Published in final edited form as:

Hippocampus. 2012 August ; 22(8): 1647–1651. doi:10.1002/hipo.22030.

Alternating Predictive and Short-term memory modes of Entorhinal Grid cells

Licurgo De Almeida^{1,4}, Marco Idiart², Aline Villavicencio³, and John Lisman^{1,*}

Licurgo De Almeida: licurgoalmeida@gmail.com; Marco Idiart: idiart@if.ufrgs.br; Aline Villavicencio: avillavicencio@inf.ufrgs.br; John Lisman: Lisman@brandeis.edu

¹Department of Biology and Volen Center for Complex Systems, Brandeis University, Waltham, MA 02454-9110

²Physics Institute, UFRGS, Porto Alegre, Brazil

³Institute of Informatics, UFRGS, Porto Alegre, Brazil

⁴Department of Neurobiology and Behavior, Cornell University. W249 Mudd Hall, Ithaca, NY 14853

Electrophysiological recordings and functional imaging show that the entorhinal cortex, a structure that provides input to the hippocampus, has a role in multi-item short-term memory (STM) ^{1–6}. The entorhinal cortex may thus act as the episodic memory buffer ⁷ that produces the persistent firing necessary to encode information into hippocampal long-term memory (LTM) stores ⁸. However, in the most extensive electrophysiological studies of the entorhinal cortex, the focus has been on grid cells and their spatial properties ⁹. It has been unclear whether the function of grid cells is purely spatial or whether their function also relates to memory processes. To examine this question, we tested whether grid cells represent positions that the animal has just experienced, as expected of a STM mode appropriate to drive memory encoding, or whether grid cells represent future locations, as expected of a prospective (predictive) mode based on the recall of stored information. Our results suggest that both functional modes exist and alternate rapidly in a network-wide manner.

The firing of grid cells occurs in many small fields (vertices) that tile the environment in a grid-like way. To understand the memory properties of grid cells, we utilized the methodological framework developed by ¹⁰ (see also ¹¹). According to this framework, the position represented by a cell is a small subregion at the center of a field (in this case, the center of each grid cell vertex). A cell is prospective (predictive) if firing occurs inbound as the rat moves toward a vertex center. Thus, firing will occur left of center if the rat is coming from the left and right of center if the rat is coming from the right. On the other hand, if coding is retrospective (STM of having been at the center), firing occurs outbound as the rat leaves the center (going in either direction) (Fig. 1A). This test was applied to grid cell data kindly provided by Mosers' laboratory.

We first analyzed data recorded as the rat foraged in a 2-D environment ¹² and considered only runs having a fairly straight and continuous trajectory (see Materials and Methods;

*To whom correspondence should be addressed: Lisman@Brandeis.edu.

Author Contributions: LDA performed the analysis, with contributions from MI and AV. Statistical Analysis was designed by MI. The initial concept was developed by JL and JL wrote the paper.

Author Information includes a reprints and permissions statement. Authors declare no competing financial interests. Correspondence and requests for materials should be addressed to J.L. (Lisman@brandeis.edu).

1,531 of ~11,000 runs). Averaging over these runs, the centers of grid vertices were defined (Fig. S1). Spikes occurred both as the rat travelled inbound to the center and outbound away from the center (outbound > inbound by 32%; Fig. S3). However, when individual runs were examined, we noted that spikes from the same cell seemed to sometimes occur mainly on the inbound leg or outbound leg (Fig. 2A). To analyze this tendency, we used the following rule: the mode of a run was classified as “outbound” if more than 2/3 of the spikes were outbound and “inbound” if more than 2/3 were inbound; otherwise, the run was classified as “indefinite”. The resulting distribution of modes combined for all cells, is shown in Fig. 1B. To determine whether this distribution could be accounted for by chance, we computed what would be expected of a random process (Fig. 1E; see caption for method). For a random process (Fig. 1E), most runs would have roughly equal inbound and outbound spikes and thus be indefinite. To the contrary, the experimental data had relatively few indefinite runs; furthermore, the measured distribution (Fig. 1B) could not be accounted for by the shuffled distribution (Fig. 1E; * $p < 0.01$; ** $p < 0.0001$, see also Fig. S4). These results thus suggest that grid cells have two modes: one in which they fire predictively of coming to the center and one in which they fire retrospectively as they leave the center. The origin of indefinite runs will be discussed later. A possible alternative explanation for Fig 1B is that grid cells fire in a short burst that sometimes occurs on the inbound leg, sometimes on the outbound leg. We have examined many variants of such models and found none they can account for the data (relatively high incidence of indefinite runs (Fig. S9) and cannot account for the high incidence of matches in the first second in the graphs of Fig. 3.

