UNIVERSITYOF BIRMINGHAM

Hexadirectional modulation of high-frequency electrophysiological activity in the human anterior medial temporal lobe maps visual space

Staudigl, Tobias; Leszczynski, Marcin; Jacobs, Joshua; Sheth, Sameer A.; Schroeder, Charles E.; Jensen, Ole; Doeller, Christian F.

[Link to publication on Research at Birmingham portal](#)

2

3   Title: **Hexadirectional modulation of high-frequency electrophysiological activity in the human**
4   **anterior medial temporal lobe maps visual space**

5   Tobias Staudigl[1,2#*], Marcin Leszczynski[3,4], Joshua Jacobs[5], Sameer A. Sheth[3], Charles E. Schroeder[3,4], Ole
6   Jensen[6], Christian F. Doeller[1,7,8*]

7

8   1 Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen, The Netherlands

9   2 Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, USA

10   3 Cognitive Science and Neuromodulation Program, Department of Neurological Surgery, Columbia

11   University College of Physicians and Surgeons, New York, New York, USA

12   4 Translational Neuroscience Division, Nathan Kline Institute, Orangeburg, New York, USA

13   5 Department of Biomedical Engineering, Columbia University, New York, New York, USA

14   6 Centre for Human Brain Health, School of Psychology, University of Birmingham, Birmingham, UK

15   7 Kavli Institute for Systems Neuroscience, Centre for Neural Computation, Egil and Pauline Braathen

16   and Fred Kavli Centre for Cortical Microcircuits, NTNU & St. Olavs Hospital, Trondheim, Norway

17   8 Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

18

19   # Lead Contact

20   *Correspondence should be addressed to T.S. (tobias.staudigl@chsh.org) or C.F.D
21   (christian.doeller@ntnu.no).

Summary

Grid cells are one of the core building blocks of spatial navigation [1]. Single cell recordings of grid cells in the rodent entorhinal cortex revealed hexagonal coding of the local environment during spatial navigation [1]. Grid-like activity has also been identified in human single cell recordings during virtual navigation [2]. Human fMRI studies further provide evidence that grid-like signals are also accessible on a macroscopic level [3-7]. Studies in both non-human primates [8] and humans [9, 10] suggest that grid-like coding in the entorhinal cortex generalizes beyond spatial navigation during locomotion, providing evidence for grid-like mapping of visual space during visual exploration - akin to the grid cell positional code in rodents during spatial navigation. However, electrophysiological correlates of the grid-code in humans remain unknown. Here, we provide evidence for grid-like, hexadirectional coding of visual space by human high frequency activity, based on two independent data sets: non-invasive magnetoencephalography (MEG) in healthy subjects and entorhinal intracranial EEG recordings in an epileptic patient. Both data sets consistently show a hexadirectional modulation of broadband high frequency activity (60-120 Hz). Our findings provide first evidence for a grid-like MEG signal, indicating that the human entorhinal cortex codes visual space in a grid-like manner [8-10] and support the view that grid-coding generalizes beyond environmental mapping during locomotion [4-6, 11]. Due to its millisecond accuracy, MEG recordings allow to link grid-like activity to epochs during relevant behavior, thereby opening up the possibility for new MEG-based investigations of grid coding at high temporal resolution.

Results

The present study set out to investigate the electrophysiological basis of grid-like, hexadirectional coding during visual exploration in humans, by simultaneously recording MEG and eye-tracking data from 35 healthy participants during free viewing of natural scenes (Fig. 1a), and simultaneously recording intracranial EEG and eye-tracking data with depth electrodes in the entorhinal cortex of one epilepsy patient (Fig. S1). Although the exact physiology of the broadband high frequency activity (BHA) remains to be discovered, it has been shown to correlate with local neural activity [12-15]. Building on this and other work demonstrating high frequency activity in the entorhinal cortex of behaving rodents [16], we hypothesized to find a grid-like modulation of neuromagnetic BHA in the anterior medial temporal lobe (MTL; Fig. 1b) and sought to verify the MEG findings in the intracranial recordings.

Grid-like modulation of source-localized broadband high frequency MEG data maps visual space

MEG data were aligned to saccade onsets (Fig. 1a) and BHA power (60-120 Hz) source-localized to the anterior MTL (Fig. 1b), was extracted during saccadic eye movements. We used a recently optimized MTL source reconstruction method [17], extending prior MEG work localizing MTL activity [18-22]. Applying a quadrature filter approach [3], we estimated the phase of hexadirectional activity as a function of saccade direction (putative 'grid orientation') and subsequently quantified BHA power aligned and misaligned to the main grid axis, in a two-fold cross-validation design (Fig. 1c). Estimation of the putative grid orientation was achieved by fitting regressors for the sine and cosine of each saccade direction Φ in the respective rotational symmetric space (e.g. 60°, 6-fold periodicity, along with biologically implausible 4-, 5-, 7- and 8-fold control periodicities) to one half of the data (set 1) in a GLM. Saccade length was included as a nuisance regressor in the analysis. The resulting phase-angle was extracted from the obtained beta coefficients ($\Phi = \arctan(\beta1/\beta2)/symmetry$), in the respective rotational symmetric space. The other half of the data (set 2) was binned according to Θ, into aligned bins (Φ +/- 15°, modulo 60°) and misaligned bins (Φ +30° +/- 15°, modulo 60°). This procedure was repeated after swapping set 1 and set 2, and power was averaged across the repetitions for aligned and misaligned bins, respectively.

