Online Methods

Subjects

All procedures were approved by the Institutional Animal Care and Use Committee at Stanford University School of Medicine. Mice were generated from a hybrid 50:50% C57BL/6J:129SVEV background (donated by Eric Kandel and Steve Siegelbaum), as described previously[51]. Briefly, loxP sites were introduced flanking the pore and S6 encoding exon of the HCN1 allele. For our experiments, restricted knockout of HCN1 was obtained by viral injection of Cre expressing AAV. Mice were bred and housed in transparent cages. Implanted mice were single housed and allowed free access to food and water for 1 week after surgery, after which they were mildly food deprived. Mice used in behavioral experiments were group housed with littermates. Mice used in *in vivo* tetrode recordings were housed on a 12-hour light-dark cycle and experiments were performed during the light phase. Mice used in behavior experiments were housed on a reverse 12-hour light-dark cycle and experiments were performed during the dark phase. Male (n = 29) and female (n = 25) mice were used for *in vivo* tetrode recordings, whereas only males were used in behavior. Adult mice (3 – 10 months) were used in all *in vivo* experiments. The experimenter was blind to the group (iWT or iCre-KO) of the mice for behavior but not for electrophysiology experiments.

In vitro whole cell patch clamp recordings

Young (p12 – p15) male floxed HCN1 knockout mice (iCre-KO) and their wildtype littermates (iWT) were slowly injected (infusion rate of 100 nl/min) with Cre-expressing AAV (AAV-DJ CMV cre-GFP; 250-400 nl injections; infection titration of 1.5e10 IU/ml) in the medial entorhinal cortex (MEC) at four sites (lateral site: medial-lateral [ML] = -3.0, anterior-posterior [AP] = 0.4, dorsal-ventral [DV] = -2.2 and -1.6 mm; medial site: ML = -2.4, AP = 0.4, DV = -2.8 and -2.0 mm). Vector plasmids contained nuclear GFP for later visual identification of infected cells. Mice recovered for a minimum of 7 days to allow for virus expression before being sacrificed.

Whole-cell patch clamp recordings were then performed on mice (p17 – p25) previously injected with virus as described above. Mice were anesthetized with isoflurane and perfused with cold artificial cerebrospinal fluid (ACSF) oxygenated by bubbling 95% O2/5% CO2 through the ACSF. After perfusion, the mouse was rapidly decapitated, and the brain removed under cold (4°C), oxygenated ACSF (ACSF concentrations in mM: 126 NaCl, 26 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 2 $MgCl_2$, 2 $CaCl_2$, 10 Glucose). For slicing, the corpus callosum was cut and each half of the brain glued with the cut side down to allow slicing of sagittal MEC sections (300 µm thick). After slicing, brain slices were held in a chamber stored at 31 - 33°C for 30 minutes followed by room temperature for at least 30 minutes. Slices were then placed in a chamber continuously perfused with oxygenated ACSF (kept at 35.0 ± 0.1°) under an upright microscope. Cells were clearly visible with a 40X water-immersion objective lens, which allowed identification of the laminar and dorsal-ventral location of MEC cells. Putative layer II stellate neurons were identified by their superficial location and their morphology, with stellate cells showing several thick branching dendrites[52]. Infected cells identified by bright GFP expression were selected for subsequent patch clamp recordings in both iCre-KO and iWT mice (Supplementary Fig. 5). The dorsal-ventral location of each recorded neuron was noted by the pipette location and a photograph taken. The border between MEC and other cortical layers and the ventral MEC end were identified based on DIC images and referenced to the Allen Brain Mouse Atlas[53]. Pixel measurements in Photoshop were then used to quantify the percent dorsal-ventral distance for each recorded neuron. Importantly, the distributions of stellate cell recording location across the DV axis did not differ between iWT and iCre-KO mice (% DV axis ± SD: iWT = 56.2 ± 21.9%, iCre-KO = 53.2 ± 28.6%, KS test, D = 0.31, p = 0.49).

For whole cell recordings, patch pipettes (4 – 6 MΩ) were pulled from 10 cm borosilicate glass capillary tubes and filled with (in mM): 120 K Gluconate, 10 HEPES, 0.2 EGTA, 20 KCl, 2 MgCl2, 7 Phosphocreatine Di-tris, 4 Na-ATP, 0.15 biocytin and 0.3 Tris-GTP. Tight seals (> 1 GΩ) were formed between the recording pipette and cell membrane, then ruptured with negative

mouth pressure. Recordings were amplified by a Multiclamp 700B sampling at 20 kHz and digitized by a Digidata 1440. The liquid junction potential was not corrected. Bridge and electrode capacitance compensations were applied. All cells were held for ≥ 5 minutes, had an input resistance of ≥ 23 MΩ, an action potential height of ≥ 60 mV from threshold to peak and a resting potential of ≤ - 58 mV. After break-in, cells stabilized for one minute during which a seal test was conducted.

To examine the sag potential, current was applied to hold the cell at -70 mV (measured at injection onset, mean ± SD; iWT = 69.2 ± 1.9 mV, iCre-KO = 69.3 ± 1.7 mV) and then 1 second current injections given. Traces which had a trough between -90 and -100 mV were analyzed. The amplitude of the sag was measured in Clampfit and quantified by dividing the trough deflection from baseline by the steady state deflection from baseline.

