Supplemental information

Task-dependent mixed selectivity
in the subiculum

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Supplementary Figures and Tables
**Figure S1 Anatomical locations of recording electrodes. Related to Fig. 1**

Nissl-stained sections for every animal recorded in the study. Animal ID number listed on top of the Nissl section marks the beginning of sections from a new animal and so applies to all following sections. Filled red arrows indicate tetrode (TT) traces at the estimated recording location (usually at the end of the tetrode track) while empty red arrowheads indicate traces of tetrodes that are visible in this section but are above (or below) the locations on the track where the recordings were conducted. The TT number above each Nissl section indicates the identity of tetrodes with filled arrowheads, arranged from left to right on the section. Dashed yellow lines indicate borders between SUB and neighboring regions like retrosplenial cortex (RSC), presubiculum (preSUB) and faciola cinerata (FC). For recording locations in SUB that are dorsal to the FC (labeled with SUB*) or the border between CA1 and SUB, special care was given to include data only from neurons that could confidently be assigned to be 50 μm or more away from the border. Scale bar, 1mm.
Figure S2 Manual cluster cutting and spatial tasks. Related to Fig. 2

(A-C) Multidimensional cluster diagrams illustrating isolation of spikes from simultaneously recorded cells in SUB (the yellow cluster is the same cell as shown in the first, left panel of Figure S5C). Scatter plots showing relationship between peak-to-peak amplitudes of spikes recorded from electrodes of a tetrode (A). Each point represents one sampled signal. Clusters are likely to correspond to spikes originating from the same cell. Distinct clusters are assigned unique colors. Spike autocorrelograms (B) showing spike frequency as a function of interspike interval (ISI) for each cluster isolated in the scatterplot. Note absence of spikes at intervals less than 2 milliseconds (gray vertical line), as expected due to refractory period for spikes originating from the same cell (number of spikes with ISI < 2 ms is indicated for each unit). Interval scale is logarithmic. Waveforms on four channels (C) for the cells isolated in the scatterplot (means ± standard deviations; scale bars: 100 µV, 1 ms; colors as in the scatter plot).

(D-E) Layout of the spatial task (D) and open field (E). The spatial task (D) consists of step one (1) in which the animal moves towards the home well. In step two (2) it searches for a random well that is filled with chocolate oat milk before (3) returning back to the home well which gets filled always after the animal has consumed the random well. In the open field (E) the animal roams freely in the search of randomly spread cookie crumbs. It takes place in the exact same environment except that the floor of the box is covered such that the holes are not perceivable for the animal. All other distal and proximal cues stay constant.

(F) Latencies to find the home well (gray) and the random well (red) for eight different rats across all trials of all sessions. Note that for animals 20382, 22295 and 20630 the home well was in a different location in every session while for the other animals it was always in the center of the environment. For these three animals therefore, home run trials have a longer latency at the beginning than at the end of the sessions while for the other animals this latency
was low from the start of every session. (Abbreviations: ST, spatial task; OF, open field; SL, stationary landmark)
Figure S3 Quantification of neural tuning and behavior in the ST and the OF. Related to Fig. 3-5

(A) Rate maps of example cells from the CA1 (left column) and the SUB (right column) in the OF (left) and ST (right). White color indicates peak firing rate, indicated also at the bottom of each map, while black colors indicate zero Hz firing rate (color bar as in Figure 2).

(B) Correlations of tuning curves for position, head direction and speed between OF-OF, ST-ST and OF-ST for position (top), head direction (middle) and speed (bottom). Correlations were calculated for every neuron between all pairs of OF and ST sessions the neuron was recorded in. The average correlation of all neurons was then compared within and across session types. Position correlations within and across session types were significantly higher for
CA1 than for SUB. For both regions, correlations between same-task sessions were larger than between sessions of different task types (Welch’s test for OF-OF vs. OF-ST comparisons in CA1: $p = 1.4 \times 10^{-16}$ and SUB: $p = 6.3 \times 10^{-27}$; Welch’s test for ST-ST vs. ST-OF comparisons in CA1: $p = 1.02 \times 10^{-15}$ and SUB: $p = 8.71 \times 10^{-30}$).