We found significantly higher 60° periodic BHA (60-120 Hz) for aligned versus misaligned directional sampling in the left anterior MTL, including entorhinal cortex (t_{34} = 4.53, p < .00007, Fig. 2a; Cohen's d = .1988, reflecting a small effect size). When inspecting the 6 aligned and 6 misaligned 30° bins, aligned directions generally elicited higher BHA power than misaligned directions (Fig. 2b), indicating that the effect is not driven by a single direction. The putative grid orientations (i.e. the angle of the hexadirectional modulation) did not cluster across participants (Fig. 2d). A 2x5 repeated-measures ANOVA with factors alignment (aligned vs. misaligned) and rotational symmetry (4-, 5-, 6-, 7-, and 8-fold), revealed a significant interaction ($F_{3.305,\ 112.3,\ \text{Greenhouse-Geisser}}$ = 3.49, p < .015). Importantly, the significant quadratic contrast (quadratic F= 10.8, p < .0025; linear and cubic contrasts not significant) indicated a u-inverted shape of the differences across the rotational symmetries, being optimal for the 6-fold symmetry. Planned post-hoc comparisons revealed that the difference for the 6-fold symmetry was bigger than for any of the other symmetries (all t_{34}'s > 2.26, all p's < .03, 2-sided, uncorrected). Moreover, the differences for 4-, 5-, 7-, and 8-fold symmetry were not significantly different from zero (all t_{34}'s < 1.2 & > -1.1, all p's> .27, 2-sided, uncorrected, Fig. 2e). A repeated-measures ANOVA for the aligned bins in the MEG data showed no significant difference ($F_{34,5}$ = 2.05, p = .075). A repeated-measures ANOVA comparing the difference

82   (aligned – misaligned) across bins indicated that the effect was more pronounced for some directions

83   ($F_{34,5}$ = 2.28, p = .049). No 6-fold modulation of BHA power was observed in right anterior MTL (t_{34} = -.4,

84   p > .68).

85   In addition, a whole-brain analysis of the 60° periodic modulation confirms the findings from the ROI-

86   based analysis and shows clustering of the highest differences between aligned and misaligned BHA

87   power in the left MTL (Fig. 2e), supporting the spatial specificity of the effect. To investigate whether the

88   hexadirectional modulation was limited to BHA, we computed the 60° periodic modulation of power in a

89   lower frequency band (20-50 Hz). There was no significant difference between aligned versus misaligned

90   directional sampling in the left anterior MTL (t_{34} = 1.179, p = .247). Furthermore, in the present analyses,

91   we investigated MEG activity during eye movements, which may be affected by oculomotor-related

92   artefacts. However, a 60° periodic BHA power modulation could not be found when oculomotor activity

93   recorded via EOG electrodes was analyzed (t_{34}= 0.296, p > .769; t_{34}= -.9347, p > .357; for horizontal and

94   vertical EOG signals, respectively; Fig. 2f). Moreover, there was no significant difference in number of

95   saccades aligned to the putative grid orientation versus number of saccades misaligned for 4-, 5-, 6-, 7-

96   and 8-fold rotational symmetries (see Fig. S2).

97   In order to investigate the possible electrophysiological origin and spatial specificity of the MEG results,

98   we analyzed intracranial data recorded from the entorhinal cortex of an epilepsy patient (Fig. S1a), while

99   the patient was performing a free viewing task. The BHA power difference (aligned – misaligned) was

100   significantly higher compared to a surrogate distribution in the 6-fold rotational symmetry (p < .011, 1-

101   sided; Fig. S1b&c). Biologically implausible 4-, 5-, 7-, and 8-fold symmetries did not show a significant

102   modulation of BHA power (all p's > .36, one-sided; Fig. S1d), neither did a spatially adjacent amygdala

103   electrode (Fig. S1e) show a 6-fold rotational symmetry of BHA (p > .36; Fig. S1f&g). The difference between

104   the hexadirectional modulation of BHA in entorhinal cortex and amygdala was significantly higher than

105   expected by chance (p < .013).

106

Discussion

108   The present results provide the first evidence for a grid-like signal in non-invasive electrophysiological

109   recordings in humans: electromagnetic activity, source-localized to the anterior MTL, revealed a

110   hexadirectional modulation of BHA power (60 -120 Hz). Confirming these findings, intracranial field

111   potentials recorded in the entorhinal cortex of a patient also showed a hexadirectional modulation of BHA

4

112 power in the same frequency band. The whole-brain MEG as well as control analyses in the intracranial

113 data point towards the spatial specificity of the effect within the anterior MTL / entorhinal cortex. The

114 millisecond accuracy of these recordings allow to link activity to epochs during the relevant behavior,

115 thereby overcoming limitations of other non-invasive techniques, such as fMRI. We found a grid-like

