain decreases and gain increases, as indicated by the low A-B cross-correlations at zero lag compared to baseline (correlation value $\pm$ SEM: gain decrease = 0.07 ± 0.03 , Wilcoxon test vs. baseline $p = 9.4e-7$; gain increase = 0.17 ± 0.03 , Wilcoxon test vs. baseline

$p = 1.7\text{e-}7$; Fig. 3b). In contrast, border cells remained remarkably stable during gain increases, but partially remapped during gain decreases (correlation value $\pm$ SEM: gain decrease = 0.34 ± 0.05 , Wilcoxon test vs. baseline $p = 0.00022$; gain increase = 0.54 ± 0.04 , Wilcoxon test vs. baseline $p = 0.16$; Fig. 3b). Even during gain decreases, however, border cells had significantly higher A-B correlations at zero lag compared to grid cells (Wilcoxon rank-sum test vs. grid cells, gain decrease $p = 7.8\text{e-}5$; gain increase $p = 4.0\text{e-}8$; Fig. 3b). These data demonstrate that while grid and border cells had similar baseline stability, grid cells remapped during gain manipulations, whereas border cells primarily remained locked to the visual cues.

Grid cells respond asymmetrically to gain increases and gain decreases

We then further considered the nature of grid cell remapping during gain increase and decrease manipulations. For gain increases, a large peak in the average A-B cross-correlation curve was evident at +12 cm, indicating a shift of the rate map forward along the track by this amount (mean correlation value $\pm$ SEM: 0.30 ± 0.04 , $n = 37$; Fig. 3b). This peak was lower than baseline, but not because cross correlation peaks were lower for each cell. Rather, cross-correlation peaks in B were similar in magnitude to those in A, but had shifted forward on the track by varying amounts (mean cross-correlation peak over individual cells $\pm$ SEM: baseline = 0.58 ± 0.02 , gain change = 0.52 ± 0.03 , Wilcoxon $p = 0.14$; mean cross-correlation shift $\pm$ SEM: baseline = -1.7 ± 0.5 cm, gain change = 14.4 ± 7.1 cm, Wilcoxon $p = 0.0099$; Fig. 3b, bottom). For gain decreases, a much smaller but still non-zero peak in the average A-B cross-correlation curve was present at -6 cm (mean correlation value $\pm$ SEM: 0.10 ± 0.03 , $n = 33$; Wilcoxon test vs zero, $p = 0.0089$; Wilcoxon test gain decrease vs gain increase peak, $p = 0.00057$; Fig. 3b). Unlike for gain increases, the peaks of individual grid cells' A-B cross-correlations were smaller during gain decrease compared to baseline, but were not shifted backward on average (mean cross-correlation peak $\pm$ SEM: baseline = 0.57 ± 0.02 , gain change = 0.37 ± 0.03 , Wilcoxon $p = 1.4\text{e-}5$; mean cross-correlation shift $\pm$ SEM: baseline = -2.2 ± 0.5 cm, gain change = 16.5 ± 9.2 cm, Wilcoxon $p = 0.050$; Fig. 3b, bottom). These results indicate that grid cells retained a coherent, but shifted, rate map during gain increases, but not gain decreases. In contrast to grid cells, some border cells shifted backward during gain decreases, whereas others were stable (Fig. 2d), leading to an average A-B cross-correlation that peaked near zero (-2 cm) but with a notable leftward skew (Fig. 3b). Practically all border cells remained highly stable with minimal shifts during gain increases (Fig. 3b).

Next, having observed stable remapping in grid cells during gain changes, with prominent phase shifts in gain increases but not gain decreases, we asked whether grid cells rescaled during gain changes. The degree of rescaling can provide insight into the relative weighting of visual and motor speed input for putative grid cell path integration calculations, with larger degrees of rescaling indicating a larger influence of motor speed cues. For example, if locomotor cues are the primary input determining grid cell firing patterns, we would expect to see a decrease in the spatial scale of the grid pattern with respect to virtual position on the track that matches the degree of gain decrease (Fig. 2b). To test this, we first computed average autocorrelations of the rate maps in the A and B periods (Fig. 3c, top). The width of the central peak of the autocorrelogram reflects the spatial scale of the grid pattern. We then

contracted or expanded the average A period autocorrelogram and correlated this rescaled map with the average B period autocorrelogram (Fig. 3c, bottom). For gain decreases, these maps maximally overlapped when the baseline was scaled by a factor of 0.61, indicating that the field size of grid firing patterns decreased and rescaled by 78% (100% scaling corresponds to a factor of 0.5; mean $\pm$ SEM over individual cells = $54 \pm 13\%$; Fig. 3c). Importantly, these scale changes were not a simple rescaling of the original firing pattern, but rather reflected new maps with smaller grid scales in virtual coordinates (Supplementary Fig. 6g, h). For gain increases, the maximum was obtained at a scale factor of 1.10, indicating that the field size of grid firing patterns increased and rescaled by 20% (100% scaling corresponds to a factor of 1.5; mean $\pm$ SEM over individual cells = $12 \pm 12\%$; Fig. 3c), significantly less than in gain decrease (Wilcoxon rank sum $p = 0.016$). On the other hand, border cells showed minimal rescaling in both manipulations (gain decrease scaling factor = 0.96, 8%, gain increase scaling factor = 0.96, -8%; Fig. 3c).

These rescaling results did not reflect rate map instability, as they were qualitatively identical when examining only the most stable cells (Supplementary Fig. 7b, c). Moreover, within-trial autocorrelations, which are not affected by drift of firing patterns across trials, showed similar results (Supplementary Fig. 7d, e), as did changes in the number and size of firing fields (Supplementary Fig. 6b-e). Grid cell responses were also relatively uniform across the environment, as grid firing patterns did not show significantly larger shifts or scale changes in different segments of the track (Supplementary Fig. 8). The fact that the amount of shift was constant over the environment during gain increases is further indication that this is a shift of the rate map rather than a rescaling, since the effects of a rescaling would grow with distance since the start of the track. Taken together, these analyses revealed that grid cells responded differently to gain increases and decreases: shifting in gain increases, with an occasional loss of fields (Supplementary Fig. 5, 6e), and remapping and rescaling in gain decreases.