Correlations for head direction tuning curves (middle) were significantly higher in SUB than in CA1 and dropped when same-task-correlations were compared to across-task-correlations (Welch’s test for comparing ST to OFST: $p = 3.8 \times 8$ and for comparing OF to OFST: $p = 1.1 \times 3$).

For speed tuning curves (bottom), correlations within and across task types were similar in CA1 and SUB except for a small but significant increase in correlation of SUB when correlating ST to ST sessions. The average of ST to ST correlations was significantly higher than OF to OF correlations ($p = 1.3e-8$) and ST-OF correlations ($p = 1.5e-11$). This might indicate that the speed tuning in ST is more stable in SUB when compared to other tasks and to CA1. In summary tuning curves in CA1 are more stable in terms of position, while in SUB stability is highest in terms of head direction and speed.

(C-D) Information content (D) and information rate (E) in OF and ST for position, head direction, speed ensemble firing and theta.

(E) Percentage of neurons selecting a 1, 2, 3, 4 or 5 covariates model in CA1 (red bars) and SUB (blue bars) during the OF (full bars) and the ST (empty bars) condition. When taking into account all the covariates, (P, H, S, T and E) a 5-covariate model was selected for a larger number of neurons in SUB than in CA1. For the majority of CA1 neurons, a 4-covariates model was selected in both the OF and the ST.

(F) Distributions of log-likelihoods (LLH) in CA1 (red violin plots) and SUB (blue violin plots) during the OF (full violin plots) and the ST (empty violin plots) condition. The mean and
the median of the LLH are comparable between CA1 and SUB neurons and between the neurons recorded in the OF and the ST. The GLM fitting process therefore performs similarly in all conditions.

(G) Model selection plot across all 32 possible models without collapsing models containing ensemble firing (E) and theta (T) with the models containing only the external covariates position (P), head direction (H) and speed (S). Separating the models also for E and T shows that the most complex model (PHS in Figure 4) which performed best for a majority of neurons in SUB, most often consists of the 5 covariate model including E and T. The model most often selected for CA1 neurons is the PHET model when the models are separated for E and T. Models missing E and T as covariates (like PHSE or PHST) were only rarely selected. Neurons that are completely independent of ensemble firing or theta were extremely rare (see groups P, H, S, PH, PS, HS and PHS).
Figure S4 Optimization of hyperparameters. Related to Fig. 4-5

(A-E) Model performance (LLH, see section ‘Learning’ in Methods) averaged across all 746 cells (325 from CA1 and 421 from SUB) for position (A), head direction (B), speed (C), ensemble firing (D) and theta phase (E). Single covariates GLMs are plotted as a function of the GLM hyperparameters: number of bins (# bins) and strength of the regularizer ($\gamma_C$). Given the maximum of the model performance, values for hyperparameters were optimized by minimizing the number of parameters without causing a significant decrease of model performance with respect to the maximum. For details, see section ‘Hyperparameters tuning’ in Methods. Blue colors indicate the lowest value and red colors indicate the largest value of the z-Axis (LLH). The bins of the surfaces are colored according to the lowest point on the z-axis that the bin touches. The range of the color-scale is normalized for every figure separately.
(F) Model selection on synthetic cells. Grey bars indicate the proportion of neurons where the model selection process selected correctly the model from which the firing rates of the cells had been generated. Coloured bars indicate the proportion of synthetic neurons that had been misclassified into a different model (colours indicate which model; see the legend). Note that the largest majority of misclassified cells stem from the group of cells that encoded speed. This might in part bee due to how the neurons had been generated since the correlation between speed and firing rate does not necessarily have to be positive or steep.
Figure S5 Comparison of proximal and distal SUB. Related to Fig. 5

Tetrode locations were divided along an approximate midline through the subiculum into a proximal and a distal group, located closer or further away from CA1, respectively (see dashed black lines in Figure S1).

(A) Mean firing rate (left) and peak firing rate (right) of neurons in CA1 (red), proximal SUB (light blue) and distal SUB (dark blue). The heights of the bars in the background and the black markers with error bars indicate the mean and S.E.M. of the respective population. While
peak firing rates show only small differences across all populations (Wilcoxon signed-rank test, between CA1 and proximal SUB $p = 0.79$, between CA1 and distal SUB $p = 0.01$ and between proximal and distal SUB $p = 0.03$), mean firing rate differs significantly between CA1, proximal and distal SUB (Wilcoxon signed-rank test, between CA1 and proximal SUB $p = 3.8e-36$, between CA1 and distal SUB $p = 2.17e-56$ and between proximal and distal SUB $p = 5.14e-11$).