116 pattern in BHA power, which has been suggested to correlate with local neural activity [12-15], indicating

117 that grid-coding is detectable with mass-electrophysiological recordings, opening up the possibility for

118 new non-invasive investigations of grid coding in cognitive neuroscience at high temporal resolution. We

119 show that the grid-like modulation of electromagnetic and intracranial electrophysiological activity is

120 related to the exploration of visual space. This is in line with work in non-human primates identifying cells

121 in the MTL that fire in relation to the animal's gaze position [8, 23-25], rather than coding location during

122 locomotion, as well as very recent fMRI work in humans showing hexadirectional modulations of

123 entorhinal BOLD activity related to the exploration of visual space [9, 10]. Given the fundamental

124 differences in sensory dominance between rodents and primates, it seems plausible that primates code

125 location during exploration by locomotion and eye movements.

126 In sum, our results support the view that grid-like coding in the anterior MTL goes beyond mapping the

127 environment during locomotion [8-10] and that the grid cell system could provide a general neural code

128 underlying core cognitive functions in humans [4-6, 11].

129

130 Acknowledgments

144

Author Contributions

146   T.S., O.J. and C.F.D. designed the experiment. T.S., O.J and C.F.D wrote the paper. T.S. collected the data.

147   T.S. performed the analyses. M.L., C.E.S. designed, conducted the iEEG part of the study and assisted in

148   writing of the manuscript. S.A.S. implanted electrodes. J.J. provided electrode imaging information.

Declaration of Interests

150   The authors declare no competing interests.

151

References

153   1.   Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., and Moser, E.I. (2005). Microstructure of a spatial map in
154   the entorhinal cortex. Nature *436*, 801.
155   2.   Jacobs, J., Weidemann, C.T., Miller, J.F., Solway, A., Burke, J.F., Wei, X.X., Suthana, N., Sperling, M.R.,
156   Sharan, A.D., Fried, I., et al. (2013). Direct recordings of grid-like neuronal activity in human spatial
157   navigation. Nat Neurosci *16*, 1188-1190.
158   3.   Doeller, C.F., Barry, C., and Burgess, N. (2010). Evidence for grid cells in a human memory network.
159   Nature *463*, 657.
160   4.   Bellmund, J., Deuker, L., and Doeller, C. (2016). Grid-cell representations in mental simulation. Elife *5*.
161   5.   Horner, A.J., Bisby, J.A., Zotow, E., Bush, D., and Burgess, N. (2016). Grid-like Processing of Imagined
162   Navigation. Curr Biol *26*, 842-847.
163   6.   Constantinescu, A.O., O'Reilly, J.X., and Behrens, T.E.J. (2016). Organizing conceptual knowledge in
164   humans with a gridlike code. Science *352*, 1464-1468.
165   7.   Kunz, L., Schroder, T.N., Lee, H., Montag, C., Lachmann, B., Sariyska, R., Reuter, M., Stirnberg, R.,
166   Stocker, T., Messing-Floeter, P.C., et al. (2015). Reduced grid-cell-like representations in adults at genetic
167   risk for Alzheimer's disease. Science *350*, 430-433.
168   8.   Killian, N.J., Jutras, M.J., and Buffalo, E.A. (2012). A map of visual space in the primate entorhinal cortex.
169   Nature *491*, 761.
170   9.   Nau, M., Navarro Schroder, T., Bellmund, J.L.S., and Doeller, C.F. (2018). Hexadirectional coding of
171   visual space in human entorhinal cortex. Nat Neurosci.
172   10.   Julian, J.B., Keinath, A.T., Frazzetta, G., and Epstein, R.A. (2018). Human entorhinal cortex represents
173   visual space using a boundary-anchored grid. Nat Neurosci.
174   11.   Wilming, N., Konig, P., Konig, S., and Buffalo, E.A. (2018). Entorhinal cortex receptive fields are
175   modulated by spatial attention, even without movement. Elife *7*.
176   12.   Ray, S., Crone, N.E., Niebur, E., Franaszczuk, P.J., and Hsiao, S.S. (2008). Neural correlates of high-
177   gamma oscillations (60–200 Hz) in macaque local field potentials and their potential implications in
178   electrocorticography. Journal of Neuroscience *28*, 11526-11536.
179   13.   Mukamel, R., Gelbard, H., Arieli, A., Hasson, U., Fried, I., and Malach, R. (2005). Coupling between
180   neuronal firing, field potentials, and FMRI in human auditory cortex. Science *309*, 951-954.

181   14.   Manning, J.R., Jacobs, J., Fried, I., and Kahana, M.J. (2009). Broadband shifts in local field potential
182   power spectra are correlated with single-neuron spiking in humans. Journal of Neuroscience *29*, 13613-
183   13620.

184   15.   Lachaux, J.-P., Axmacher, N., Mormann, F., Halgren, E., and Crone, N.E. (2012). High-frequency neural
185   activity and human cognition: past, present and possible future of intracranial EEG research. Progress in
186   neurobiology *98*, 279-301.

187   16.   Chrobak, J.J., and Buzsaki, G. (1998). Gamma oscillations in the entorhinal cortex of the freely behaving
188   rat. J Neurosci *18*, 388-398.