What mechanism might drive the grid rescaling we observed during gain manipulations? If grid cells estimate position by integrating self-motion input over time, then grid spacing should be inversely proportional to the magnitude of the velocity input to grid cells. In our experiments, this velocity input would represent a combination of the available locomotor and optic flow cues. The observed grid cell rescaling of 78% in gain decrease but only 20% in gain increase suggests that locomotor inputs exert a greater influence on grid cells in gain decrease compared to gain increase, leading to asymmetrical, contextually-dependent integration of locomotor cues by grid cells. We next sought to investigate possible sources of this asymmetry by examining MEC speed signals during gain manipulations.

Multiple MEC speed signals respond asymmetrically to gain increases and gain decreases

Multiple signals in MEC carry information about running speed, the two most prominent being speed cells^{7,8,25} and theta oscillations^{7,26}. We first analyzed MEC speed cells, which could provide speed-tuned input to grid cells⁸. We analyzed speed cells with respect to real running speed on the ball, not the speed of the visual cues; in the previous section, grid and border cells were analyzed with respect to virtual position on the track. This change was made as the mouse's real running speed did not change during gain manipulations, making it

more straightforward to compare speed cells in this reference frame (Supplementary Fig 2). Thus, for these analyses, if locomotor cues are the primary input determining speed cell responses, we would expect the slope of firing rate as a function of speed to remain constant between baseline and gain conditions. On the other hand, if visual cues are the primary input determining speed cell responses, we would expect speed cell slope to increase with gain increases and decrease with gain decreases (Fig. 4a).

Speed cells were identified based on open field and VR recordings ($n = 33$ gain decrease, 41 gain increase; Fig. 4b, c and Supplementary Fig. 9; Online Methods). The mean firing rate of speed cells during running (speed > 2 cm/s) significantly increased in gain increases but did not change in gain decreases, suggesting that like grid cells, speed cells respond asymmetrically to gain manipulations (firing rate (Hz) $\pm$ SEM: gain decrease, $A = 9.65 \pm 1.63$, $B = 10.21 \pm 1.71$, Wilcoxon $p = 0.16$; gain increase, $A = 10.83 \pm 1.51$, $B = 13.31 \pm 1.96$, Wilcoxon $p = 7.1e-5$). To explore this further, we estimated instantaneous firing rate by smoothing vectors of spike counts across time bins (~ 16.7 ms) and then linearly regressed instantaneous firing rate against running speed for each speed cell individually. Linear regression slopes significantly increased in gain increase but did not change in gain decrease (slope (Hz/cm/s) $\pm$ SEM: gain decrease, $A = 0.095 \pm 0.014$, $B = 0.085 \pm 0.013$, Wilcoxon $p = 0.17$; gain increase, $A = 0.115 \pm 0.016$, $B = 0.142 \pm 0.020$, Wilcoxon $p = 0.0013$; Fig 4d-e). Intercepts of the linear fits did not change in either case (intercept (Hz) $\pm$ SEM: gain decrease, $A = 6.67 \pm 1.40$, $B = 6.69 \pm 1.35$, Wilcoxon $p = 0.99$; gain increase $A = 7.79 \pm 1.29$, $B = 7.86 \pm 1.35$, Wilcoxon $p = 0.79$; Fig 4d-e). Quadratic fits, which account for firing rate saturation, showed a similar effect (Supplementary Fig. 10). No changes were found between the A period and the first 10 trials of the A' period, ruling out effects from differing numbers of trials or slow changes over time (Supplementary Fig. 10). To estimate the percent weighting of visual cues by speed cells during gain changes, we converted plots of B period slope versus A period slope into polar coordinates and computed the average angle of the response (Fig. 4f). This method is robust to the over-influence of speed cells with large slopes. Here, full locomotor weighting corresponds to an angle of 45° , and full visual weighting corresponds to $\arctan(0.5)$, or 26.6° , in gain decrease, and $\arctan(1.5)$, or 56.3° , in gain increase. Angles were not significantly different from 45° during gain decrease, but were significantly larger than 45° during gain increase (mean $\pm$ SEM: gain decrease = $42.8 \pm 2.2^\circ$, $p = 0.37$; gain increase = $51.0 \pm 1.6^\circ$, $p = 0.0011$; Wilcoxon test vs. 45° ; Fig. 4g-h). Expressed as a percentage, speed cell visual weights were significantly larger during gain increase than gain decrease (mean $\pm$ SEM: gain decrease = $11.9 \pm 12.0\%$, gain increase = $52.9 \pm 13.8\%$, Wilcoxon test $p = 0.0503$; Fig. 4i). Together, these findings show that as a population, speed cells weighed visual cues more highly in gain increase than in gain decrease, mirroring the asymmetry found in grid cells.

Another MEC signal that is positively modulated by increases in running speed is theta frequency of the local field potential (LFP)^{26,27}. To examine whether the modulation of theta frequency by running speed is altered in gain changes, we identified sessions with clear theta oscillations (power in 6–10 Hz band > 3 times power in 10–14 Hz band; 158/187 gain manipulation sessions) and concatenated them by mouse. First we found that, compared to baseline, peak theta frequency was significantly higher in gain increase, but did not change in gain decrease (peak frequency (Hz) $\pm$ SEM: gain increase $A = 7.51 \pm 0.10$, $B = 7.82$

± 0.13 , $n = 16$ mice, Wilcoxon $p = 0.0016$; gain decrease $A = 7.50 \pm 0.09$ Hz, $B = 7.45 \pm 0.07$, $n = 17$ mice, Wilcoxon $p = 0.59$; Supplementary Fig. 11a). Second, we found that the slope of theta frequency with respect to running speed significantly changed in gain increase but not gain decrease (Supplementary Fig. 11b, c). These results show that visual cues affect LFP theta frequency in MEC more for gain increases than gain decreases, which parallels the asymmetric effect of visual cues on speed cells during gain manipulations.

