Supplemental Information

Cell Type-Specific Differences in Spike Timing and Spike Shape in the Rat Parasubiculum and Superficial Medial Entorhinal Cortex

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Figure S1: Classification of medial entorhinal cortex layer 2 neurons, Related to Figure 1.

(A) Polar plot of theta strength and theta phase angle, $\phi$, of the spiking activity of calbindin+ pyramidal cells (Pyr, green dots) and calbindin- stellate cells (Stel, black dots) identified in freely moving rats. Lines indicate mean theta phase angle and median theta strength.

(B) Polar plot of theta strength and preferred theta phase angle, $\phi$, for nonidentified MEC L2 cells, which were classified as putative calbindin+ cells (pCb+, white dots) and putative calbindin- cells (pCb-, dark grey dots). The background colors indicate the two classification groups (light green and light grey for putative calbindin+ (pCb+) and putative calbindin- (pCb-)) and the guard zone around the classification boundary (white). No nonidentified cell fell within the guard zone.
Figure S3: Classification of medial entorhinal cortex layer 2 cells does not explain cell-type specific differences in burstiness, Related to Figure 3.

(Legend on next page)
Figure S3: Classification of medial entorhinal cortex layer 2 cells does not explain cell-type specific differences in burstiness, Related to Figure 3

(A) Graphical representation of three fitted generalized linear models to investigate if burstiness is related to theta strength (Model 1), putative cell type (Model 2) or both (Model 3).

(B) Comparison of the Akaike information criterion (‘AIC’) of the three models. P-values indicate theoretical likelihood ratio tests between nested models. Both the AIC and the likelihood ratio tests suggest that Model 2 is superior to the other models.

(C) ANOVA tables with F-statistics for the three fitted models. Model 1 indicates a significant relationship between theta strength and burstiness ($P_{\text{Strength}} = 0.00015$), but this effect disappears when we add cell type as another independent variable (Model 3: $P_{\text{Type}} = 0.014$, $P_{\text{Strength}} = 0.54$). The best model depends only on cell type (Model 2: $P_{\text{Type}} = 0.0000076$).

(D) Left: Comparison of the burstiness for the four different neuron types as in Figure 3C, i.e. including MEC L2 cells classified as putatively pyramidal and putatively stellates (Same as Fig 3C). Right: Same plot, but only including identified MEC L2 cells. P-values indicate results of $t$-tests (assuming equal (Cb- vs. L3) or unequal (PaS, Cb+ vs. Cb-, L3) variances). Due to the low number of Cb+ cells, we report the P-value both with and without the statistical outlier (indicated by arrow).
Figure S4. Spike shape is a feature of cell type rather than burstiness, Related to Figure 4.

(A) Scatterplot of the peak-to-trough time as a function of burstiness for the four neuron types defined in Fig. 4A; symbols indicated by legend. Lines indicate the estimated peak-to-trough times as a function of burstiness and cell type from the generalized linear model 1 (GLM1, dashed grey line) and GLM2 (best model, four solid horizontal lines, color code as for symbols).

(B) Comparison of degrees of freedom (“params”) and Akaike information criterion (AIC) for four GLMs in (A). P-values indicate theoretical likelihood ratio tests for nested models. According to the AIC, the best model is GLM2 (Peak-to-trough time depends only on neuron type, not on burstiness). Similarly, a likelihood ratio test rejects the inclusion of burstiness as an extra predictor variable (p = 0.32 for GLM2 vs. GLM3). There is no indication of a statistical interaction between cell type and burstiness (GLM4).

| GLM      | Model                           | ANOVA: p_{burstiness} | F(1,126) | F(3,124) | p     |
|----------|---------------------------------|------------------------|----------|----------|-------|
| GLM1     | Peak-to-trough ~ 1 + Burstiness | 0.0087                 | 7.11     | n.s.     | ***   |
| GLM2     | Peak-to-trough ~ 1 + Type       | 0.0015                 | 5.43     | n.s.     | ***   |
| GLM3     | Peak-to-trough ~ 1 + Burstiness + Type | 0.22          | 1.50     | n.s.     |       |
| GLM4     | Peak-to-trough ~ 1 + Burstiness + Type * Burstiness | 0.38          | 0.77     | n.s.     |       |
(C) Results of ANOVA for four statistical models relating the spike peak-to-trough time to cell type and burstiness.
Figure S5. Comparison of rhythmicity and cycle-skipping with and without including classified medial entorhinal cortex layer 2 cells show similar patterns, Related to Figure 5.

(A) Left: Same plot as Figure 5C, proportions (above) and counts (below) of non-rhythmic, rhythmic and cycle-skipping neurons among the cell types, i.e. including MEC L2 cells classified as putatively pyramidal and putatively stellate. Right: Same plot, but only including identified MEC L2 cells. (P values indicate $\chi^2$ tests of equal proportions among cell types).
Supplemental experimental procedures

Juxtacellular recordings

In this paper, we analyzed a data set of juxtacellular recordings from the superficial medial entorhinal cortex and the parasubiculum which we have previously published (Ray et al. 2014, Tang et al. 2014, Tang et al. 2015, Tang et al. 2016). Below, we present a summary of the recording procedure from these previous papers.