189   17.   Backus, A.R., Schoffelen, J.M., Szebenyi, S., Hanslmayr, S., and Doeller, C.F. (2016). Hippocampal-
190   Prefrontal Theta Oscillations Support Memory Integration. Curr Biol *26*, 450-457.

191   18.   Kaplan, R., Doeller, C.F., Barnes, G.R., Litvak, V., Düzel, E., Bandettini, P.A., and Burgess, N. (2012).
192   Movement-related theta rhythm in humans: coordinating self-directed hippocampal learning. PLoS biology
193   *10*, e1001267.

194   19.   Fuentemilla, L., Barnes, G.R., Duzel, E., and Levine, B. (2014). Theta oscillations orchestrate medial
195   temporal lobe and neocortex in remembering autobiographical memories. Neuroimage *85 Pt 2*, 730-737.

196   20.   Staudigl, T., and Hanslmayr, S. (2013). Theta oscillations at encoding mediate the context-dependent
197   nature of human episodic memory. Curr Biol *23*, 1101-1106.

198   21.   Heusser, A.C., Poeppel, D., Ezzyat, Y., and Davachi, L. (2016). Episodic sequence memory is supported by
199   a theta-gamma phase code. Nat Neurosci *19*, 1374-+.

200   22.   Guitart-Masip, M., Barnes, G.R., Horner, A., Bauer, M., Dolan, R.J., and Duzel, E. (2013).
201   Synchronization of Medial Temporal Lobe and Prefrontal Rhythms in Human Decision Making. The
202   Journal of Neuroscience *33*, 442-451.

203   23.   Rolls, E.T., Robertson, R.G., and Georges-François, P. (1997). Spatial view cells in the primate
204   hippocampus. European Journal of Neuroscience *9*, 1789-1794.

205   24.   Wirth, S., Baraduc, P., Planté, A., Pinède, S., and Duhamel, J.-R. (2017). Gaze-informed, task-situated
206   representation of space in primate hippocampus during virtual navigation. PLoS biology *15*, e2001045.

207   25.   Meister, M.L., and Buffalo, E.A. (2018). Neurons in primate entorhinal cortex represent gaze position in
208   multiple spatial reference frames. Journal of Neuroscience *38*, 2430-2441.

209   26.   Berens, P. (2009). CircStat: A MATLAB Toolbox for Circular Statistics. J Stat Softw *31*, 1-21.

210   27.   Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J.M. (2011). FieldTrip: Open source software for
211   advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci *2011*,
212   156869.

213   28.   Penny, W.D., Friston, K.J., Ashburner, J.T., Kiebel, S.J., and Nichols, T.E. (2011). Statistical parametric
214   mapping: the analysis of functional brain images, (Elsevier).

215   29.   Avants, B.B., Tustison, N., and Song, G. (2009). Advanced normalization tools (ANTS). Insight j *2*, 1-35.

216   30.   Staudigl, T., Hartl, E., Noachtar, S., Doeller, C.F., and Jensen, O. (2017). Saccades are phase-locked to
217   alpha oscillations in the occipital and medial temporal lobe during successful memory encoding. PLoS Biol
218   *15*, e2003404.

219   31.   Stolk, A., Todorovic, A., Schoffelen, J.M., and Oostenveld, R. (2013). Online and offline tools for head
220   movement compensation in MEG. Neuroimage *68*, 39-48.

221   32.   Engbert, R., and Kliegl, R. (2003). Microsaccades uncover the orientation of covert attention. Vision Res
222   *43*, 1035-1045.

223   33.   Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., Mazoyer, B.,
224   and Joliot, M. (2002). Automated anatomical labeling of activations in SPM using a macroscopic
225   anatomical parcellation of the MNI MRI single-subject brain. Neuroimage *15*, 273-289.

226   34.   Nolte, G. (2003). The magnetic lead field theorem in the quasi-static approximation and its use for
227   magnetoencephalography forward calculation in realistic volume conductors. Phys Med Biol *48*, 3637-
228   3652.

229   35.   Gross, J., Kujala, J., Hamalainen, M., Timmermann, L., Schnitzler, A., and Salmelin, R. (2001). Dynamic
230   imaging of coherent sources: Studying neural interactions in the human brain. Proc Natl Acad Sci U S A
231   *98*, 694-699.

232   36.   Jacobs, J., Miller, J., Lee, S.A., Coffey, T., Watrous, A.J., Sperling, M.R., Sharan, A., Worrell, G., Berry,
233   B., Lega, B., et al. (2016). Direct Electrical Stimulation of the Human Entorhinal Region and Hippocampus
234   Impairs Memory. Neuron *92*, 983-990.

235   37.   Maris, E., and Oostenveld, R. (2007). Nonparametric statistical testing of EEG- and MEG-data. J Neurosci
236   Methods *164*, 177-190.