In vivo survival surgery

Anesthesia was induced by an injection of buprenorphrine (0.1 mg/kg) and maintained with isoflurane (0.5 – 3%). Anesthetized mice first underwent injections of Cre-expressing AAV using a 1 µL Hamilton syringe. Bilateral 750 nl injections (infection titration of 1.5e10 IU/ml) were made 0.4 mm anterior to the transverse sinus at multiple ML positions and DV depths (ML = 2.8, DV = -3.0 and -2.2 mm; ML = 3.4, DV = -2.4 and -1.6 mm). Virus was delivered at a slow rate (100 nl/min) and the injection needle left in place for 5 min after the end of the infusion. For *in vivo* recordings, mice were then unilaterally implanted with a 2 (MEC) or 4 (hippocampus) tetrode Microdrive connected to 17µm polyimide-coated platinum–iridium (90%-10%) wire tetrodes (plated to impedances of 150 to 250 kΩ at 1 kHz). For MEC recordings, tetrodes were implanted at 0.4–0.5 mm AP from the transverse sinus, 3.25–3.45 mm ML, and 0.8–1.0 mm below the dura, angled 0–4 degrees in the posterior direction in the sagittal plane. For hippocampal recordings, tetrodes were implanted at -1.8 mm AP from bregma, 1.8 mm ML, and 0.6–0.8 mm below the dura. Small screws and dental cement were used to affix the microdive to the animal's skull.

Beginning 3 days after implantation, mice were habituated to the training arena (See *In vivo single-unit data collection)*. *In vivo* recordings began two weeks after surgery to allow time for the virus to express.

In vivo single-unit data collection

For all recording sessions, mice were connected to the equipment via AC-coupled unity-gain operational amplifiers attached to a counterbalanced cable that allowed free movement through the environment. Recorded signals were amplified 8,000 to 25,000 times and bandpass filtered between 0.8 and 6.7 kHz. Triggered spikes were stored to a disk at 48 kHz (50 samples per waveform, 8 bits/sample) with a 32 bit time stamp (clock rate at 96 kHz). EEG was recorded from one of the electrodes and amplified 3,000-10,000 times, lowpass filtered at 500 Hz, sampled at 4,800 Hz, and stored with the unit data. To track the mouse's position, two light-emitting diodes, one small and one large, were attached to the head stage (sampling rate 50 Hz) and detected by an overhead camera.

After all data collection for a given set of cells was complete, tetrodes were moved ventral by 25 μm. For comparison of cells across multiple testing days, tetrodes were not moved between days. For these comparisons, only cell pairs in which the waveforms on each day appeared identical were considered. After close manual inspection of each cell pair, we computed the normalized center of mass shift in the spike clusters, and excluded cell pairs for which this value exceeded two standard deviations above the mean.

Data collection in 2D open field arenas

Data was collected during 20-60 min sessions, while mice foraged for scattered food (crumbled chocolate cereal) in one of several 50 cm tall square arenas (box width and length; MEC recordings: 70 cm, 90 cm or 100 cm, Hippocampal recordings: 50 or 62 cm). Box sizes were selected on a per animal basis in order to maximize coverage. Mice implanted in the MEC, with

two-tetrode drives, had an average running speed of 12.72 ± 2.49 cm/s (mean ± SD), and there was no difference between iWT and iCre-KO mice ($t(27) = 0.067$, $p = 0.95$, unpaired t-test). Mice implanted in the hippocampus, with four-tetrode drives, had an average running speed of 8.20 ± 0.98 cm/s (mean ± SD), and there was no difference between iWT and iCre-KO mice ($t(17) = 0.031$, $p = 0.98$, unpaired t-test). The animal was habituated to the testing arena ≥ two weeks before data collection began. Black curtains surrounded the black recording boxes, with a white cue located midway between the corners of one wall. Mice were exposed to the open field not more than twice a day, with sessions separated by at least three hours, and rested in their home cage between testing. The test box was cleaned with soapy water followed by odor remover (Nature's Miracle) between each trial.

Histology and reconstruction of recording positions

After the final recording session, electrodes were not moved. In 22 MEC mice and in all hippocampus mice, small electrolytic lesions were made to mark the end of the tetrode track by passing 20 μA current for 16 s on two channels of each tetrode. In the 7 MEC mice that did not receive lesions, tetrode tracks were used to identify the recording site. Mice were then killed with an overdose of pentobarbital and transcardially perfused with 1X phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were extracted and stored overnight in 4% paraformaldehyde before being transferred into 30% sucrose solution. For slicing, brains were rapidly frozen, cut into 40 μm sagittal sections with a cryostat, mounted and stained. To determine tetrode locations, every other slice was stained for Cresyl Violet, with the positions of the tips of the recording electrodes determined from digital pictures of the brain sections. In the case of electrolytic lesions, the tetrode location was marked as the center of the lesion. The border between MEC and other cortical regions was determined from post-hoc Nissl stained sagittal brain sections and based on the reference Allen Brain Atlas and The Mouse Brain in Stereotaxic Coordinates[54]. Dorsal-ventral measurements were made using Axiovision and the laminar

location of recordings in MEC determined based on cytoarchitecture criteria.