A coupled-oscillator attractor network model elucidates principles for the integration of landmarks and self-motion

Combined, our experimental data point to the presence of an asymmetry in the integration of locomotion and visual cues by grid and speed cells during gain changes. What underlying principles govern this cue-integration process? Previous work has shown that grid cells rely on both self-motion input¹⁰, which can reflect locomotion and optic flow cues, as well as an error correcting signal provided by environmental landmarks¹². However, gain changes alter the relationship between distance traveled and the locations of known landmarks, as well as the relationship between locomotion and optic flow. Therefore, the responses observed in our electrophysiological data likely reflect a complex interaction between the effects gain changes have on these different relationships. To better understand these complex dynamics, we modeled the integration of self-motion with landmark input in a 1D attractor network (Fig. 5).

We added external landmark inputs to standard attractor-based path integration machinery, in which grid cells are modeled as a one-dimensional periodic network of neurons with short-range excitatory and long-range inhibitory synaptic weight profiles (Fig. 5a, and see Appendix)^{28–30}. In the absence of any external input, this neural architecture yields a family of steady state bump activity patterns, in which single grid cell responses are generated when the velocity of the animal is used to drive phase advance in the attractor network^{29,31}. External landmark inputs drive neuronal activity that changes as a function of the animal's immediate position relative to external landmark cues and serve to reinforce the phase of the attractor network corresponding to the learned locations of landmarks (Fig. 5b, c)^{12,28}. In this framework, gain changes correspond to a mismatch between the phase, or position estimate, of the path integrator (red arrow, Fig. 5a, c) and the phase of the landmark input (blue arrow, Fig. 5b, c). To model this situation, we varied the constant k_A , which describes the gain between the velocity input and the change of the attractor phase due to path integration. During gain changes, landmark inputs exert a corrective force on the attractor phase, pulling it towards the landmark phase (Fig. 5d). Importantly, this force grows with the difference between the path integrator estimate and the landmark position estimate (Appendix). The dynamics governing this process are analogous to a coupled-oscillator system, in which the two oscillators are grid cells, described by the attractor phase, and landmark inputs to grid cells, described by the landmark phase. Coupled-oscillator systems are well-studied in physics³² and provide a clarifying analogy for the cue-integration process studied here.

In the model, as in other coupled-oscillator systems, gain manipulations lead to two response types depending on whether path integration and landmark inputs disagree by a small or

large amount. In what we call the “sub-critical” regime, when the two disagree by a small amount, landmark inputs continuously correct the evolving phase of the attractor network and force it to keep pace with the landmarks, albeit at a constant phase difference (Fig. 5e, f). In this situation, an equilibrium is reached where the grid pattern shifts, but does not change in scale. By contrast, in the “super-critical” regime, when path integration and landmarks disagree by a large amount, the phase advance of the attractor network due to path integration enables the attractor phase to break free of the forces exerted by landmark inputs. A consequence of this freedom, or decoherence, is that the resulting grid firing patterns are both remapped and rescaled (Fig. 5e, f). We derived a formula for the point at which grid cell responses transition from sub-critical to super-critical (Appendix):

$$D = \frac{(k_A - k_L) \cdot v}{\omega}$$

where D is a decoherence constant demarcating the two regimes. The sub-critical regime corresponds to $-1 < D < 1$. Outside of this range, responses are super-critical. Here, $k_A - k_L$ is the difference between baseline and gain-manipulated velocity input, which depends on the percent weighting of locomotor and optic flow cues (A stands for attractor, L for landmarks); v is the running speed of the mouse (which was assumed to be constant along the track in the model); and ω is the strength of the landmark input. Thus, there are three determinants of whether the response will be sub- or super-critical: the degree of mismatch between the velocity-driven attractor advance and landmark inputs ($k_A - k_L$), the running speed of the animal itself (v), and the strength of the landmark input (ω). For example, $|D|$ will increase with larger gain changes and faster running speeds, and decrease with stronger landmark input. Importantly, k_A corresponds to the velocity input received by the attractor network. This does not need to be proportional to the VR gain, if velocity signals are processed in a nonlinear manner (i.e. if the percent weighting of locomotor vs. visual speed cues changes with VR gain). In this way, we have derived a quantitative framework for understanding the interactions between velocity signals, path integration, and landmarks in MEC codes.

Strikingly, our grid cell gain decrease data resembled the model’s super-critical regime, and gain increase data resembled the sub-critical regime (compare Fig. 3b, c and Fig. 5f, g). The model’s super-critical regime also explains the small bump in the cross-correlation during gain decreases (Fig. 3b) as well as the partial rescaling (Fig. 3c; Fig. 5g). What determinants could drive this asymmetry in regimes across the two gain changes? As our model offers a principled framework for understanding how different cues interact to drive grid responses, we can combine our model with experimental measurements to answer this question. First, the mouse’s running speed (v) was not different between baseline and either gain change (Supplementary Fig. 2), leaving landmark strength (ω) and velocity input (k_A) as candidate sources of the asymmetry. Second, landmark strength (ω) could increase or decrease during gain manipulations. Landmark input could come from border cells, other spatially stable MEC cells, or from outside the MEC, such as from hippocampal place cells. While the possibility remains that the partial remapping of border cells we observed during gain decreases contributed to a reduction in the strength of landmark input (Fig. 3b), the firing