237

Figure legends

Figure 1. Hexadirectional mapping of visual space: Procedure and Analysis. (A) Paradigm: Free viewing of 200 indoor / outdoor scenes with simultaneous eye tracking (left). MEG data are aligned to saccade onsets, defining events of interest (middle). (B) Region-of-interest (highlighted), comprising bilateral anterior portions of the hippocampus and parahippocampal gyrus. (C) Analysis rationale. Data was split into halves (set 1, set 2) to estimate the putative grid orientation (angle of hexadirectional activity) separately from computing aligned and misaligned BHA power, in a two-fold cross-validation design. Estimation of putative grid orientation was done by fitting regressors for sine and cosine of saccade directions (Θ) in the respective rotational symmetric space (here: 60° periodicity) to set 1 BHA power (the general linear model included a constant and saccade duration as nuisance regressors). Resulting beta estimates were used to derive the putative grid orientation angle (Φ). Trials in set 2 were split according saccade directions aligned vs. misaligned to Φ. The difference in BHA power (aligned – misaligned) reflects the grid-like modulation of BHA power.

Figure 2. Grid-like modulation of BHA MEG activity during visual exploration. (A) BHA power (60-120 Hz) aligned to the putative grid orientation is significantly higher than misaligned BHA power in the left anterior MTL. (B) 6-fold symmetric modulation of the BHA power, visualizing the effect in (A). The x-axis depicts the difference between saccade directions and the estimated putative grid orientations. (C) Other rotational symmetries (4-, 5-, 7- and 8-fold) do not show significant differences between aligned and misaligned BHA power (D) Putative grid orientations across participants did not show clustering. (E) Whole-brain analysis shows clustering of highest differences (aligned vs. misaligned, 60-120 Hz, 6-fold symmetry) in the left temporal lobe. (F) No significant difference between aligned vs. misaligned BHA power, in horizontal nor vertical EOG data (available in 32 participants). Dots show data from all participants; Error bars show S.E.M. See also Figures S1 and S2.

9

Star Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Tobias Staudigl (tobias.staudigl@cshs.org).

Experimental Model and Subject Details

36 young healthy adults were included in the MEG study. Initially, 48 participants were recruited; however, 12 dropped out due to not completing the study (7 participants did not come back for one of the sessions, see below), excessive movement artifacts (2 participants) and technical problems during the recordings (3 participants). The 36 participants included in this study (24 females; mean age 23.1 years, range 18-30 years; 35 right handed) reported no history of neurological and/or psychiatric disorders and had normal or corrected-to-normal vision. One participant was excluded from the analysis due to insufficient number of trials. Parts of this data have been published in Staudigl, et al. [30] with respect to independent research questions and analyses. All participants gave written informed consent before the start of experiment in accordance with the Declaration of Helsinki. The study was approved by the local ethics committee (commission for human related research CMO-2014/288 region Arnhem/Nijmegen NL). Additionally, one male patient (age range 25-45) with a history of drug resistant epilepsy was included in the study. The patient, who volunteered to participate in the study at Columbia University Medical School, had depth electrodes implanted for diagnostic reasons. All procedures were approved by the Institutional Review Board at Columbia University Medical School. The patient provided informed consent before participating in the study and was free to withdrawn from the study at any point. The study was approved by the Institutional Review Board at Columbia University Medical School, New York City, US.

Method Details

Design, Procedure and Materials.

The design for the healthy participants comprised an MEG and an fMRI (not reported here) session. The session order was counterbalanced across participants. Three stimulus sets, consisting of 100 photographs each, were constructed for each session. Half of the photographs were outdoor scenes, the other half indoor scenes (see Fig. 1a, for an example). The photographs were presented on a 39 x 46 cm back-projection screen in the MEG chamber, subtending a visual angle of approximately 27° × 32°. Two stimulus sets were presented during encoding, all three sets during test. Assignment of set to encoding or

10

297   test was counterbalanced across participants. Nine additional scenes were presented during a short

298   practice session before encoding and test in order to explain the task. Participants were made aware

299   about the memory test before the start of the experiment. During the study phase, photographs were

300   presented for 4 s. The order was randomized with the constraint that no more than four scenes of the

301   same type (indoor / outdoor) were shown consecutively. The participants were instructed to judge the

302   scene type (indoor / outdoor) via a button press during the fixation cross (variable duration of $1 - 2$ s)

303   following each scene (mean accuracy = .954, std = .068). This encoding task was chosen to ensure

304   attention to each scene. Participants were not expected to fixate, i.e. they freely viewed the scenes. The

305   study phase was followed by a distracter phase (solving simple mathematical problems for ~ 1 min), ~5

306   min of fixation to different locations on the screen used to evaluate eye tracker accuracy, and ~1 min of

307   eyes open and ~1 min of eyes closed. Subsequently, participants performed a recognition memory test

308   followed. Only data from the study phase are presented here.