Immunohistochemistry

A subset of brains were stained for NeuN to visualize neuronal nuclei. After intracardial perfusion with 4% paraformaldehyde in PBS, brains were post-fixed overnight and transferred to 30% sucrose in PBS. After the brains sank, sagittal slices (40 µm) were prepared using a cryostat and mounted onto glass slides. Slices were washed in PBS (3 × 10-min each) and then blocked for one hour (10% Normal Goat Serum, 0.1% BSA, 0.05% Triton X-100 in PBS). Primary antibody was dissolved in carrier solution (1% Normal Goat Serum, 0.1% BSA, 0.05% Triton X-100 in PBS) and applied overnight (rabbit anti-NeuN, diluted 1:1,000; Millipore ABN78). Slices were then washed in PBS (3 × 10-min each) and incubated for two hours in secondary antibody solution (Alexa Fluor 568 goat anti-rabbit; 1:500 dilution; Invitrogen A-11011). Sections were washed in PBS three more times before the slides were cover slipped with Vectashield with DAPI.

Virus infection and flat maps

Virus efficiency near the injection site was quantified in 24 regions of interest across 12 slices from 6 mice. Images of slices stained for NeuN were captured at 20× using a fluorescent microscope (Nikon Eclipse E800M). Image J software was used to calculate the percentage of NeuN-expressing cells infected with GFP.

To quantify the percentage of MEC infected, we defined a region as virus-infected if the mean fluorescent intensity was ≥ 2 standard deviations above the mean value of the background signal, using Image-Pro Plus software. The length of the infected area was measured and the percentage of MEC infected calculated for each slice. Note this results in a conservative estimate, as layers containing few cells (I and IV) are included in the calculation of MEC area. The percentage of total MEC infected by our injection coordinates was quantified by examining GFP

expression in 9 mice (one hemisphere each, Fig. 1b). All implanted and behavior-performing mice included were also examined for MEC GFP expression (see below).

For inclusion in the study, MEC-implanted mice were required to have infection of ≥ 20% of MEC in slices near the recording region (± 160 µm from the slice containing the tetrode track; range: 21–60%, mean ± SD: 41 ± 11%) (Supplementary Fig. 1). For inclusion of hippocampus-implanted and behavior-performing mice, ≥ 20% of total MEC had to be significantly infected (Hippocampus mice: range, 22–76%, mean ± SD, 47 ± 16%; Behavior mice: range = 21–89%, mean ± SD, 59 ± 24%) (Supplementary Fig. 2-3). GFP expression was largely restricted to the MEC. However, if GFP was detected in hippocampus, the area of infection was quantified in Image Pro Plus using the same procedure as described above. The percent area of CA1 or dentate gyrus infected was subsequently calculated (for details see Supplementary Figs. 1-2). Because HCN1 reduction in the hippocampus has been linked to enhanced spatial navigation[55], behavior mice with virus leakage into the hippocampus were excluded from all analyses (n = 4 iWT, 5 iCre-KO mice). The amount of virus expressed in the LEC was quantified from sagittal sections containing MEC in four animals, using Image Pro Plus. Boundaries of LEC were determined from cytoarchitectonic criteria[56] and the Mouse Brain atlas[54]. The percentage of LEC with detectable virus expression was 5.0 ± 3.5% (mean ± SD) (Supplementary Fig. 4).

Position estimates in 2D open field arenas

For recordings in open arenas, data was speed filtered and only epochs of instantaneous running speeds ≥ 2.5 cm/s included. Spike sorting was performed offline using graphical cluster-cutting software (TINT, Axona). To identify cells, clustering was performed manually in two-dimensional projections of the multidimensional parameter space, using autocorrelation and cross-correlation tools as additional separation tools. Cluster separation was assessed by calculating distances, in Maholonobis space, between clustered spikes from different cells on the same tetrode[57]. The cluster quality of cells recorded in either MEC or hippocampus was

comparable between iWT and iCre-KO (MEC: n = 527 iWT cells, 600 iCre-KO cells, median Mahalonobis distance, iWT = 14.03, iCre-KO = 15.65, WRS Z = -1.50, p = 0.10; Hippocampus: n = 323 iWT cells, 303 iCre-KO cells, median Mahalonobis distance, iWT = 40.31, iCre-KO = 55.66, Z = -1.72, p = 0.086) (Supplementary Fig. 13). Putative excitatory cells were separated from putative interneurons using a combination of spike width and average firing rate. Only cells with ≥ 100 spikes and sessions where the animal covered ≥ 70% of the environment were used in analyses. Position data was sorted into 2.5 × 2.5 cm bins and smoothed with a 21 sample boxcar window filter (400 ms, ten samples on each side). Time and spiking maps were smoothed separately using a quasi-Gaussian kernel over the adjacent 5 × 5 bins[58]. Firing rates were calculated from smoothed maps by dividing spike number by the time spent in each bin. The rate in the bin with the highest rate was defined as the peak rate. An adaptive smoothing method was used before the calculation of grid and border scores[58, 59].

Cell-type classification

To classify grid cells, we generated a grid score by taking a circular sample of the autocorrelation centered on the central peak and compared it to rotated versions of the same circular sample (60 and 120 versus 30°, 90° and 150°)[58]. Grid field size was calculated from the radium of an inner circle fit around the center autocorrelation peak. Grid spacing was defined as the mean spacing between the central autocorrelation peak and the nearest six peaks. As the grid spacing measurement is sensitive to noise in the autocorrelation, spacing measurements from cells in which the standard deviation of the three grid spacing measurements fell above 2 standard deviations of the mean were excluded (WT n = 3 cells; iCre-KO n = 4 cells). Because grid stability correlates with grid spacing (r(97) = 0.45, p = 3.2e-6, Pearson's correlation), we compared stability in grid cells while controlling for scale. For grid cells, as well as all functionally-defined neurons, within-trial stability was calculated by generating smoothed rate maps from even

and odd time bins and quantifying the correlation in rates of firing between the two rate maps[60].