rates of border cells and other spatially stable MEC cells did not change during gain manipulations (Supplementary Figs. 6, 12 and 13), and place cell firing rate did not change during gain reduction in another study²³. In addition, effects of non-uniformity in landmark input, such as in the average border cell map (Fig. 1e), could cause larger decoherence during the gain decrease condition than the gain increase condition. However, this would also lead to a corresponding non-uniformity in grid cell responses, which we did not observe (Supplementary Fig. 8). Thus, our data and previous work suggest that the asymmetry in grid cell gain change responses likely does not reflect differences in the strength of the landmark input (ω). Finally, non-linear integration of visual and locomotor speed cues in the velocity input to the attractor, as was observed in speed cells, could lead to asymmetrical grid responses (Fig. 5h, i). Using our experimentally measured speed cell data, we can derive an estimate of k_A in the model (12% visual, gain decrease; 53% visual, gain increase; Fig. 4i, See Appendix). Keeping ω constant, we calculate that $|D_{g,d}|$ in the gain decrease (g.d.) condition exceeds $|D_{g,i}|$ in the gain increase (g.i.) condition by a factor of 1.9 (95% CI: 1.2 – 3.0) (Appendix). This difference in $|D|$ thus drives the grid cell responses into the super-critical regime during gain decreases, while grid cell responses remain in the sub-critical regime during gain increases, explaining the asymmetric grid response to the two gain manipulations. The model also correctly predicts that grid scale should not strongly impact the amount of shift in the sub-critical regime (Supplementary Fig. 14).

The asymmetry of the grid cell response to gain increases and gain decreases can now be understood intuitively as follows. In the gain increase condition, speed cells are more strongly driven by optic flow than by locomotion (Fig. 4). This makes the phase advance of the attractor more similar to the rate of advance of visual landmarks. Thus, there is only a weak disagreement between path integration and landmarks, leading to the sub-critical, coherent regime ($|D| < 1$) in which grid cells shift relative to baseline. In contrast, in the gain decrease condition, speed cells are more strongly driven by locomotion than by optic flow. Thus, there is a larger disagreement between path integration and landmarks, leading to the super-critical, decoherent regime ($|D| > 1$) where the attractor phase breaks free of landmark correction and grid firing patterns rescale and remap.

However, despite the match between experimental observations and model predictions based on grid and speed cell population responses, the responses of individual pairs of speed and grid cells were uncorrelated. Specifically, there was no significant correlation of the percent weighting of visual speed cues between co-recorded grid and speed cells (Pearson's $r = 0.065$, $p = 0.58$, $n = 75$ pairs, 25 gain decrease, 50 gain increase; Supplementary Fig. 15b), or between co-recorded speed and grid cells when averaged by session (Pearson's $r = 0.048$, $p = 0.79$, $n = 33$ sessions, 12 gain decrease, 21 gain increase; Supplementary Fig. 15c). Importantly, however, despite the lack of one-to-one correspondence between co-recorded grid and speed pairs, these co-recorded grid and speed cells together showed the same asymmetric weighing of locomotion versus visual cues during gain manipulations as that observed for the entire grid and speed cell population (Supplementary Fig. 15b, c). This suggests that grid cells either integrate input from many MEC speed cells, such that the correlation with any individual speed cell is very weak; integrate input from a specific subset of speed cells that our methods could not identify; or combine speed information from multiple sources.

Generalization of our model to 2D (Appendix Part C and Appendix Fig. 5), where 1D patterns are considered as slices through a 2D grid³³, gave similar results to the 1D model: gain changes lead to sub-critical (shifts) and super-critical (rescaling and remapping) responses depending on the size of the gain change, the speed of the animal, and the strength of the landmark input. An interesting distinction between the 1D and 2D models is that in the 2D super-critical regime, the landmarks also exert a pull on the attractor state that is *orthogonal* to the path of the animal. This orthogonal pull can lead to the disappearance and appearance of firing fields, which we often observed in our data (Supplementary Fig. 5 and 6e).

Grid cell responses to an intermediate gain change follow the predictions of the model

To further test our attractor model framework, we recorded grid cells in a third gain change condition, gain=0.75 (n=11 grid cells from 5 mice, Fig. 6). We can now use our model to predict how grid cells should respond to this intermediate gain change. First, we define the “percent mismatch” between landmarks and self-motion as $\frac{|k_A - k_L|}{k_L} \times 100$. This mismatch, when large enough, drives grid cells into the super-critical regime. Since visual and locomotor cues were weighted roughly equally by speed cells in gain=1.5 (Fig. 4), we estimate that our gain increase experiments created a 25% mismatch between landmarks and self-motion, rather than the 50% mismatch that would have been created if speed inputs were purely locomotor at this gain value. While we are unsure of the exact weighting of locomotor and visual speed inputs at gain=0.75 (Fig. 6a, left), the maximum amount of mismatch that could be generated by a gain change of 0.75 is 25%, which would occur if speed inputs were 100% locomotor. Thus, the maximum possible decoherence number for gain=0.75 is equal and opposite to the decoherence number for gain=1.5 (Fig. 6a). Therefore, since responses to gain=1.5 were within the coherent regime, the model strongly predicts that gain=0.75 will also be within the coherent regime, and that grid fields will shift backwards on the track by an amount less than or equal to the amount that they shifted forwards during gain=1.5, without rescaling. Strikingly, this is exactly what we observed in our data (Fig. 6b-e), providing further support for our model.

Integration of visual and locomotor cues in a VR path integration task

Finally, we asked whether the asymmetrical weighting of visual and locomotor cues observed in grid and speed cell physiology is also reflected in behavior. We developed a VR path-integration task wherein mice were trained to run 200 cm along a virtual track for an automatic water reward (Fig. 7a). Trials were interleaved with periods of complete darkness that varied in length (30 – 130 cm long). No fixed visual landmarks were present on the track but black and white squares on the floor, ceiling and walls provided optic flow. The pattern of squares was randomized each trial to prevent the animal from using these cues as landmarks. This landmark-free task design enabled us to ask whether path integration shows the same asymmetrical weighting of visual and motor speed cues as we found in speed cells (Fig. 4), which, in the framework of our model (Fig. 5), explains the asymmetry between gain increase and gain decrease responses that we observed in grid cells (Fig. 3). Recordings from a similar VR track showed that grid cells remained stable for up to 200 cm in the absence of landmarks but presence of optic flow, and that population speed cell signals

remained intact, suggesting that these neural signals are available in this task (Supplementary Fig. 16).