309

310   **MEG Acquisition and Preprocessing.**

311   MEG was recorded in a magnetically shielded room, using a 275 whole-brain axial gradiometer system

312   (VSM MedTech/CTF MEG, Coquitlam, Canada). The data were sampled at a rate of 1200 Hz following a

313   low-pass anti-aliasing filter with a cutoff at 300 Hz. In addition, we recorded vertical and horizontal

314   electro-oculograms from bipolar Ag/AgCl electrodes (<10kΩ impedance; available for 32 participants)

315   placed below and above the left eye and at the bilateral outer canthi. 3 head coils placed at anatomical

316   landmarks (nasion and both ear canals) were used to track the position of the head relative to the MEG

317   helmet during the recordings. The head position was continuously monitored using a real-time head

318   localizer [31]. Each participant's nasion, left and right ear canal, and head shape were digitized with a

319   Polhemus 3Space Fasttrack. Data preprocessing was done using the Fieldtrip [27] toolbox. Data were

320   divided into single epochs, ranging from 0 to 4 s after scene onset, and corrected for cardiac artifacts using

321   Independent Component Analysis (ICA).

322

323   **Eye Tracking Acquisition, Analyses and Trial Definition.**

324   Eye tracking data were recorded simultaneously with MEG data. We tracked the horizontal and vertical

325   movements of each participant's left eye with an Eyelink 1000 (SR Research) eye tracker. The eye tracker

326   was calibrated before recording data, by collecting gaze fixation samples from known target points to map

327   raw eye data onto screen coordinates. Participants fixated nine dots sequentially appearing on a 3 by 3

328 grid. During the subsequent validation run, the difference between current gaze fixations and fixations

329 during the calibration were obtained. If this difference was smaller than 1 degree visual angle, the

330 calibration was accepted. Vertical and horizontal eye movements were transformed into velocities.

331 Velocities exceeding a given threshold (velocity > 6 x the standard deviation of the velocity distribution,

332 duration > 12 ms, see Engbert and Kliegl [32]) were defined as saccades. Saccade onsets during scene

333 presentation in the study phase defined the events of interest (trials). Only trials that were free of other

334 saccades and blinks in a 200 ms interval after saccade onset were included. On average, 558 (std = 196.9)

335 trials remained for the analysis. The trials were zero-padded to a length of 0.6 s (i.e., adding 200 ms of

336 zeros before and after the 200 ms of data). One participant was excluded from the analysis due to

337 insufficient number of trials for the hexadirectional analysis.

338 We focused our analysis on high frequency activity because it has been shown to correlate with local

339 neural activity [12-15] and was reported in the entorhinal cortex of behaving rodents [16]. We did not

340 analyze lower frequencies (e.g., theta) because our data epochs were too short to obtain a reasonable

341 frequency resolution in these bands.

342

343 **Source Reconstruction.**

344 Based on our a priori hypothesis on the origin of the grid signal in the entorhinal cortex, we performed a

345 region-of-interest based source reconstruction. To account for the spatial resolution of MEG, we

346 constructed two anterior medial temporal ROIs, comprising the anterior portions of the hippocampus and

347 parahippocampal gyrus in the left and right hemisphere, respectively (Figure 1b). For each hemisphere,

348 we aimed at computing one leadfield generated by the entire ROI, rather than averaging across multiple

349 point sources constructed within each ROI, applying a recently optimized MTL source reconstruction

350 method [17].

351 To construct the anterior medial temporal ROIs, we created 5 mm grids covering the voxels inside the

352 anterior half (median split) of anatomical masks including the labels 'Hippocampus_L',

353 'ParaHippocampal_L' and 'Hippocampus_R', 'ParaHippocampal_R', respectively, based on the Automatic

354 Anatomical Labeling atlas in Montreal Neurological Institute space [33].

355 For each participant, the MNI grid was warped onto each participant's anatomy bases on individual

356 structural MR images (1 mm isotropic voxels), acquired on a 3T Siemens Magnetom Prisma MRI system

357 (Siemens, Erlangen, Germany), after aligning the structural images to the MEG coordinate system, utilizing

358 the fiducials (nasion, left and right preauricular points) and individual head-shapes recorded after the

359   experiment. A realistic single-shell brain volume conduction model [34] was constructed for each

360   participant, based on these structural MRIs.

361   On the basis of this model, the contribution of dipolar sources at each grid point to the sensor level data

362   was estimated. Singular value decomposition was then used to reduce the columns of this sensor-by-grid

363   point leadfield matrix. The vector explaining most variance was selected, resulting in a leadfield matrix

364   consisting of one spatial component for each anterior medial temporal lobe ROI.

365   The cross-spectral density for the construction of the spatial filters was derived from the Fourier

366   transformation of all trials (epoched 0 to 200 ms from saccade onset) at the frequency of interest (90 Hz)

367   with 30 Hz spectral smoothing using a multitaper approach with 11 tapers from discrete prolate spheroidal

368   sequences (dpss). The cross-spectrum was regularized prior to matrix inversion by loading the diagonal of

369   the matrix with 5% of the average sensor power. We employed the Dynamic Imaging of Coherent Sources

370   (DICS) beamformer [35] to construct a spatial filter for each specified location. The sensor level single-trial

371   data was projected into source space by multiplying it with the spatial filter of each ROI, allowing for

372   further analysis to be conducted in virtual sensor space.

373   For the whole brain analysis (see Figure 2e), the same source estimation procedure was repeated for all

374   unique labels of the Automatic Anatomical Labeling atlas that include cortical brain areas.