Border cells were identified using a border score[61]. Border cell firing fields were detected from the smoothed rate map as ≥ ten connected bins with a firing rate in 75th percentile of firing rates for that rate map[62]. The edge of the firing field was determined by finding the bins that neighbored non-field bins and the mean distance of these edge bins from the nearest boundary defined as the width of the border cell.

To define head direction cells, we plotted the firing rate of a neuron relative to the mouse's directional heading, divided into 0.5 degree bins and smoothed with a 14.5 degree mean window filter. Peak firing rates were determined as the bin with the highest rate in the polar firing rate map. The strength of directional tuning was calculated from the length of the mean vector for the circular distribution of firing rate. Tuning width was defined as the width (degrees) of the tuning curve of preferred orientation at half of the peak firing rate.

To define speed cells[63], we calculated the instantaneous firing rate and running speed for 20 ms time bins, which were smoothed using the smoothing procedure in Kropff et al., 2015. We then computed the speed score as the Pearson correlation coefficient between firing rate and running speed for bins with a running speed < 2 cm/s. To further restrict our analyses to cells with a stable firing rate-running speed relationship, we computed a speed score stability metric. To compute this metric, we generated a firing rate-running speed tuning curve for each quarter of a recording session[63]. Running speed was binned into 2 cm/s wide bins, starting at 5 cm/s and ending at 50 cm/s, and the mean firing rate for each bin was calculated. We then determined the Pearson correlation coefficient between each tuning curve (6 comparisons in total) and took the average correlation coefficient as the stability score. Speed cells were then defined as neurons with speed and speed stability scores above the 95th percentile of shuffled data.

To define place cells, we first identified putative excitatory pyramidal neurons based on a waveform analysis (longest waveform peak to trough > 0.20 ms). Place cells were then defined as neurons with a spatial information value exceeding the 95th percentile of the shuffled

distribution, with greater than 100 spikes and a peak firing rate of at least 0.1 Hz. Spatial information content in bits per spike was calculated as: Spatial information = $\sum_i p_i \frac{\lambda_i}{\lambda} log_2 \frac{\lambda_i}{\lambda}$ Where *i* is the bin number, λ_i is the mean firing rate of a unit in the *i*-th bin, λ is the mean firing rate and p_i is the probability of the animal being in the *i*-th bin (occupancy in the *i*-th bin/total recording time)[9]. If a place cell was recorded over multiple sessions, the session with the highest spatial information was used for all within-session analyses. Spatial selectively, a measure of the specificity of in-field versus out-of-field firing, was expressed as $\log_{10}(FR_{in\text{-}field}/FR_{out\text{-}field})$.

For the analysis of place cell field location relative to boundaries, we ensured that border and center fields were sampled evenly between groups. The proportion of fields in the center of the box did not differ between iWT and iCre-KO mice (binomial test, Z = 1.50, p = 0.13). In addition, the distribution of field distances did not differ between groups (KS test, *D* = 0.13, p = 0.43). Place field size was determined from binned firing rate maps, with place fields quantified as portions of the map with at least 5 contiguous bins (31.25 cm^2) that reached 20% of the peak firing rate.

Across-session place cell stability was defined as the Pearson correlation coefficient between firing rate maps for two consecutive sessions, separated by 24 hours, over which the cell was recorded. If the cell was recorded over more than two consecutive days, the two consecutive days with the highest average spatial information were used, unless otherwise stated. The shift in place maps across consecutive days was determined by first computing the cross-correlogram between z-scored firing rate maps from each day, and then finding the shift of the bin with the highest correlation from center. This method avoided the need to match individual fields across days, which becomes difficult when multiple place fields are detected in at least one of the recording sessions. Normalized changes in firing rate across days were expressed as |(rate day1 – firing rate day2)|/(rate day 1 + rate day 2).

Shuffling procedures for functionally-defined neurons

For classification of cell types, a shuffling procedure was performed[58, 64]. Shuffling was run separately for each genotype (WT, iCre-KO) and brain region (MEC and hippocampus). For each cell, the spikes were time shifted along the animal's recorded trajectory by a random interval > 20 s and < the total trial length minus 20 s, with the end of the trial wrapped to the beginning. This procedure was repeated 100 times, with a firing rate map and relevant score (grid score, border score, mean vector length, speed score, speed stability score, spatial information score, spatial coherence score, or stability score) computed for each permutation. Cells were classified as a particular functional cell type when their score exceeded the 95th percentile of scores generated in the shuffled data set. Neurons classified as speed cells had a speed score higher than the 95th percentile of the shuffled data, as described above, but also had a speed score stability metric higher than the 95th percentile of the shuffled data. Neurons were defined as stable spatial cells if their stability and coherence scores were higher than the 95th percentile of the shuffled data, and they were not classified as grid or border cells (Supplementary Fig. 6).

LN model framework

Full details of the model framework used here are described in Hardcastle et al. 2017 and custom MATLAB code generated to fit the series of linear-nonlinear-Poisson (LN) models can be found at: https://github.com/GiocomoLab/ln-model-of-mec-neurons. Briefly, these models quantify the dependence of spiking on one or more variables (e.g. position, head direction or speed) by estimating the spike rate (r_t) of a neuron during time bin t as an exponential function of the sum of variable values (e.g. the animal's position at time bin t, indicated through an 'animal-state' vector) projected onto a corresponding set of parameters. Mathematically, this is expressed as:

$$r = \exp\left(\sum_i X_i^T w_i\right)/dt$$

where r is a vector of firing rates for one neuron over T time points, i indexes the variable ($i \in$

$[P, H, S]$), X_i is a matrix where each column is an animal-state vector x_i for variable i at one time bin, w_i is a column vector of learned parameters that converts animal state vectors into a firing rate contribution, and dt is the time bin length (20 ms).

