ONLINE METHODS

Hippocampal CA1 pyramidal cells form functionally distinct sublayers

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Animals and surgery

Ten male Long-Evans rats (250-400 g) were implanted with a 4- or 8-shank silicon probe in the right dorsal hippocampus under isoflurane anesthesia (1-1.5%) and recorded from dorsal CA1 pyramidal layers. In four of the rats, another 4-shank silicon probe was also implanted in the right dorsocaudal medial entorhinal cortex[27]. The silicon probes were attached to micromanipulators and moved slowly to the target. Each shank had 8 recording sites (160 μm^2 each site; 1-3 MΩ impedance) and inter-shank distance was 200 µm. Recordings sites were staggered to provide a two-dimensional arrangement (20 µm vertical separation). The EC probe was positioned so that the different shanks recorded from different layers[27]. Histological and electrophysiological localization of recording sites in the entorhinal cortex was described previously[27]. Two stainless steel screws inserted above the cerebellum were used as indifferent and ground electrodes during recordings. All protocols were approved by the Institutional Animal Care and Use Committee of Rutgers University.

Behavioral testing

Physiological signals during waking were recorded during six different tasks. (1) The task on the linear track (250 cm x 7 cm), (2) the task on the open field (180 cm x 180 cm, or 120 cm x 120 cm). (3) the wheel running task, and (4) The alternation task in the T-maze (100 cm x 120 cm) with wheel running delay were described previously[27]. (5) On an elevated plus maze (100 cm x 100 cm), the animal was motivated to run to the ends of 4 corridors, where water was given every 30 sec. (6) On a zigzag maze (100 cm x 200 cm) with 11 corridors, the animals learned to run back and forth between two water cups placed in the first and last corridors. Theta periods from all maze behaviors were lumped together as 'RUN'. The task was regarded as "novel" when the animal performed it for the first time and "familiar" after at least 3 testing sessions.

Data collection and cell type classification

Detailed information about the recording system and spike sorting has been described[27]. Briefly, signals were amplified (1,000X), bandpass-filtered (1 Hz-5 kHz) and acquired continuously at 20 kHz. LFP was down-sampled to 1,250 Hz for additional analysis. Spike sorting was performed by KlustaKwik (http://klustawik.sourceforge.net), followed by manual adjustment of the clusters ("Klusters" software package; http://klusters.sourceforge.net). CA1 pyramidal cells and interneurons were separated as described[27]. Cell type classification of entorhinal cortical neurons was described previously[27]. A total of 3541 (CA1), 491 (EC2), 576 (EC3) and 559 (EC5) principal neurons and 468 (CA1), 85 (EC2), 217 (EC3) and 94 (EC5) interneurons were identified and used for analyses. Median isolation distances[30], reflecting the quality of cluster

isolation, were similar across the deep, middle and superficial CA1 pyramidal cells (28.7, 28.3, 28.7, respectively; $P > 0.13$, Wilcoxon rank sum test).

Detection of brain states

Theta periods during task performance (RUN), REM epochs (REM) and slow wave sleep (SWS) were detected using the ratio of the power in theta band (5-11 Hz) to delta band (1-4 Hz) of LFP, followed by manual adjustment with the aid of visual inspection of whitened power spectra and the raw traces[27]. REM periods were cross-validated with experimenter notes taken while observing theta activity on-line in sleep session and verifying that the rat was sleeping.

To detect gamma epochs, a recording site with the highest ripple power in a given shank was used. LFPs were band-pass filtered (30-80Hz) and the power (root mean square) of the filtered signal was calculated in 80 msec time windows. Gamma phase of spiking was computed within gamma oscillatory periods. Gamma oscillatory periods were defined as epochs with gamma power larger than 85 percentile of gamma power. If the gap between epochs was shorter than 30 msec, these flanking epochs were combined, generating a single epoch. Epochs less than 40 msec were discarded. The detected gamma periods represented 19.8 ± 3.4 % of RUN, 13.2 ± 3.3 % of REM sleep, 12.8 ± 2.0 % of SWS and 17.9 ± 2.8 % of awake non-theta epochs (mean ± S.D.).

To detect ripple events, LFP in CA1 pyramidal layer during non-theta periods was band-pass filtered (140-230Hz), and the power (root mean square) was calculated in 17 msec time windows. Ripple epochs were defined as periods during which ripple power was continuously greater than mean + 3S.D., and peak of power in the periods were greater than mean + 7 S.D. Events shorter than 15 msec were discarded.

DOWN-UP transitions in the entorhinal cortex (EC) were detected by using spiking activity of EC neuron population during slow wave sleep. All the simultaneously recorded single EC neurons were combined as a multiunit activity (MUA), and smoothed with a Gaussian kernel (S.D. = 10 msec, kernel size = 60 msec). Upstate onsets were detected if the following criteria were all fulfilled: 1) the smoothed MUA reached above the "upstate threshold", defined to be the geometric mean of all non-zero MUA; 2) the mean MUA in a 150 msec window before the onset of the candidate event was below the "downstate threshold", which was defined as 0.16 times upstate threshold; and 3) the mean MUA in both 100 msec and 200 msec window after the onset candidate was above the upstate threshold. The frequency of the detected DOWN-UP transitions was 0.45 ± 0.14 Hz (mean ± S.D.). To compare the magnitude of DOWN-UP modulation between cell groups, we computed modulation index for each neuron.

$$Modulation\ Index = \frac{NspikesUP - NspikesDOWN}{NspikesUP + NspikesDOWN}$$

where NspikesDOWN = number of spikes during –200~0 msec, and NspikesUP = number of spikes during 0~200 msec (time 0 = DOWN-UP transition). One-way ANOVA or t-test was used to test the significance.