375

376   **Hexadirectional analysis.**

377   The estimation of the hexadirectional signal followed a two-step procedure (see Fig. 1c): First, the putative

378   grid orientation was estimated on one half of the trials (a trial was defined by the onset of individual

379   saccades, see above). Second, aligned and misaligned (to the estimated putative grid orientation)

380   broadband high frequency activity (BHA) (60-120 Hz) power was computed. The procedure was repeated

381   with inversed assignment of data sets to the two steps, and aligned and misaligned BHA power was

382   averaged across the repetitions (two-fold cross-validation design).

383   Because of a horizontal bias in the distribution of saccade directions, we removed trials such that the

384   distribution of saccade directions in the analyses did not differ from a uniform distribution within

385   participants (Rayleigh-test, all p-values > .05). Performing a 4-,5-,6-,7- and 8-fold analyses of the saccades

386   directions yielded no significant difference between the number of saccades aligned and misaligned to

387   the putative grid orientation (4-fold: t_{34} = -1.673, p=.104; 5-fold: t_{34} = -1.202, p=.238; 6-fold: t_{34} = -.392,

388   p=.698; 7-fold: t_{34} = -.82, p=.418; 8-fold: t_{34} = -.986, p=.3310; see Fig. S2).

389   The remaining data was split into halves (set 1, set 2), and BHA power was computed for each set in virtual

390   sensor space by applying a sliding time window approach with a window length of 44 ms length in steps

391   of 10 ms across the data to each trial (epoched from saccade on- to offset, individual for each trial). After

392   multiplying a hanning taper to each window, the Fourier transformation was calculated at the frequency

393   of interest (90 Hz) with 30 Hz spectral smoothing using a multitaper approach with 2 dpss tapers. BHA

394   power was averaged across time bins within each trial, in cases where more than one BHA value resulted

395   from the sliding time window approach.

396   To estimate the putative grid orientation, regressors (β1, β2) for sine and cosine of saccade directions (Θ)

397   in the respective rotational symmetric space (6-fold symmetry = 60° periodicity) were fitted to set 1 BHA

398   power using a general linear model including the saccade length (sl) as a nuisance regressor:

399    $y = \beta_0 + \beta_1 * \cos(6 * \theta) + \beta_2 * \sin(6 * \theta) + \beta_3 * sl + \varepsilon$

400   Resulting beta estimates were used to derive the putative grid orientation (Φ):

401    $\Phi = \arctan(\beta_1 + \beta_2)/symmetry$

402   The other half of the data (set 2) was binned according to each trial's saccade direction Θ, into aligned

403   bins (Φ +/- 15°, modulo 60°) and misaligned bins (Φ +30° +/- 15°, modulo 60°). BHA power was averaged

404   for aligned and misaligned bins, respectively.

405   After repeating the procedure with inversed assignment of data sets to the two steps, power was

406   averaged across the repetitions for aligned and misaligned bins, respectively. The difference in BHA power

407   (aligned – misaligned) reflects the grid-like modulation of BHA power. Biologically implausible 4-, 5, 7- and

408   8-fold periodicities were computed with the same approach and compared to the 6-fold periodic

409   modulation of BHA power.

410

411   **Intracranial data.**

412   One male patient (age range 25-45) with a history of drug resistant epilepsy was included in the study.

413   The patient was implanted with intracranial EEG electrodes for diagnostic purposes. Recordings were

414   performed at the Department of Neurological Surgery, Columbia University, USA. All procedures were

415   approved by the Institutional Review Board at Columbia University Medical School. The patient provided

416   informed consent before participating in the study and was free to withdrawn from the study at any point.

417 The procedure and design of the study was similar to the MEG procedure and design. The patient
418 performed a free viewing task with 80 coloured images (indoor and outdoor sense, faces, animals, etc.)
419 as stimuli. Each image was presented for 6 s in the center of a screen at a distance of about 65 cm,
420 subtending a visual angle of approx. 18° x 12°. The patient was requested to freely view each image. After
421 the stimulus offset a gray screen was displayed with five possible response options. The participant was
422 asked to indicate how he liked the last image on a scale ranging from 1 (very little) to 5 (very much) via
423 button press. The next image was displayed with a jitter interval of 0.1 to 0.5 s.

424 The locations of the electrodes were determined using pre- and post-operative MRIs and CTs, respectively
425 (for details see Jacobs, et al. [36]). One contact was identified to be fully located within the left entorhinal
426 cortex (indicated by red crosshair in Fig. S1A) and field potentials from this contact were used for further
427 analysis. To investigate spatial specificity of the hexadirectional modulation, a contact in the amygdala,
428 neighboring the entorhinal cortex (see Fig. S1E), was used to as a control site.

429 Intracranial EEG was recorded from depth electrodes (PMT Corporation) with multiple recording sites
430 (inter-contact spacing = 5 mm), using a Blackrock system (Blackrock Microsystems, Inc., Salt Lake City,
431 USA), with voltages referenced to an intracranial electrode site with least signal (2000 Hz sampling rate).
432 Data was re-referenced offline using a bipolar montage. Entorhinal data was re-referenced to the
433 contact's medial neighbor. Amygdala data was re-referenced to its lateral neighbor. A bipolar montage
434 provides high spatial specificity with respect to the underlying electric source and low susceptibility to
435 volume conducted artifacts (e.g. oculomotor artifacts).