Each animal-state vector denotes a binned variable value. All elements of this vector are 0, except for a single 1 element that corresponds to the bin of the current animal state. To learn the variable parameters w_i, we use MATLAB's fminunc function to maximize the Poisson log-likelihood of the observed spike train (n) given the model spike number ($r * dt$) and under the prior knowledge that the parameters should be smooth. Model performance for each cell is computed as the increase in log-likelihood of the model compared to the log-likelihood of a flat-line mean firing rate model. Performance is quantified through 10-fold cross-validation, where each fold is a random selection of 10% of the data.

To identify which variable, or set of variables, a neuron encodes, we used a heuristic forward-search algorithm that determines whether adding variables significantly improves model performance ($p < 0.05$ for a one-sided signed rank test, Hardcastle et al., 2017). For the purposes of this study, we considered three navigational variables (position, head direction and speed) rather than the four variables (position, head direction, speed and theta) considered in Hardcastle et al., 2017.

For cells that significantly encoded S, we calculated two metrics: curvature and speed-modulation. The curvature of each speed tuning curve was quantified as the sum of the squared second-order derivative of the smoothed speed-firing rate tuning curve (smoothed with a 5-bin Gaussian boxcar filter). The speed-modulation was computed as the range divided by the mean of the speed tuning curve.

Information metrics

Angular and speed information were computed following spatial information: $\sum_i p_i \frac{\lambda_i}{\lambda} log_2 \frac{\lambda_i}{\lambda}$,

where *i* is the bin number, λ_i is the mean firing rate of a unit in the *i*-th bin of either the smoothed head direction or speed tuning curve, λ is the mean firing rate of that bin, and p_i is the probability of the animal being in the *i*-th bin (occupancy in the *i*-th bin/total recording time). Speed was divided into 25 bins and smoothed with a 5-bin Gaussian boxcar filter, while head direction was divided into 180 bins and was adaptively smoothed.

Theta rhythm, theta modulation of running speed, and gamma rhythm

The degree of theta modulation for individual neurons was determined from the fast Fourier transform-based power spectrum of the spike train autocorrelation functions of the cells. Theta power in the unfiltered LFP was quantified as the ratio between power in the theta band (4-12 Hz) and all other frequencies between 0 and 125 Hz. Slow (25-50 Hz) and fast (65-125 Hz) gamma power were computed by calculating for each the power in the LFP for the given band compared to the power across all other frequencies between 0 and 125 Hz. We quantified modulation of theta frequency by running speed as follows. EEG was filtered for theta offline, as previous described[58] (3 – 5 Hz low passband and stopband and 11 – 12 Hz passband and stopband frequencies). The peaks of the filtered theta signal were detected and the frequency determined on a cycle by cycle basis. Instantaneous running speed was calculated for 20 ms time bins from position samples. The data was then pooled for portions of the trial corresponding to speeds between 2.5 cm/s and 50 cm/s and the relationship between theta frequency and speed reported as the slope of a regression line fit to the data. The mean theta frequency for the session was reported as the Y intercept of this linear fit. Statistics were carried out using each mouse as a statistical unit. For each analysis, we considered only the session with the highest theta power, intercept, slope, or gamma power.

Linear track recordings and analysis of phase precession and sharp wave ripples

To assess theta phase precession, a separate group of CA1-implanted iWT and iCre-KO mice were trained to both explore the standard 2D arena and to run back and forth on a linear track. The linear track, constructed of black-painted wood, was 100 × 7 cm (L × W), elevated 23 cm above the floor, and surrounded by black walls (31 cm). A white polarizing cue was placed at one end of the track. The track was placed in the same arena used for open field recordings. Mice were first encouraged to explore the track by randomly scattering food rewards along length of the track. Over the course of 2–3 weeks, rewards were gradually moved outwards towards the ends of the track. Place cell recordings began once mice consistently ran back and forth with little hesitation. Sessions lasted between 15 and 30 min. Leftward and rightward runs were analyzed separately, and individual fields were treated as separate statistical units.

Place cells were first identified in the open field, and then examined on the linear track for theta phase precession. If the same cell was recorded on multiple linear track sessions, the session with the highest theta power was analyzed. Fields on the linear track were defined as follows: first, the peak bin rate in a field was required to be at least 1 Hz and exceed 20% of the peak rate for that cell (bins = 2.5 cm). The field was then extended in either direction as long as the firing rate remained above 20% of the field's peak rate. Field size was defined as the distance between the two field boundaries. As an additional measure of field size, population vector cross-correlation matrices were constructed from all place cells in the iWT and iCre-KO groups. Population vectors were defined for each 2.5-cm bin of the rate maps spanning the analyzed portion of the linear track (central 70 cm, 28 bins). Leftward and rightward rate maps were constructed separately for each cell, and the peak firing rate in each map was required to reach at least 1 Hz for inclusion in the population vector analysis. To maintain independence between neighboring bins, firing rates were not smoothed. Field size was estimated from the cross correlation matrix of the population vectors by determining the average number of consecutive bins flanking the diagonal with correlations exceeding 0.2. Linear track analyses were restricted

to fields whose boundaries fell within the central 70 cm of the track, where the mice ran consistently without stopping, and at high speeds (see Supplementary Figure 11). Segments over which the animal ran below 3 cm/s were omitted. Fields in which less than 30 spikes were emitted were excluded from analysis of theta phase precession.