In the VR path integration task, mice spontaneously slowed down in anticipation of the reward (Fig. 7b, Supplementary Movie 1). Leveraging this spontaneous behavior, we omitted rewards on a subset of trials (1 in 5). In these reward-omission trials, the running speed profile of the mouse provided a readout of its expected reward location, with the slight overshoot beyond 200 cm likely reflecting the mouse's inertia on the running wheel (location of minimum running speed in reward omission trials, mean $\pm$ SEM: mouse 1 = 243 ± 4 cm, $n = 41$ sessions; mouse 2: 237 ± 2 cm, $n = 41$ sessions; mouse 3: 268 ± 3 cm, $n = 49$ sessions; Fig. 7c and Supplementary Movie 1). In reward omission trials, distance from the onset of the visual cues was more predictive of running speed than time (all $p < 1e-6$, $n = 3$ mice; Supplementary Fig. 17). Therefore, in this task, the mice performed spontaneous path integration, integrating some combination of visual and locomotor speed to compute distance traveled.

Next, to test the contributions of visual and locomotor speed to this linear path integration behavior, we interleaved a subset of trials in which the gain was either increased or decreased by differing amounts (1 in 5 gain change trials, counterbalanced with reward omission trials; Fig. 7a). These gain change values were chosen such that the average real distance run on the track remained constant, to avoid retraining the mouse to run a different distance (gain decreases: 0.92, 0.86, 0.8, 0.75; gain increases: 1.09, 1.2, 1.33, 1.5). On a subset of these gain manipulation trials (1/5) the reward was also omitted, allowing us to use the running speed profile of the mouse as a readout of whether it used locomotion or visual (optic flow) cues to estimate the expected reward location. Running speed profiles in baseline reward omission trials were then stretched and correlated with running speed profiles in gain change reward omission trials. We converted these optimal stretch values to percentages, with 0% reflecting fully locomotion-based behavior and 100% reflecting fully visual behavior. Like grid cells, speed cells, and theta, mice weighed visual cues significantly more during gain increases than gain decreases (% visual $\pm$ SEM: gain decrease = $19 \pm 9\%$, gain increase = $56 \pm 6\%$, $n = 12$ [4 conditions $\times$ 3 mice], Wilcoxon $p = 4.8e-4$, Fig. 7d, e). These values closely matched the cue weights estimated from speed cells (gain decrease = $12 \pm 12\%$ visual, gain increase = $53 \pm 14\%$ visual, Fig. 4i). This demonstrates that the asymmetrical gain change responses observed in the electrophysiological data extend to behavior.

Discussion

Here, we revealed principled regimes under which both behaviorally-measured position estimates and MEC grid and speed neural codes differentially weigh the influence of visual landmark and self-motion cues. First, we found that conflicts between locomotion and visual cues caused grid cells to remap whereas border cells primarily remained locked to visual landmarks. However, gain increases and decreases elicited qualitatively different grid cell responses, with gain increases causing phase shifts, and gain decreases causing grid scale changes. This asymmetry was mirrored by multiple MEC speed signals, which weighed visual input more highly in gain increases compared to gain decreases. Second, we

developed a coupled-oscillator attractor model that combined path integration with landmark input to explain how grid responses during gain manipulations could arise from competition between conflicting self-motion and landmark cues. This model successfully predicted grid cell responses to an intermediate gain change. Finally, we used a virtual path-integration task to demonstrate a behavioral asymmetry in the weighting of visual versus locomotor cues that matched grid and speed cell responses. Taken together, these findings provide a new framework for understanding the dynamics of cue combination in MEC neural codes and navigational behavior.

Our combined experimental and modeling results indicate that asymmetric speed input is likely to be the cause of the asymmetric grid response to gain manipulations. However, the strategic reasons for the asymmetry in speed responses, from the point of view of behavior, remain to be determined. The asymmetry in speed responses corresponds to increased reliance on optic flow in the gain increase versus decrease condition. Intriguingly, it is precisely in the gain increase condition that optic flow is a more salient cue for speed, compared to locomotion, suggesting that this increased reliance on visual cues for computing speed may be consistent with optimal Bayesian principles for cue combination³⁴. For example, VR high gain conditions could correspond to scenarios in which the predominant sensory inputs come from nearby objects, in which case speed estimates based on visual input may carry more information than speed estimates based on locomotion.

The mechanistic origins of the speed cell asymmetry are also unknown. Information about speed in MEC could partially originate in visual cortex, where both locomotion and optic flow influence the speed tuning of neurons³⁵. Visual inputs compose a significant fraction of cortical inputs to MEC³⁶, reaching MEC through either direct projections from primary visual areas^{36,37}, or indirectly via projections from the postrhinal cortex^{36,38}. These visual inputs could include signals that are asymmetric with respect to VR gain. For example, visual cortex receives thalamic projections that selectively respond to the degree to which running speed exceeds visual speed³⁹, information that could then indirectly influence MEC speed coding. In addition, MEC receives projections from extrastriate visual cortical regions that preferentially respond to fast-moving stimuli^{37,40,41}. These inputs are presumably important for processing visual stimuli during running, and could be most highly activated during gain increase conditions. While it is unlikely that vision provides the primary source of speed information to MEC, as speed cells retain their basic tuning features in the absence of visual cues¹⁶, visual cortex remains a likely substrate for modulating the strength of speed responses to changes in optic flow, an idea that future experiments could more directly assess. In addition, at the behavioral level, we show that mice can accurately integrate optic flow to estimate distance traveled. This offers intriguing directions for future study regarding the connections between parahippocampal spatial coding and the visual system, such as the dependence of task performance and concomitant entorhinal codes on specific visual inputs or features of the visual stimulus.