436 Eye movements were monitored with a Tobii TX300 eye tracker. The left and right eye positions were
437 sampled at 300Hz. A five-point calibration was performed prior to experimental session. Intracranial EEG
438 data was offline downsampled to 1000 Hz and eye tracking data was interpolated to match sampling rate
439 at 1kHz. Subsequently, eye tracking and intracranial EEG data were co-registered and segmented into
440 epochs with 0.1 sec of prestimulus interval and 6 sec of stimulus presentation. All epochs were visually
441 inspected for artifacts (e.g. epileptiform spikes). Contaminated epochs were excluded from the analyses.
442 The eye tracking data was low-pass filtered at 30 Hz using a zero-phase forward and reverse butterworth
443 infinite impulse response filter.

444 Vertical and horizontal eye movements of the left eye were transformed into velocities. Velocities
445 exceeding a given threshold (velocity > 6 x the standard deviation of the velocity distribution, duration >
446 12 ms, see Engbert and Kliegl [32]) were defined as saccades. Saccade onsets during scene presentation

447   in the study phase defined the events of interest (trials). Only trials that were free of other saccades and

448   blinks in a 200 ms interval after saccade onset were included.

449   Intracranial EEG data were aligned to saccade onsets and BHA power was extracted during saccadic eye

450   movements. The trials were zero-padded to a length of 0.6 s (i.e., adding 200 ms of zeros before and after

451   the 200 ms of data). The estimation of the hexadirectional signal was identical to the procedure described

452   above (see Hexadirectional Analysis).

453

454   **QUANTIFICATION AND STATISTICAL ANALYSIS**

455   We tested the null-hypothesis that there is no difference between BHA power for aligned versus

456   misaligned saccade directions, in the left and right ROIs for the 6-fold rotational symmetry, using 2-sided

457   t-tests. To control for multiple comparisons, we adopted a significance level of 0.025. To measure effect

458   size Cohen's d was computed as

459
$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s_1^2 + s_2^2 - 2rs_1s_2}/\sqrt{2(1-r)}}$$

460   with r denoting Pearson's correlation coefficient.

461   A 2x5 repeated-measures ANOVA with factors alignment (aligned vs. misaligned) and rotational symmetry

462   (4-, 5-, 6-, 7-, and 8-fold) was used to investigate the effect in the left anterior MTL. Planned post-hoc

463   comparisons (2-sided t-tests, uncorrected) were used to test the difference for the 6-fold symmetry versus

464   the other symmetries (4-, 5-, 7-, and 8-fold) and to test whether BHA power in the left anterior MTL were

465   different from zero for the biologically implausible 4-, 5-, 7-, and 8-fold rotational symmetries.

466   A further repeated-measures 1-way ANOVA with the factor rotational symmetry (4-, 5-, 6-, 7-, and 8-fold)

467   was used to test for differences among aligned bins in the MEG BHA. To investigate whether the

468   hexadirectional modulation was limited to BHA, a post-hoc t-test (2-sided, alpha level = .05) was

469   performed to test the 60° periodic modulation of power in a lower frequency band (20-50 Hz). As a further

470   post-hoc control analysis, two t-test (2-sided, alpha = .05, uncorrected) were used to test a 60° periodic

471   BHA power modulation recorded on EOG electrodes (for horizontal and vertical EOG signals, respectively).

472   Five post-hoc t-tests (2-sided, alpha = .05, uncorrected) were used to test the difference in number of

473   saccades aligned to the putative grid orientation versus number of saccades misaligned to the putative

474　grid orientation (for 4-, 5-, 6-, 7- and 8-fold rotational symmetries, respectively). All of the above analyses

475　were performed on the group level with N = 35.

476　We did not include a whole brain statistical approach (as for example implemented in the MEG/EEG

477　Fieldtrip Toolbox), because a significant outcome in this kind of test would only speak to the null

478　hypothesis (no difference between conditions) being rejected and not provide information on the exact

479　spatial extent of the effect [37].

480　The intracranial BHA power differences (aligned – misaligned) in one patient were statistically quantified

481　by comparing them to a distribution of surrogate BHA power differences. The surrogate distribution of

482　BHA power differences was constructed by randomly assigning trials to the aligned and misaligned

483　condition, respectively. 50000 surrogate BHA power differences were computed. Intracranial BHA power

484　differences were compared to the 50000 surrogate BHA power differences, and considered to be

485　significant if they were larger than the 95 % of the surrogate BHA power differences (one-sided test). This

486　procedure was used to quantify the 6-fold periodic modulation of BHA power, as well as the biologically

487　implausible 4-, 5-, 7- and 8-fold periodicities. Additionally, the difference between the hexadirectional

488　modulation of BHA in entorhinal cortex (aligned – misaligned) and amygdala (aligned – misaligned) was

489　compared to a distribution of 50000 surrogate BHA power differences. Differences were considered to be

490　significant if they were larger than the 95 % of the surrogate BHA power differences (one-sided test).

491

492

493　**Data and Software Availability**

494　Data and custom-built MATLAB scripts are available from the authors upon request.

495