EEG was filtered offline. An acausal (zero phase shifted), frequency domain, finite impulse response bandpass filter was applied to the signals. For theta filtering, 4 Hz and 5 Hz were chosen for the stopband and passband, respectively, for the low cutoff frequencies; 10 Hz and 11 Hz were chosen for the passband and stopband high cutoff frequencies. The peak of the EEG was defined as 0/360° and the trough defined as 0°. Spikes between the peak and tough were interpolated linearly so that a spike at time t was 360 X (*t*-*t*0)/(*t*1 – *t*0), with t0 indicating the time of the preceding point and *t*1 indicating the time of the succeeding point.

For analysis of theta phase precession, field size was first normalized to extend from 0 to 1, and the relationship between distance through the field and the theta phase of spikes emitted on all passes through the field was tested using linear-circular correlations. The correlation coefficient ρ_c is given by: $\rho_c = \frac{\sum_{j=1}^{n} \sin(\phi_j - \bar{\phi}) \sin(\theta_j - \bar{\theta})}{\sqrt{\sum_{j=1}^{n} (\sin(\phi_j - \bar{\phi}))^2 \, (\sin(\theta_j - \bar{\theta}))^2}}$, where n is the number of spikes, ϕ_j is the phase angle of the j^{th} spike, $\bar{\phi}$ is the circular sample mean given by $\bar{\phi} = arctan \, \frac{\sin(\phi_j)}{\cos(\phi_j)}$, $\bar{\theta} = arctan \, \frac{\sin(\theta_j)}{\cos(\theta_j)}$, and $\theta_j = 2\pi|\hat{a}|x_j (\mathrm{mod}\, 2\pi)$ converts the animal's position x_j to a circular variable θ_j through a slope $\hat{a}$ that is computed from the data. The slope $\hat{a}$, which is in units of cycles per normalized field size, is computed by fitting a linear model of the form $\widetilde{\phi_j} = 2\pi a x_j + \phi_0$. Here, ϕ_0 is the phase-offset, or intercept, of the linear function, and $\widetilde{\phi_j}$ is the estimate of the function. Both a and ϕ_0 can be computed by minimizing the distance metric $d(\phi_j, \widetilde{\phi_j}) = 2(1 - \cos(\phi_j - \widetilde{\phi_j}))$.

Place-phase firing rate maps were constructed as follows: for each field, normalized position was divided into 20 bins, and theta cycles were divided into 36 bins. Instantaneous firing rate was smoothed with a Gaussian kernel with a standard deviation of 0.5 s, and the average

instantaneous firing rate in each bin of the position-phase map computed. Mean place-phase firing rate maps were then constructed for iWT and iCre-KO mice by averaging each individual map and smoothing using a 2-D Gaussian kernel with a standard deviation of 3 bins. To determine whether the difference between groups in any position-phase bin exceeded that expected by chance, we performed the following shuffling procedure: for each bin of each individual map, the group assignment was randomly assigned. Next, an average position-phase firing rate map was constructed for the two groups, and the difference between groups at each bin computed. This procedure was performed 500 times to establish a 95% confidence interval for the group difference at each bin. The observed difference between groups at a given bin would be considered significant if it fell outside of the 95% confidence interval at that bin.

Linear track sessions during which at least one place cell was recorded were examined for sharp wave ripple (SWR) events. For each session, the raw EEG signal (sampled at 4800 Hz) recorded on one electrode was denoised using a 60 Hz notch filter and then filtered at ripple frequency range (100-240 Hz) using a fifth-order Butterworth band-pass fitler[65]. The envelope of the signal, obtained from the absolute value of the Hilbert transform of the band-passed EEG, was smoothed with a Gaussian kernel (SD = 5 ms) and then z-scored. Ripples were defined as events that exceeded 5 SD above the mean of non z-scored envelope for longer than 3 ms, and ripples separated by less than 20 ms were merged together. The beginning and end of each ripple were defined as the time points on either side of the ripple at which the signal envelope crossed its mean. Mice were considered to be immobilized when their head speed was less than 0.5 cm/s, and only ripples that occurred during periods of immobility were analyzed.

Winner-Take-All Model of Place Cells

General Winner-Take-All Framework: To simulate place cell remapping, we implemented a winner-take-all model of place cells. In this model, place cells receive input from grid cells and cells with large-scale spatial input that varies between sessions (i.e. “unstable spatial inputs”).

Grid cell rate maps were generated using a combination of three constructively interfering sine waves oriented 60 degrees apart from each other. The orientation of the first sine wave was fixed at 7 degrees[66, 67], and the phase was randomly chosen such that all grid cells of a single spacing uniformly tiled the entire arena. The location of intersection of all three sine waves marked the center of each grid field. For a given grid cell, a pre-determined peak firing rate and variance was then selected and used as parameters for the 2-dimensional Gaussian fields placed at each grid location. The peak firing rate was randomly selected from a distribution of firing rates generated from experimentally recorded iWT and iCre-KO grid cells with peak firing rates < 10 Hz. The variance was determined from field size, which was computed based on the best linear fit to the field size and grid spacing data of a large WT dataset (see section below on 'determining the linear fits for grid field size versus spacing'). In other words, for each grid scale, we computed the field size of each field; we then used this value to find the variance of the Gaussian that would return the appropriate field size if we used the field detection algorithm previously described. In each simulation, grid cells contained five different scales (modules), which scaled up by a factor of square root of two[70]. To investigate the effects of grid scale, we varied the spacing of the smallest module, g, by increments of 5, with the first simulation beginning at 25 cm; the ratio of grid spacing between modules was preserved such that increasing the scale of the smallest module also increased that of the subsequent four.