We checked whether the velocity on the inbound leg of a run as compared to the velocity on the outbound leg on the same run might account for the difference in spike counts and, thus, for modes. To evaluate this possibility, we calculated the difference between the velocities on the two legs divided by the average velocity. If this was larger than 2/3 for outbound legs or less than -2/3 for inbound legs, velocity difference alone would explain the modes, but the calculated values were very small ($-0.03 \pm \text{s.d.} 0.28$ for inbound runs; $0.12 \pm \text{s.d.} 0.27$ for outbound runs), with only 4 of 216 (< 2%) inbound runs and 9 of 343 (< 3%) outbound runs having the necessary discrepancy. Thus, differential velocity on the inbound/outbound legs of a run cannot account for the spike differences (see also Fig. S7). We also checked if a particular mode occurred when the rat was running in a particular direction or when crossing a particular vertex. Fig. S10 and Fig. 2 show that this is not the case.

We next examined whether modes could be seen in all cells. We used a single cell test (see Materials and Methods), comparing the number of inbound and outbound spikes to shuffled data for that cell. By this test, 78% of the cells had modes, whereas 22% did not. An example of a modeless cell is shown in Fig. 2B. Eliminating modeless cells, the distribution of inbound, outbound, and indefinite runs (Fig. 1D) becomes even more different from the shuffled data (Fig. 1E).

Modes might be single cell property or a network property. In the latter case, different cells firing within a short time interval should have matching modes. Fig. 3A shows how the percent match varies as a function of the interval between the vertex crossings for cell pairs (a pair was not considered if either crossing was categorized as indefinite). If the crossings occurred <1 s apart, the percent match was 68.5% ($p < 0.05$), higher than expected by chance (57%). With the removal of modeless cells, the percent match was 71% ($p < 0.03$). We conclude that modes are a network property.

The experiments discussed above were done while the rat explored an open environment. To further study modes, we utilized a second data set obtained while rats traversed a linear track¹³. Similar evidence for modes was found (Fig. 1C), and this could not be accounted for by the inbound leg vs. outbound leg velocity difference (the normalized velocity

difference was $-0.11 \pm \text{s.d.}0.25$ for inbound runs and $-0.02 \pm \text{s.d.}0.28$ for outbound runs). We again found (Fig. 3B) that the percent of matches for times < 1 s was greater than expected by chance (75.0%; $p < 0.05$). Thus, modes are a network property both in the open field and on a linear track.

In both the open field and linear track data (Fig. 3) the probability of match fell below chance at 3 seconds; combining this data, the probability of this occurring by chance is low (0.059). This time-dependent fall in probability of match from greater than chance to chance (or below chance) is expected if the network mode alternates on the time scale of seconds. To demonstrate this, we used the actual path of rats and simulated alternating modes (inbound 1.6 s; outbound 2.5 s). As seen in Fig. S5, the percent match starts high and falls within 1–2 s to chance or below chance, similar to the experimental data (Fig. 3). This timing is consistent with what is expected from the transition rates incorporated into the simulation and it demonstrates the adequacy of the method used to analyze the experimental data. We conclude that the network mode of the entorhinal cortex alternates on the second time scale. Given this alternation, a perfect distinction between run types (all spikes either inbound or outbound) is not expected because the mode may change during a run; indeed, 70% of indefinite runs can be accounted for by such changes (see supplementary material).

We have interpreted the tendency of firing to occur either before or after the vertex center as evidence for functional modes. Alternatively, variation in firing position in entorhinal network might reflect mislocalization. For example, the path integrator by which position is determined might momentarily have a low value, causing all cells to fire at a position before their field center. However, the mislocalization model (Fig. S5B) does not predict the fall below chance in Fig. 3, whereas the mode model does (Fig. S5A). Furthermore, as discussed below, the time scale of the mode changes that we identified in the entorhinal cortex is similar to those observed for hippocampal modes¹⁴. The possibility that modes arise because of uncertainty of determining where the animal is (this is done using lights on the head) is unlikely given the very large effect of modes (~ 15 cm) on firing position (Fig. S6). Finally, if modes exist, there may be a behavioral correlate. The open field data we analyzed was obtained while the rat foraged. We tested the only behavioral variable recorded (velocity) to see if it varied in the two modes (note that this is velocity over the whole run, but specific for mode, a variable that is different from the velocity of inbound (or outbound) legs analyzed above). The results show that the rat's velocity was 10% slower in the outbound mode (inbound $v = 41.1 \pm \text{s.d.}9.7$ cm/s with a small acceleration; outbound $v = 36.3 \pm \text{s.d.}8.0$ cm/s with a small deceleration, Fig. S8; $p < 10^{-8}$; no significant velocity difference was found on the linear track, perhaps because there was no foraging). We considered and rejected the hypothesis that overcompensation of the path integrator's response to acceleration accounts for modes (Figs. S8E and S8F). The most likely explanation of our findings is that modes are functional states of the brain and that this state has a modest ($\sim 10\%$) effect on running speed.