While our data also show that the population responses of grid and speed cells to gain manipulations clearly corresponded, this registration was not clear at the level of single, co-recorded grid and speed cell pairs. This absence of one-to-one correspondence in co-recorded grid and speed cells during gain manipulations raises the important possibility that grid cells

integrate input from many speed cells. In line with this idea, several recent works have demonstrated that speed cells show high degrees of heterogeneity, with their firing rates showing positive or negative linear, saturating and even non-monotonic relationships with running speed^{7,8,25}. These levels of heterogeneity present a significant challenge for attractor-based grid cell network models, which often require inputs that are positively and linearly modulated by running speed. One way to overcome this challenge would be to assume grid cells integrate a population vector of speed inputs. This population vector could consist of signals from MEC speed cells as well as speed signals such as changes in theta frequency or speed-tuned inputs from outside MEC, like medial septal glutamatergic axons⁴². Such a population code that, across many speed inputs, created a positively modulated linear speed input to the grid cell network would be highly consistent with our current experimental results. As another alternative, grid cells could integrate inputs from only a subset of speed cells.

The degree to which plasticity of the landmark inputs influences the set point for sub-critical (phase shift) versus super-critical (spatial frequency change) grid network regimes remains to be established. Since our mice underwent extensive pre-training before experiencing any gain manipulations, our data reflect the dynamics of self-motion and landmark integration after mice have learned the sensory features of our VR environment. The possibility remains that these dynamics could differ during learning, or change based on the salience of sensory landmarks. Even so, our model offers a novel, principled approach for future work to quantify the expected set-point for sub-critical and super-critical grid network regimes during or after different learning paradigms. This type of framework could be useful in interpreting the responses of grid cells to different environmental geometries, in which varying degrees of distortion^{13,19}, shearing¹⁵, spatial frequency changes^{14,43} or remapping^{44,45} in the grid pattern may depend on complex relationships between the animal's experience and the nature of the sensory landmark or self-motion inputs the grid network receives^{13,18}.

Our data show that grid and speed codes, as well as behavioral path integration-based position estimates, differentially weigh self-motion and landmark cues in a principled, context-dependent manner. The ability of the path integration system to operate in sub-critical and super-critical regimes likely serves an adaptive purpose during navigation in the real world. For example, the sub-critical regime is appropriate when landmark input is close enough to path integration to be used for error correction¹². However, if landmarks change location or become unreliable, creating a large disagreement between landmark input and path integration, the network can enter the super-critical regime and pull free from the influence of landmarks. The decoherence threshold could therefore reflect the animal's expectations about the reliability of landmark input³⁴.

This idea that non-linear cue integration serves an adaptive purpose during navigation may be a more general principle of parahippocampal computation. Recent work used VR gain changes to show that hippocampal place cells integrate visual and locomotor information nonlinearly²³. Specifically, during VR gain reduction, place cells shifted backward by an amount that did not grow with distance since the start of the track. These data strongly resemble the sub-critical regime of our model, raising the intriguing possibility that some of

the principles we reveal governing the integration of different information sources by MEC neural codes may generalize to other brain regions that support spatial navigation^{46–49}.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Functionally-identified MEC cell types in real and virtual environments. **a)** Schematic of cue sources and types in VR. Cues can come from visual or locomotor input (cue source). Visual cues can provide information regarding visual landmarks (cue type) or self-motion, in the form of optic flow (cue type). Locomotor cues also provide information regarding self-motion (cue type). **b)** The open field (OF) environment consisted of a 90 × 90 cm square box with a single polarizing cue card. **c)** Following OF recordings, mice were immediately transferred to a head-fixed VR setup. Mice ran on a Styrofoam ball that was constrained to rotate in one dimension. At the end of the 400 cm linear track, the mice received a water reward and were teleported to the beginning of the track. **d)** Example grid, border, and speed cells recorded in OF (top row; peak firing rate listed above rate maps) and VR (bottom row). Grid and border cell VR data show firing rate (top panel) and spikes over trials (raster plots, bottom panel). Speed cell data are plotted as a heat map of instantaneous firing rate with respect to running speed, with colors indicating the percentage of time bins with the corresponding firing rate and running speed. **e)** Average stable grid cell activity (top) and border cell activity (bottom) in baseline VR sessions. Stable cells defined as those with a stability > 0.2. Border cell activity peaked near landmarks in the environment (rmANOVA for dependence of firing rate on track location, Greenhouse-Geisser correction, $p = 0.016$, $n = 87$) whereas grid cell activity did not ($p = 0.18$, $n = 85$). Vertical green lines show the locations of landmarks, and horizontal dashed black lines show the 5th, 50th, and 95th percentiles of a shuffled distribution of firing rates. Asterisks indicate firing rate peaks that exceeded the 95th percentile of the shuffled distribution. **f)** Grid and border cells had significantly higher half-and-half stability than shuffled distributions on the VR track (mean ± SEM: grid = 0.40 ± 0.024 , shuffle = 0.0055 ± 0.0010 , $p < 1e-30$; border = 0.54 ± 0.023 , shuffle = 0.0026 ± 0.0011 , $p < 1e-30$; Wilcoxon rank-sum tests). Border cells had significantly higher stability than grid cells ($p = 4.4e-5$, Wilcoxon rank-sum test).

Figure 2.

Gain manipulations place visual and locomotor cues in conflict. **a)** Experimental design. Mice ran 15 trials of baseline gain (A period) followed by 5 or 10 trials of gain decrease or gain increase (0.5x or 1.5x; B period). Gain decrease changed the real distance the animal had to run to reach the end of the track from 400 cm to 800 cm; gain increases changed this distance to 267 cm. Following the gain change period, mice ran a second baseline period of 15 trials (A' period). **b)** Illustration showing two potential responses of grid or border cells to gain manipulations, with firing patterns plotted with respect to virtual position on the track. If the spatial firing pattern only reflects the influence of visual cues, no change in the spatial pattern would be observed during gain changes. If the spatial firing pattern only reflects the influence of locomotor cues, the frequency of the spatial pattern would decrease in gain decreases and increase in gain increases. **c)** We considered the following possible response types to gain manipulations: No change, degeneration, phase shift, and rescaling. The last two are subtypes of coherent remapping. **d)** Example grid cell (left) and border cell (right) responses to baseline (A; top and bottom row), gain decrease (B, top row) and gain increase (B, bottom row). For analyses, the A period was defined as the n baseline trials immediately preceding the B period, where n is the number of gain change trials. Firing patterns were analyzed relative to virtual position on the track.