Modeling of unstable spatial inputs: "Unstable spatial inputs" inputs were modeled as large, 2-dimensional Gaussian fields that varied from day to day, and thus formed the direct mechanism of place cell re-mapping between days. The location of each unstable spatial input was randomly chosen according to a uniform distribution over a 100 cm x 100 cm area in which a 50 cm x 50 cm box is centered. The variance of each Gaussian distribution was chosen uniformly to be between l to l+2000 cm^2, where l is a model parameter. In separate analyses (see Supplementary Fig. 15), we tested the effects of two different scales of unstable spatial input inputs, where l = {1000, 3000}. All results presented in Fig. 4 used the smallest parameter for

unstable spatial input scale. The peak firing rate was drawn from the same distribution as the simulated grid cell population. We generated 3,000 grid cells per dataset (divided evenly among the 5 modules), and 5,000 random unstable spatial inputs total.

Simulations of place cells: Each place cell rate map was then modeled as a combination of m randomly chosen grid cells and p randomly chosen unstable spatial inputs, where m varies from 800 to 1200 in steps of 200, and p varies from 100 to 300 in steps of 100. The strength of each input to each place cell was drawn from a uniform distribution over [0,1] [71, 72]. Following Lyttle et al. (2013) [71], we then implemented a winner-take-all mechanism, in which the firing rate F for every cell i at every spatial bin r was computed as:

$$F_i(r) = [I_i(r) - (1 - E) * I_{max}(r)]_+$$

where $I_i(r)$ is the summed grid and unstable spatial input to that place cell at location r, $I_{max}(r)$ is the maximum summed grid and unstable spatial input at that location across all grid cells, and E = 0.1. If the value inside the brackets is less than 0, $F_i(r)$ = 0. Activity at every spatial bin, for every place cell, is then normalized by dividing by the maximum observed activity, across all place cells, at that bin. Using this framework, we generated two sets of place cell rate maps for every value of m, p, g, and l: day *N* place cells and day *N-1* place cells. The only difference between day *N* and day *N-1* place cell rate maps in each group was the location and size of the unstable spatial input fields. For each group of place cell rate maps, we identified place fields as contiguous regions larger than 100 cm^2 with a firing rate equal to or exceeding 20% of the maximum firing rate. All simulations were repeated 10 times (with re-generated grid rate maps, unstable spatial input rate-maps, and weights to place cells to enforce statistical independency) to ensure robustness of the results.

Determining the linear fits for grid field size versus spacing: The best linear fit to the grid spacing and grid field size was determined from large datasets of grid cells recordings. For simulations describing how grid scale impacts place stability, we selected grid field sizes

according to a linear fit between grid spacing and grid field sizes observed in a large dataset of widtype (WT) grid cells. For the simulation (Figure 4b) in which we determined how well the simulations quantitatively recapitulated the experimental results, we ran two separate simulations in which the spacing of the smallest grid cell module was set to either the median of the smallest grid spacings observed in iWT mice (27 cm) or iCre-KO mice (37 cm). In addition, for this latter simulation, we selected grid field sizes according to separate linear fits for grid spacing and field size observed in a large dataset of WT and a large dataset of HCN knockout grid cells. The use of these large datasets allowed us to calculate fits for the grid spacing/field size relationship across a larger portion of the dorsal-ventral axis (0 – 2000 microns) compared to the much smaller dorsal-ventral range of the current iWT and iCre-KO dataset. These datasets were composed of of 435 WT grid cells (which included both the current iWT data and grid cells from a database of 38 non-injected WT mice; 6 from Giocomo et al., 2011[64]; 6 from Eggink et al., 2014[68], 7 from Hardcastle et al., 2017[69]; 19 unpublished) and 275 KO grid cells (which included both the current iCre-KO data and grid cells from a database of 12 non-injected HCN knockout mice; 6 from Giocomo et al., 2011[64], 5 from Eggink et al., 2014[68], 1 unpublished). For the databases, all grid cells (grid score > 0.35) were recorded as animals randomly foraged in 90x90 or 100x100 cm open arenas, following identical methods to those used to record the current dataset in iWT and iCre-KO mice.

Maximum-likelihood Decoding

Decoding position within sessions: Rate maps for simulated iWT (siWT) and iCre-KO (siCre-KO) populations were constructed using 2-dimensional Gaussian functions, each with a randomly chosen center and a variance chosen to return the field size of a cell randomly selected from the experimentally recorded iWT or iCre-KO datasets. The peak of each Gaussian was identical and matched the average peak firing rate across the combined iWT and iCre-KO datasets. Once the rate maps were constructed, we simulated spike trains for the siWT and siCre-KO place cell populations. We selected the first 20 minutes of the position trajectory (the animal's

position over time) from the session with highest coverage, and used this trajectory in conjunction with each cell's rate map to generate an estimated average observed spike number ($r_t^i * dt$) at every time bin t along the trajectory for each cell i (dt = 20 ms). Poisson spikes (n_t^i) for each cell were then drawn for each time bin independently according to the mean firing rate in that bin.