Localization of neuronal somata in the CA1 pyramidal layer

For each silicon probe shank, one recording site was chosen first and used to detect ripple epochs as described above. LFP recorded from each recording site was band-pass filtered (140-230Hz) and the mean power (root mean square) during ripple epochs was calculated. The site with the largest power of ripples, reflecting the middle of the pyramidal layer[28], was determined for each shank for each session and served as the reference depth. The site with the largest average spike amplitude for each unit was regarded as the approximate location of the cell body[26]. The error of cell body assigment was 20 μm, due to the limitation of the vertical distance between recording sites.

Theta and gamma phase modulation

Three methods were used to determine the phase of band-pass filtered theta (5-11 Hz) or gamma (30-80 Hz) waves y(t)[27]. (1) Instantaneous phase was derived from Hilbert transform of y(t). (2) Peaks (0^o) of filtered waves were identified as the positive to negative zero crossings of the derivative dy/dt, and phase was linearly interpolated between the peaks. (3) Troughs (180^o) of filtered waves were identified as the negative to positive zero crossings of the derivative dy/dt, and phase was linearly interpolated between the troughs. A phase value was assigned to each action potential using linear interpolation. Peaks are at 0, 360^o and troughs at 180^o throughout the paper. The results obtained by the three methods were consistent and the peak method was used for both theta and gamma phases. The mean direction and mean resultant length of the phases of a given neuron's spikes were taken as the preferred phase and modulation depth of that neuron, respectively. For the circular statistics of theta phase of single cells, only neurons with at least 50 spikes during theta epochs (RUN or REM) were used, and $P < 0.01$(Rayleigh test) was used to define significantly theta modulated neurons. For gamma modulation of single cells, only neurons with at least 20 spikes during gamma oscillatory periods in relevant brain state (RUN, REM, SWS or awake non-theta) were used and $P < 0.05$ (Rayleigh test) was used to define significantly gamma modulated neurons[20]. To avoid gamma phase variability as a function of depth[45], the recording site with the largest ripple power (i.e., the middle of the CA1 pyramidal layer) for each probe shank was used for detecting gamma phase.

Interspike interval analysis

Burst index was defined as the ratio of bursting spikes to all spikes. A bursting spike was defined as a spike associated with ISI at least either before or after that spike smaller than 6 msec. To compute the circular statistics of theta phase at different ISIs, we first sorted the spikes of a neuron according to their ISIs. A spike associated with an ISI either before or after that spike less than 6, 8, 10, 15 msec was sorted into < 6 msec, < 8 msec, < 10 msec or < 15 msec ISI groups, with allowing less stringent groups to redundantly contain spikes in more stringent groups. Therefore, the < 15 msec ISI group contains the groups of < 6 msec, < 8 msec and < 10 msec. A spike associated with ISIs both before and after that spike larger than 20, 30, 50 or 100 msec was sorted into > 20 msec, > 30 msec, > 50 msec or > 100 msec ISI groups. Therefore, the > 20 msec ISI group contains the > 30 msec, > 50 msec and > 100 msec groups. The preferred phase, modulation depth and p-value of Rayleigh test were calculated for each ISI group of each neuron. If the number of spikes from a given cell in a given ISI group was greater than 50 and associated p-

value was less than 0.01, the cell was regarded as significantly theta modulated in that ISI group.

Spatial tuning of spiking activity

The data recorded on the open field (180 cm x 180 cm) and linear track (250 cm) were used for the analysis of spatial tuning of spiking activity. Only the data during theta epochs were used. Position of the animal was estimated by recording LEDs on the head stage at 30 Hz. For the linear track, the positions were projected onto the track axis. The position and spiking data were sorted into 5 cm x 5 cm (open field) or 5 cm (linear track) bins, generating the raw maps of spike number and occupancy. For the linear track, spatial representation (rate map, number of place fields, spatial information[39], spatial coherence[37], stability[41] and phase precession[42]) was analyzed for each direction separately. A raw rate map was constructed by dividing a raw spike map by a raw occupancy map, and used to compute spatial coherence[37]. Peak firing rate, number of place fields, stability[41] and spatial information[39] were computed from the smoothed rate map. To construct smoothed rate map for open field, an adaptive smoothing[18,40] was used. The firing rate at each bin was estimated by expanding a circle around the bin until

$$Nspikes > \frac{\alpha}{Nocc^2 r^2}$$

where Nocc is the occupancy time (second) within the circle, Nspikes is the number of spikes emitted within the circle, r is the radius of the circle in bins and α is a scaling parameter, set at 40,000. The firing rate at that bin was then set to

$$\frac{Nspikes}{Nocc}$$

For linear track, Gaussian kernel (S.D. = 5 cm) was applied for both raw maps of spike and occupancy, then smoothed rate map was constructed by dividing the smoothed spike map by the smoothed occupancy map. Area at 0-25 cm (starting point) was excluded from the analysis to exclude the effect of behavioral variability. A place field was defined as a contiguous region of at least 225 cm^2 (9 bins) for the open filed and 15 cm (3 bins) for the linear track where the firing rate was above 10 % of the peak rate in the maze and the peak firing rate of the area was > 2 Hz. Using a threshold of 20 % of the peak rate gave similar results (not shown). The results using rate threshold of 0.5, 1, 2, 4 Hz for the peak firing rate of place field were essentially similar and shown in **Supplementary Figure 8**. Place map stability[41] was defined by the bin-by-bin correlation coefficient between the firing rate maps of the first and second half of the recording session. Theta phase-position correlation and mean theta phase in place field were calculated as described previously[27,38].