Figure 3.

Structural analyses of gain change responses reveal an asymmetry in the weighting of visual and locomotor cues by grid cells. **a)** To analyze degeneration, we compared half-and-half stability between the A period and B period. Except for grid gain increase, which had high baseline stability, stability did not decrease from the A period to the B period (all $p > 0.5$), indicating that degeneration was minimal. Only gain manipulations in which rate maps had stability > 0.2 in both the A period and B period (upper right-hand quadrant) were kept for further analysis. Each data point represents a single gain manipulation. Sometimes multiple gain manipulations were run for a single cell. In this case, statistics such as cross- and auto-correlations were averaged by cell over all stable gain manipulations (stability > 0.2 in A period and B period). **b)** Top panels: Cross-correlations of firing rate maps. Baseline traces (black) show the average cross-correlation between adjacent blocks of 5 baseline trials that were both stable (stability > 0.2). Colored traces (red for grid cells, blue for border cells) show the cross-correlation between the A period (baseline trials immediately preceding gain change) and the B period (gain change). Grid cells responded differently to gain decreases and gain increases, showing a pronounced phase shift in gain increases but near complete remapping in gain decreases with a small peak to the left of zero. There was partial remapping in border cells during gain decrease, but less than in grid cells ($p = 1.3e-5$). Border cells were highly stable during gain increase. Error bars: mean $\pm$ SEM. Bottom panels: Peak AB cross-correlation vs. location of peak for all cells individually. These data show that the reduction in peak average cross-correlation in grid cells during gain increase was caused by forward shifts of varying amounts, rather than a reduction in individual cross-correlation peaks (Wilcoxon p for shift = 0.0099; p for change in peak = 0.14). In contrast, cross-correlation peaks during gain decrease were smaller than baseline ($p = 1.4e-5$) and did

not consistently shift backwards. Some border cells shifted backwards during gain decrease, while others were stable (mean shift $\pm$ SEM: baseline = -0.3 ± 0.9 cm, gain change = -27.4 ± 7.1 cm, Wilcoxon $p = 0.00086$), and peak cross-correlations were only marginally lower than baseline (p for change in peak = 0.055). Border cells were remarkably stable during gain increase (p for shift = 0.16; p for change in peak = 0.94). **c)** Top panels: Average autocorrelations in the A and B periods for grid and border cells in gain decrease and gain increase revealed rescaling in grid cells during gain decreases. Bottom panels: To estimate the amount of rescaling, we stretched baseline autocorrelograms and correlated them with gain change autocorrelograms.

Figure 4.

Speed cells asymmetrically integrate visual and locomotor cues. **a)** Illustration showing two potential responses of speed cells to gain manipulations, with firing patterns plotted with respect to real running speed on the track. If the speed slope (running speed $\times$ firing rate) only reflects the influence of locomotor cues, no change in the slope would be observed during gain changes. If the speed slope only reflects the influence of visual cues, the speed slope would decrease in gain decreases and increase in gain increases. **b)** Two example speed cell responses to gain decrease. Plots show instantaneous firing rate vs. running speed, with colors indicating occupancy over time bins. White dashed lines show least-squares linear fits to data. **c)** Slope and intercept of linear fits did not significantly change for speed cells during gain decrease ($n = 33$). **d, e)** Same as (b,c), but for gain increase. In contrast to gain decrease experiments, slope of linear fit increased during gain increase ($n = 41$; $p < 0.01$). Intercepts did not change. **f)** Schematic showing the angle of a speed cell response to gain manipulation, based on the slope of the linear fit in the A period and the B period. The response of an individual neuron is illustrated as a black dot and the angle measured as Θ . **g)** Distribution of angles for speed cells during gain decrease. Dotted lines show locomotion response (45°) and visual response (26.6°). **h)** Same as (f), but for gain increase. Dotted lines show locomotion response (45°) and visual response (56.3°). **i)** Angles from (f) and (g) converted to percentages. Visual weights were significantly higher during gain increase (g.i., green) than gain decrease (g.d., pink) ($p = 0.0503$, Wilcoxon rank-sum test). * $p = 0.0503$, ** $p < 0.01$, *** $p < 0.001$.

Figure 5.

A coupled-oscillator attractor network model of the integration of landmarks and self-motion input by grid cells. **a)** Without landmarks, the model is a 1D ring attractor, with short-range excitation and long-range inhibition, as captured by translationally symmetric synaptic weights $J(u'-u)$ from position u to u' on the ring. This synaptic weight profile creates a set of symmetrical steady-state “bump” patterns which can be described by the phase angle θ_A of peak activity. When conjunctive velocity tuning is added, and the synaptic weight profiles are offset in the direction of preferred velocity, velocity inputs move the bump around the attractor at a rate given by $k_A \cdot v$, where v is the running speed of the mouse and k_A is the “gain” of the network. Note that k_A (the gain between mouse velocity and attractor phase advance) should not be equated with the VR gain (the gain between mouse locomotion and advance in virtual position on the VR track). **b)** Efferent synapses from landmark inputs to the attractor network will pin the attractor phase to a certain phase θ_L that depends on the position of the landmark. **c)** Hebbian learning of these synapses will alter the learned landmark pinning phase θ_L (blue arrows) to make it consistent with attractor phase θ_A (red arrows), which advances due to path integration (black arrow). Thus, after learning, landmarks and path integration agree. **d)** Illustration of a situation in which