To decode the animal's position P^t at each time point t, we found the animal position that maximized the summed log-likelihood of the observed simulated spikes from $t - L$ to t:

$$\widehat{P^{(t)}} = \text{argmax}_{P^t} \sum_{l=0}^{L} \sum_{i=1}^{N} \log \text{P}\left(n_{t-l}^i \middle| r_{t-l}^i * dt\right)$$

where N= 112 and L = 15 time bins (300 ms). Decoding error was then computed as the mean absolute difference between the estimated and actual position ($|\widehat{P^{(t)}} - P^{(t)}|$). We repeated this process (simulating spike trains and decoding position) 10 times. The number of cells in each simulated population was varied in steps between 20 and 1000).

Decoding using Gaussian fields, across sessions: Gaussian maps for Day *N* were first generated in the same manner as described above. Gaussian maps for Day *N-1* were then generated by shifting the center of each Gaussian field by a shift value randomly chosen from the experimentally recorded dataset, with the requirement that the field shifted to another location inside the environment. To decode the animal's position, we used these spike trains in conjunction with the average firing rate in each time bin derived from Day *N-1* maps.

Shuffling procedure to determine if the difference in position estimate error changes with simulation cell number: First, we computed the slope of the best-fit line to the average error difference (computed pair-wise across iterations) across the number of cells. We then compared this to a null distribution of slopes, which was generated by computing the slopes of error differences of 5,000 null data sets. Each null data set was generated by first pooling the errors for each population across number of cells (i.e. creating a set of 20 14-element vectors; 14 arises from the number of tested cell numbers), randomly assigning each vector to either the siWT or

siCre-KO population, and then computing slope of the difference in decoding error between the two populations.

Behavior methods

The same mice underwent watermaze and Y-maze tasks. Mice observed to be consistently floating instead of swimming were excluded from all analyses ("floaters;" n = 1 iWT, 1 iCre-KO). Watermaze tasks were conducted in a 178 cm diameter dark-colored tank filled with opaque water (22°C) by an experimenter blind to the group (iWT or iCre-KO). On the Morris water maze, mice underwent four trials separated by 60 min for 5 days. In each trial, the mouse was released in a pseudorandom location and navigated to a submerged (1 cm) circular platform (12 cm diameter). Mice were guided to the platform if they failed to find it by the trial's end (60 s). After 10 s mice were removed from the platform and placed in a dry cage. On day 6, the platform was removed and a 60 s probe trial conducted. Mice rested for two days before the DMP task. On the DMP, mice were given a series of 4 trials per day, separated by 10 min. At the start of each day, the platform (17 cm diameter) was moved to a new, pseudorandom location. For the cued trials, a white ball was erected from the submerged platform. The animal's swim paths and velocity were recorded with Ethovision (Noldus Information Technology). Perseveration was defined as the frequency of visits on the first trial of each day (when path lengths were comparable) to either the training platform, or the previous day's target platform.

The Y-maze task was conducted in an opaque apparatus containing three arms (arm A: 51.62 cm L × 32.26 cm H × 19.35 cm W; arms B and C: 38.71 cm L; arm angle = 120°). Mice were placed into the maze facing arm B. An entry was recorded when all legs of the mouse entered a new maze arm. The data was broken into triads of entries. Alternation entries, defined as sequences in which the mouse entered each of the three arms, and non-alternation entries were scored manually by an experimenter blind to the genotype. Animals were given one 5 min trial.

Statistical Analysis and Reproducibility

All statistical tests were two-sided unless otherwise stated. All experiments (with the following exception, described below) were performed once using the N stated in the figure legends; attempts at experimental replication were not attempted. Exception: during the review process, we successfully replicated one of our main results, which is that iCre-KO mice have larger scaled place cells. At the suggestion of the reviewers, we examined place cell scale on a linear track, in addition to the open arena. During this process we found that place scale was larger in iCre-KO mice on the linear track, but we also successfully replicated the main finding that place scale was increased in the open arena. Quantification of gradients using slope and Y intercept measures were based on a univariate analysis of covariance (ANCOVA) in SPSS. We first tested for an interaction between the main factors to determine whether the regression lines showed significantly different slopes. Next, to test for a significant difference in the Y intercept, we used the presence or absence of a significant slope difference to set up the ANCOVA model. If no significant difference in slope was detected, the ANCOVA was run without an interaction term, making the assumption of equal slopes between the two groups. If a significant difference in slope was detected, the analysis was run with the interaction term, making the assumption of unequal slopes between the two groups. Effect size is reported as η^2. For testing differences in distributions and means, non-parametric tests (KS tests and Wilcoxon rank sum tests) were used when normality assumptions were violated (as assessed by Shapiro-Wilk tests). For behavioral analyses ANOVA with repeated measures were performed in SPSS. Bootstrapping methods were used to determine whether the difference in place field size in boundary versus center regions was significant between iWT and iCre-KO mice. For each group, boundary and center-place fields were randomly selected with replacement, and the difference in average field size computed. This procedure was repeated 10,000 times. A t-test was then computed between the iWT and iCre-KO bootstrapped differences. For additional information regarding sample sizes, statistics, and

reproducibility, please see the accompanying Life Sciences Reporting Summary.

Data availability

Data are available from authors upon request.

Code availability

Code for the winner-take all model is available at https://github.com/GiocomoLab

Methods References

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