Juxtacellular recordings and tetrode recordings in freely moving animals were obtained in male Wistar and Long-Evans rats (150-250 g). Experimental procedures were essentially performed as recently described (Tang et al., 2014a; Tang et al., 2014b). Briefly, for juxtacellular recordings, glass pipettes with resistance 4-6 MΩ were filled with extracellular (Ringer) solution containing (in mM) NaCl 135, KCl 5.4, HEPES 5, CaCl$_2$ 1.8, and MgCl$_2$ 1 (pH = 7.2) and Neurobiotin (1-2%). The glass recording pipette was advanced into the brain by means of a miniaturized micromanipulator (Tang et al 2014b) while rats explored open field arenas (70 x 70 cm or 1 x 1 m square black box, with a white cue card on the wall). Juxtacellular labeling was attempted at the end of the recording session according to standard procedures (Pinault, 1996). Unidentified recordings in parasubiculum and MEC were either lost before the labeling could be attempted, or the recorded neurons could not be unequivocally identified, as described in Tang et al., 2014a, Tang et al 2015, Tang et al., 2016. After the experiment, the animals were euthanized with an overdose of ketamine, urethane or pentobarbital, and perfused transcardially with 0.1 M phosphate buffer followed by 4% paraformaldehyde solution. The juxtacellular signals were amplified by the ELC-03XS amplifier (NPI Electronics, Tamm, Germany) and sampled at 20 kHz by a data-acquisition interface under the control of PatchMaster 2.20 software (HEKA, Ludwigshafen, Germany). The animal’s location and head-direction was automatically tracked at 25 Hz by the Neuralynx video tracking system and two head-mounted LEDs.

Tissue preparation, immunohistochemistry, and image acquisition

Rats were anaesthetized by isoflurane and euthanized by an intraperitoneal injection of 20% urethane. Animals were then transcardially perfused with 0.9% phosphate-buffered saline, followed by PFA.
Subsequently, brains were removed from the skull and postfixed in PFA overnight. Brains were then immersed in 10% sucrose and then in 30% sucrose for at least one night for cryoprotection. The brains were embedded in Jung tissue Freeing Medium (Leica Microsystems Nussloch, Germany), and mounted on a freezing microtome (Leica 2035 Biocut) to obtain tangential and parasagittal sections at 60 microns.

Tangential sections of the medial entorhinal cortex and parasubiculum were obtained as previously described (Ray et al., 2014; Naumann et al., 2016) by separating the entorhinal cortex from the remaining hemisphere by a cut parallel to the face of the medial entorhinal cortex (Ray & Brecht, 2016) and sectioning with the surface of the entorhinal cortex attached to the block face of the microtome.

Immunohistochemical stains were performed on tangential and sagittal sections. The sections were pre-incubated in a blocking solution containing 0.1 M PBS, 2% Bovine Serum Albumin (BSA) and 0.5% Triton X-100 (PBS-X) for an hour at room temperature (RT). Following this, primary antibodies were diluted in a solution containing PBS-X and 1% BSA. Primary antibodies against the calcium binding proteins Calbindin (Swant: CB300, CB 38; 1:5000), the transmembrane protein Wolframin (Proteintech: 11558-1-AP; 1:200), and the calmodulin binding protein Purkinje cell protein 4 (Sigma: HPA005792; 1:200) were used. Sections were incubated in primary antibodies for at least 24 hours under mild shaking at 4 degrees centigrade. Subsequently sections were incubated in secondary antibodies coupled to different fluorophores (Alexa 488, 546; Invitogen; 1:500). For multiple antibody labelling, antibodies raised in different host species were used.

Images were acquired with a Leica DM5500B epifluorescence microscope with a Leica DFC345 FX camera. Alexa fluorophores were excited using the appropriate filters (Alexa 488- L5; Alexa 546- N3). Fluorescent images were acquired in monochrome, and color maps were applied to the images post acquisition. Post hoc linear brightness and contrast adjustment were applied uniformly to the image under analysis.
Quality control across juxtacellular recordings

We checked explicitly for systematic differences in LFP power across recordings. First, we calculated the global theta power in the LFP of all recordings, defined as the mean power spectral density of the theta-peak in the LFP spectrogram ± 0.3 Hz. We did not find any significant differences in LFP theta power among cell types (P > 0.05, one-way ANOVA). We also did not find any correlation between the LFP theta power and burstiness in our data (P > 0.05, Spearman correlation).

If spikes are missed during bursts, this would bias a recording towards low burstiness. Under the juxtacellular recording configuration, however, spikes are well above the noise level (signal-to-noise typically an order of magnitude higher than tetrode recordings) and thus unlikely to fall below the detection threshold. It is the case, however, juxtacellular recordings might potentially be more disruptive for the recorded neurons due to the close proximity of the glass tip and the membrane; recordings (or portions of recordings) where signs of cellular damage were observed (e.g. action-potential broadening, increase in firing rate; see Pinault et al., 1996; Herfst et al., 2012) were excluded from the analysis. As a measure of ‘recording quality’, we estimated the signal-to-noise ratio of the spikes (Joshua et al. 2007), and we found no difference between cell types (P > 0.05, Kruskal-Wallis test). We also found no correlation between recording quality and burstiness, spike shape or phase precession (all P > 0.05, Spearman correlations).
Supplemental references

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