landmark input and path integration disagree ($\theta_A - \theta_L$). When a landmark cell with pinning phase θ_L fires, it exerts a force on the attractor phase (black arrow) that grows with the level of disagreement $\theta = \theta_A - \theta_L$ as $-\sin(\theta)$ (See Appendix). Thus, a landmark cell draws the attractor phase θ_A towards its pinning phase θ_L . **e)** Solution to the model equations illustrate how the disagreement θ between the attractor and the landmark phase evolves as the mouse moves through a sequence of landmarks. When $|\text{ID}| < 1$, θ settles into a steady-state, in which the attractor phase either leads or lags the current landmark phase at a constant phase shift. When $|\text{ID}| > 1$, no steady-state exists, and the difference between the attractor phase and the landmark phase will continually change. **f)** In the “sub-critical” regime ($0 < |\text{ID}| < 1$), the phase advance due to path integration (red arrows) and the pinning phase due to landmarks (blue arrows) do not disagree too much, leading to a constant phase lag, and grid firing patterns that phase shift but do not rescale. Top row: Baseline (path integration matches landmarks). Bottom row: Gain change with landmark input. The evolving pinning phases of the landmarks continuously correct, or drag along, the slower evolving attractor phase. Right: Cartoons of the expected A-B cross correlation and autocorrelations in the sub-critical regime. Note the striking similarity to the gain increase response (Fig. 3b₃). **g)** Same as (f), but in the “super-critical” regime ($|\text{ID}| > 1$), where landmark pinning phases and attractor phase due to path integration advance at such different rates that the grid pattern breaks free of landmark inputs and rescales. Note, however, that even in this regime the influence of landmarks is still present. For super-critical gain decrease, when the advancing attractor phase is *behind* the landmark phase, the pull from landmarks *accelerates* the relative precession rate. When the attractor phase is *ahead* of the landmark phase, the pull from landmarks *slows* the relative precession. This waxing and waning of the precession rate results in the network spending more time in an advanced attractor phase, leading to a small peak in the cross-correlation and an incomplete rescaling of the pattern (right). We observed this exact same effect in grid cells in the gain decrease condition (Fig. 3b₁). **h-i)** Asymmetric speed input explains the asymmetry in the grid response to gain manipulations. If ω is constant (Supplementary Fig. 8), then a speed input which linearly integrates locomotion and visual speed can only lead to both super-critical or both sub-critical responses to symmetric gain manipulations (h). On the other hand, nonlinear integration of visual and locomotor cues, as we observed in our speed cell data (Fig. 4), can explain the asymmetric grid cell response to gain manipulations (i).

Figure 6:

Grid cell responses to an intermediate gain value follow the predictions of the model. **a)** Model predictions for gain=0.75. Using our speed cell data (left-hand diagram), the model predicts that the decoherence number during gain=0.75 is bounded above by $-D_{1.5}$, where $D_{1.5}$ is the decoherence number for gain=1.5 (right-hand diagram; see main text for argument). This predicts that gain=0.75 should be within the sub-critical regime, and that grid cell firing patterns should shift backwards on the track, without rescaling, by an amount less than or equal to the forward shifts during gain=1.5. **b)** Example grid cell responses to gain=0.75. **c)** Top: average cross-correlations between rate maps generated from the A and B periods of gain=0.75 manipulations (red), and adjacent blocks of 5 trials within baseline periods (black). Average A-B cross-correlations for gain=0.5 manipulations are shown in gray for comparison. Black dots indicate locations at which the red and black traces significantly differed (one dot, paired t-test $p < 0.05$; two dots, $p < 0.01$; no correction for multiple comparisons). Bottom: Locations of the peaks of the A-B and baseline cross-correlations of individual cells (red: gain=0.75, black: baseline). Cross correlation peaks were shifted backwards compared to baseline (mean shift $\pm$ SEM, gain=0.75: -9.3 ± 4.0 cm, baseline: -2.5 ± 4.4 cm, one-sided Wilcoxon test $p = 0.027$). **d)** Grid cell shifts during gain=0.75 and gain=1.5. Note that the signs of gain=1.5 shifts (which were forward along the track) have been flipped in order to directly compare them with gain=0.75 shifts (which were backward along the track). The model predicts that backward shifts during gain=0.75 should be less than or equal to forward shifts during gain=1.5. That is exactly what we

observed in the data (mean shift $\pm$ SEM, gain=0.75: -9.3 ± 4.0 cm, $n = 11$; mean sign-flipped shift $\pm$ SEM, gain=1.5: -14.4 ± 7.1 cm, $n = 37$; Wilcoxon rank sum $p = 0.14$). **e)** Average rate map autocorrelations during the B period of gain=0.75 manipulations (red), the B period of gain=0.5 manipulations (gray), and baseline (black). As predicted by the model, we did not observe significant rescaling in gain=0.75.

Figure 7.

Integration of visual and locomotor cues in a path integration task. **a)** Schematic of the task. Animals were trained to run along an infinite-perspective landmark-free VR track for water reward. During training, the distance to reward was gradually increased to 200 cm. After 400 cm (or 2 x reward distance during training), the animal was teleported to a random starting position between -130 and -30 cm and the screens were turned off. When the animal reached 0 cm, visual cues came on. Visual cues consisted of black and white squares on the floor, ceiling and walls that were randomized each trial. After training, normal trials were interleaved with trials in which the reward was omitted (1/5) or the gain was changed (1/5, counterbalanced with reward omission). **b)** Behavior from an example session. Mice spontaneously slowed down prior to the reward zone. In reward omission trials, the speed profile of the mouse could be used to estimate its perceived location on the track. **c)** Average running speed profiles in gain change, reward omission trials for 3 mice. **d)** Optimal stretch values for correlating baseline speed profiles with gain changed speed profiles. The 1:1 line represents fully visual behavior, and the $y = 0$ line represents fully locomotor behavior. **e)** Optimal stretch factors from (d) were converted to the percent weighting of visual cues. Mice used visual cues significantly more in gain increase trials than gain decrease trials (Wilcoxon $p = 4.8e-4$). Note the striking similarity with the percentages estimated from speed cells (Fig. 4i). *** $p < 0.001$.