(B) Representative path plots (left) and rate maps (right) from neurons in the proximal (left column) and the distal SUB. Rate maps show firing rates color-coded from 0 (black) to the neuron’s peak firing rate (pFR – white), indicated below each rate map. Color scale as in Fig. 2A. Path plots show the path of the animal during the entire session in gray and the emitted spikes of the respective neuron overlaid in red.

(C-D) Mean and S.E.M of Information content (C) and Information rate (D) of the different covariates P (position), H (head direction), S (speed), E (ensemble firing) and T (theta phase) in the three different cell populations from CA1 (red), proximal SUB (light blue) and distal SUB (dark blue). The $p$ values for the differences in information content (C) between proximal and distal SUB populations were $p = 1.09e-05$ for P, $p = 0.0040$ for H, $p = 0.0036$ for S, $p = 0.0210$ for E and $p = 0.0814$ for T. For the differences in information rate (D) the $p$-values for the differences between the populations were: $p = 0.0250$ for P, $p = 0.0446$ for H, $p = 0.0054$ for S, $p = 0.0033$ for E and $p = 0.0024$ for T.

(E) Bar graphs showing fractions of neurons from proximal SUB (light blue bars) and distal SUB (dark blue bars) selecting the different models. Models were not selected at significantly different frequencies by the two populations (none of the differences between the two populations where larger than 99.9 percentile of a shuffled distribution. When considering differences higher than the 99.0 percentile of a model-selection shuffled distribution, there was a significant difference in the P-only model).
(F) rSCC mean and S.E.M. across the populations of neurons in proximal SUB (light blue) and distal SUB (dark blue) for all covariates P, H, S, E and T. Differences between proximal and distal SUB populations where significant for P.

(G) Three-dimensional plot of the rSCCs for the behavioral covariates P, H and S. Each neuron is represented by one datapoint in light blue for proximal SUB and in dark blue for distal SUB.

(H) Mixed selectivity score (MS score) defined as the product between rSCC(P), rSCC(H) and rSCC(S). Mean MS score across the population in proximal SUB (pSUB; light blue bars) and distal SUB (dSUB; dark blue bars; differences between the two populations not significant; p = 0.99).
Figure S6. **Significant difference in decoding error between SUB and CA1 and between OF and ST when compared to the differences in a shuffled population. Related to Fig. 6**

(A) The blue histograms show the frequency of occurrence for differences in decoding error (left) and frequency of exactly decoded bins (FED) when decoding from populations of neurons with randomized and equalized anatomical location (CA1 or SUB). Shuffling was performed on data in the open field (left column) or the spatial task (right column). The red bar indicates the difference of decoding error between the two neuronal populations recorded in the CA1 or the SUB, respectively. As we shuffled 1000 times, the p value of the red bar is smaller than 10e^{-3}.

(B) The blue histograms show the frequency of occurrence for differences in decoding error (left) and frequency of exactly decoded bins (FED) when decoding from populations of neurons with randomized task identity (OF or ST). Shuffling was performed on data from the CA1 (left column) or the SUB (right column). The black bar indicates the difference of decoding error between the two neuronal populations recorded during OF or ST, respectively. As we shuffled 1000 times, the p value of the red bar is smaller than 10e^{-3}. 

16
Figure S7. Controls for decoding: Equal sampling from the different bins of speed and goodness-of-fit of the GLM. Related to Fig. 6

(A) Decoding in comparable time windows for SUB and CA1. Decoding of position, head direction and speed is maximized in similarly long time-windows for SUB and CA1. However, in SUB the decoder reaches bin size decoding at these decoding window lengths while in CA1 data it does not (particularly for head direction and speed).
(B-D) Accurate decoding of position, head direction and speed when the data was equally sampled from the different speed bins. Decoding error (left column) and percentage of exactly decoded bins (right column) at a population size of 150 neurons for position (A), head direction (B) and speed (C). Note that the difference in decoding error for position between OF (full bars) and ST (empty bars) remains after equally sampling from different speed bins between OF and ST.