In the entorhinal cortex information is organized by theta and gamma oscillations (and theta phase precession)^{13,14}; Fig. S6 shows that this is true in both modes. The retrospective mode represents positions in the recent past and is thus a form of STM; the phase coding of different positions is consistent with the Lisman-Idiart model¹⁵ of multi-item STM, as modified by Koene and Hasselmo to have first-in, first out properties¹⁶. Such dual oscillation models have been shown to account for a range of psychophysical and physiological properties of STM^{17–24}.

Our results suggest that the entorhinal cortex has alternating functional modes. Although we have tested many alternative explanations of our results that do not assume modes, we cannot rule out that a non-mode explanation of our data is possible. It will therefore be

important evaluate the mode model with other lines of evidence. A study of gamma oscillations in CA1¹⁴ also points to alternating modes (of unknown function). One of these CA1 gamma modes involves strong interaction with the entorhinal cortex; it is thus of interest that these gamma modes alternate on the time scale of seconds, similar to the alternation of retrospective/prospective modes that we have observed in the entorhinal cortex. Both this evidence and ours points to idea that the theta state during locomotion, which has generally been assumed to be homogenous, is actually composed of two very different modes. Our work points to potential functions of these modes. One is retrospective, representing positions of the recent past. This firing, which is repeated on multiple theta cycles, has the properties of an STM buffer appropriate for encoding information into long-term memory by an LTP-like process⁸. The other mode is predictive of upcoming positions or events and may thus arise from the recall of stored information¹⁰. Such functional specialization probably requires different network properties and so cannot be done at the same time²⁵; consistent with this, retrieval interferes with encoding²⁶. Therefore, it would make sense for the hippocampal region to have specialized read/write modes similar to computer memory. A long duration write mode would be problematic because the brain needs access to stored information with minimal delay. The rapid mode alternation we have observed may provide a solution to this problem. If modes exist, there are likely to be pathways that are turned on in one mode and off in the other. Experimental results showing such pathway modulation on the time scale of seconds would provide definitive evidence for modes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Mosers' laboratory and particularly Torkel Hafting, Dori Derdikman, and Laura Colgin for information about the data files and how to analyze them. We thank Edvard Moser for providing access to relevant data and for his suggestions and encouragement. We thank Edvard Moser, Ole Jensen, Torkel Hafting, and Andre Fenton for comments on the manuscript. The work was supported by NIH grants MH060450 and NS27337, Fapergs-Pronex 10/0008-10, CNPq PQ 305256/2008-4, 479824/2009-6 and PQ 309569/2009-5.

References

1. Suzuki WA, Miller EK, Desimone R. Object and place memory in the macaque entorhinal cortex. *J Neurophysiol.* 1997; 78:1062–1081. [PubMed: 9307135]
2. Young BJ, Otto T, Fox GD, Eichenbaum H. Memory representation within the parahippocampal region. *J Neurosci.* 1997; 17:5183–5195. [PubMed: 9185556]
3. van Vugt MK, Schulze-Bonhage A, Litt B, Brandt A, Kahana MJ. Hippocampal gamma oscillations increase with memory load. *J Neurosci.* 2010; 30:2694–2699. [PubMed: 20164353]
4. Axmacher N, et al. Cross-frequency coupling supports multi-item working memory in the human hippocampus. *Proc Natl Acad Sci U S A.* 2010; 107:3228–3233. [PubMed: 20133762]
5. Axmacher N, et al. Sustained neural activity patterns during working memory in the human medial temporal lobe. *J Neurosci.* 2007; 27:7807–7816. [PubMed: 17634374]
6. Schon K, Hasselmo ME, Lopresti ML, Tricarico MD, Stern CE. Persistence of parahippocampal representation in the absence of stimulus input enhances long-term encoding: a functional magnetic resonance imaging study of subsequent memory after a delayed match-to-sample task. *J Neurosci.* 2004; 24:11088–11097. [PubMed: 15590925]
7. Baddeley A. The episodic buffer: a new component of working memory? *Trends Cogn Sci.* 2000; 4:417–423. [PubMed: 11058819]
8. Hasselmo ME, Stern CE. Mechanisms underlying working memory for novel information. *Trends Cogn Sci.* 2006; 10:487–493. [PubMed: 17015030]

9. Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. Microstructure of a spatial map in the entorhinal cortex. *Nature*. 2005; 436:801–806. [PubMed: 15965463]
10. Battaglia FP, Sutherland GR, McNaughton BL. Local sensory cues and place cell directionality: additional evidence of prospective coding in the hippocampus. *J Neurosci*. 2004; 24:4541–4550. [PubMed: 15140925]
11. Muller RU, Kubie JL. 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*. 1989; 9:4101–4110. [PubMed: 2592993]
12. Derdikman D, et al. Fragmentation of grid cell maps in a multicompartiment environment. *Nat Neurosci*. 2009; 12:1325–1332. [PubMed: 19749749]
13. Hafting T, Fyhn M, Bonnevie T, Moser MB, Moser EI. Hippocampus-independent phase precession in entorhinal grid cells. *Nature*. 2008; 453:1248–1252. [PubMed: 18480753]
14. Colgin LL, et al. Frequency of gamma oscillations routes flow of information in the hippocampus. *Nature*. 2009; 462:353–357. [PubMed: 19924214]
15. Lisman JE, Idiart MA. Storage of 7 +/- 2 short-term memories in oscillatory subcycles. *Science*. 1995; 267:1512–1515. [PubMed: 7878473]
16. Koene RA, Hasselmo ME. First-in-first-out item replacement in a model of short-term memory based on persistent spiking. *Cereb Cortex*. 2007; 17:1766–1781. [PubMed: 17030561]
17. Jensen O, Lisman JE. An oscillatory short-term memory buffer model can account for data on the Sternberg task. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 1998; 18:10688–10699. [PubMed: 9852604]
18. Miller GA. The magical number seven plus or minus two: some limits on our capacity for processing information. *Psychol Rev*. 1956; 63:81–97. [PubMed: 13310704]
19. Sternberg S. High-speed scanning in human memory. *Science*. 1966; 153:652–654. [PubMed: 5939936]
20. Raghavachari S, et al. Gating of human theta oscillations by a working memory task. *J Neurosci*. 2001; 21:3175–3183. [PubMed: 11312302]
21. Canolty RT, et al. High gamma power is phase-locked to theta oscillations in human neocortex. *Science*. 2006; 313:1626–1628. [PubMed: 16973878]
22. Fuentemilla L, Penny WD, Cashdollar N, Bunzeck N, Duzel E. Theta- coupled periodic replay in working memory. *Curr Biol*. 2010; 20:606–612. [PubMed: 20303266]
23. Rutishauser U, Ross IB, Mamelak AN, Schuman EM. Human memory strength is predicted by theta-frequency phase-locking of single neurons. *Nature*. 2010; 464:903–907. [PubMed: 20336071]
24. Siegel M, Warden MR, Miller EK. Phase-dependent neuronal coding of objects in short-term memory. *Proc Natl Acad Sci U S A*. 2009; 106:21341–21346. [PubMed: 19926847]
25. Albasser MM, Poirier GL, Aggleton JP. Qualitatively different modes of perirhinal-hippocampal engagement when rats explore novel vs. familiar objects as revealed by c-Fos imaging. *The European journal of neuroscience*. 2010; 31:134–147. [PubMed: 20092559]
26. Allan K, Allen R. Retrieval attempts transiently interfere with concurrent encoding of episodic memories but not vice versa. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2005; 25:8122–8130. [PubMed: 16148220]

Fig. 1. Grid cells have modes

A. Test for retrospective or prospective coding. Grey bar is grid cell vertex (star is vertex center). Modes are defined by whether firing occurs mainly on the inbound leg or on the outbound leg of a run through the vertex. **B.** Histogram of run types in the open field classified according to the 2/3 rule (total of 1,531 runs from 147 neurons in 13 different rats.) cannot be accounted for by the shuffled data in E (* $p < 0.01$; ** $p < 0.00001$). **C.** The same as B, but for unidirectional runs on the linear track (753 runs from 15 neurons in 7 different rats). **D.** The same as B, but with 33 modeless cells removed. **E.** Shuffled data. In order to have a model for random distribution of spikes, we preserved the number of spikes

of each experimental run but randomly assigned them as inbound or outbound, according to the experimental proportion of inbound and outbound spikes (Fig. S3). The shuffled distribution made in this way is statistically different from the experimental data (* $p < 0.00001$).

Fig. 2. Firing properties of grid cells with and without modes

Raster plots of spikes (the number in parenthesis indicates which vertex was crossed) during runs classified according to the 2/3 rule for **A.** a cell with modes and **B.** one without modes (11 of 14 experiments had modeless cells; they were found both in layer 2 and layer 3) . The time interval between runs is not fixed. Black circles represent inbound spikes; grey circles outbound spikes. The total number of runs through any given vertex was generally less than 5. The data in A and B are from different animals, but each were from the same experimental session. The grid spacing for the cells depicted in A and B were nearly identical.

Fig. 3. Modes are a network property that alternates in seconds

A. Runs (open field environment) across vertices of cell pairs have a high percent of mode match (both inbound or both outbound) if crossings occurred within 1 s but a low percentage for crossings that occurred between 1 and 3 s (42 cell pairs). The dashed line is the probability of match by chance (57%) computed from shuffled data (* $p=0.05$). **B.** Similar plot for linear track. (Note that for both plots the number of data points per time bin falls with time because the rat may turn or stop.)