(E-F) Average across all the cross-validation folds of the difference in the log-likelihood per time bin of the best model M* and the average firing rate model – LLH(M*) defined in section Learning of the Methods (D) and cross-validated explained Deviance per time bin – exD(M*) defined in section Learning of the Methods (E) for the same population of neurons recorded in the open field (left column) and the spatial task (right column). Spike trains from neurons in CA1 and SUB are equally well modelled by the GLM by goodness-of-fit measures of explained deviance (D) or difference in likelihood between the best performing and the average firing rate model (E). The superiority of decoding in SUB is therefore unlikely to result from the GLM as a modeling framework. Black dots and error-bars indicate the sample mean and s.e.m., respectively. P-values were computed using the Mann–Whitney U test.

(G) Spatial autocorrelation of rate maps from SUB neurons (left panel), CA1 neurons (right panel). The size of the region in which the correlation is highest (autocorrelation larger than 0.6) is smaller in in SUB (675 cm$^2$) than in CA1 (1125 cm$^2$).

(H) Average population vector autocorrelation as a function of the distance between spatial bins for CA1 (red) and SUB (blue) in open field (left) and spatial task (right). SUB is less correlated over short distances, (tested using an autoregressive model for the firing rates in one bin based of the 8 neighbouring spatial bins F-test: p= 0.0048).
### Table S1 Number of recorded neurons for every tetrode of the dataset. Related to Fig. 1

In order to approximate the anatomical distribution of the recorded cells within CA1 and SUB, the respective regions were subdivided into proximal (p), middle (m) and distal (d) parts. The boundary between the respective subregions was approximated by dividing CA1 into three and SUB into two equally large subregions in the plane that the brains were sectioned.

| Animal ID | Tetrode # | Recording area | Number of neurons | Animal ID | Tetrode # | Recording area | Number of neurons |
|-----------|-----------|----------------|-------------------|-----------|-----------|----------------|-------------------|
| 20382     | 8         | Dist. SUB      | 8                 | 22295     | 1         | Mid.CA1        | 13                |
|           | 10        | Dist. SUB      | 7                 |           | 3         | Dist. CA1      | 25                |
|           | 11        | Dist. SUB      | 1                 |           | 4         | Prox. SUB      | 16                |
|           | 12        | Prox. SUB      | 47                |           | 5         | Prox. SUB      | 10                |
| 20630     | 8         | Prox. SUB      | 3                 | 23783     | 1         | Dist. SUB      | 8                 |
|           | 9         | Prox. SUB      | 19                |           | 7         | Prox. SUB      | 3                 |
|           | 11        | Prox. SUB      | 6                 |           | 9         | Dist. SUB      | 5                 |
|           |           |                |                   |           | 11        | Dist. SUB      | 5                 |
| 21012     | 1         | Dist. SUB      | 5                 | 24101     | 1         | Mid.CA1        | 21                |
|           | 2         | Dist. SUB      | 20                |           | 3         | Mid.CA1        | 20                |
|           | 3         | Prox. SUB      | 13                |           | 7         | Mid.CA1        | 0                 |
|           | 4         | Prox. SUB      | 34                |           | 8         | Mid. CA1       | 56                |
|           | 5         | Prox. SUB      | 31                |           | 10        | Prox. CA1      | 5                 |
|           | 6         | Dist. CA1      | 15                |           | 11        | Prox. CA1      | 30                |
|           | 7         | Mid.CA1        | 2                 |           |           |                |                   |
|           | 9         | Mid.CA1        | 18                |           |           |                |                   |
|           | 10        | Dist. CA1      | 18                |           |           |                |                   |
|           | 12        | Prox. SUB      | 9                 |           |           |                |                   |
| 22098     | 3         | Prox. SUB      | 11                | 24116     | 1         | Mid. CA1       | 3                 |
|           | 4         | Prox. SUB      | 11                |           | 2         | Mid.CA1        | 52                |
|           | 5         | Prox. SUB      | 32                |           | 3         | Mid.CA1        | 18                |
|           | 6         | Dist. CA1      | 9                 |           | 4         | Mid.CA1        | 20                |
|           | 8         | Prox. SUB      | 18                |           |           |                |                   |
|           | 9         | Dist. SUB      | 33                |           |           |                |                   |
|           | 10        | Dist. SUB      | 22                |           |           |                |                   |