Three separable roles for context in breaking environmental symmetries for place cells

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Running title: Contextual symmetry-breaking in place cells

Key words: place cells, head direction cells, context, hippocampus, spatial cognition, olfaction

Conflict of interest statement: The authors declare the following competing interests: K.J.J is a non-shareholding director of Axona Ltd.

Acknowledgements

This research was funded in part by the Wellcome Trust [Grant number WT103896AIA]. For the purpose of open access, the author has applied a CC BY public copyright licenses to any Author Accepted Manuscript version arising from this submission. The work was also supported by a grant from the Biotechnology and Biological Sciences Research Council (BB/Joo9792/1) to K.J.J, and an A*STAR (Singapore) National Science Scholarship to HYC.

Author contributions: Han Cheng: Conceptualization, Methodology, Software, Investigation, Formal analysis, Data curation, Visualization, Writing – Original Draft, Review & Editing; Dorothy Overington: Conceptualization, Methodology, Software, Investigation, Formal analysis, Data curation; Kate Jeffery: Conceptualization, Methodology, Data curation, Supervision, Resources, Funding acquisition, Writing – Original Draft, Review & Editing.

The authors are grateful to Joanna Holeniewska for technical support during the project's execution.

Data and materials availability

Summarized datasets are documented in an online repository xxx, and the analysis code can be found on xxx (xxx).

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Abstract

We investigated how place cells resolve spatial ambiguities, such as identical compartments and rotational symmetry, by recording from hippocampal CA1 in rats exploring a visually ambiguous environment, the '2-box', comprising two identical, connected rectangular compartments with directionally opposing layouts. The layouts distinguish the two ends of each box, while the boxes themselves are distinguished by their odor context. We found that firing field ensembles were usually oriented consistently relative to the odor cues, indicating use of odor context to break the overall global symmetry. Some place cells also maintained a consistent context-specific relationship to the layout cues, thus 'remapping' between boxes. Other place cells ignored both the context and the layout cues but maintained the same relationship to walls of given orientations, thus repeating their fields in the two boxes. No cells ignored orientation altogether by rotating independently of the ensemble. And finally, sometimes the whole ensemble rotated its firing *en bloc*, decoupling from the odor context cues. To explain these observations we propose that odor establishes a context signal having three simultaneous but separable roles, to: (i) orient the global directional signal via an association with layout cues, (ii) gate the spatial inputs onto place cells, and (iii) organize the configural association of head direction with layout. This configuration can in turn regenerate the context signal if the original sensory input becomes unavailable, explaining how spatial firing patterns originally established by the odor context can become independent of it. We speculate that these interactions occur in retrosplenial cortex.

Significance statement

Can the hippocampal place cells resolve spatial ambiguities such as identical compartments and rotational symmetry? We found that place cells used context information in conjunction with the environment structure. The pattern of findings suggests three simultaneous but separable roles for the context cues: to orient the overall pattern, to position place fields, and to guide formation of stable associations between viewpoints and head direction. This network of interactions may be located in a multi-sensory cortical region such as retrosplenial cortex.

Introduction

Place cells fire in specific locations in an environment, forming the neural basis for the brain's internal representation of space ('cognitive map'; O'Keefe and Nadel, 1978). The spatial pattern of place cell firing locations (place fields) is unique to each environment.

Given this, how does the system distinguish environments that have repeated identical elements, and locate the animal within each one?

We have been exploring this issue using environments possessing an inherent symmetry, broken only by sensory cues of interest. Symmetry refers to a system's property of invariance under a transformation. For a spatial environment, symmetries of relevance are translational, rotational and scale (Jeffery, 2023). If place cell firing patterns break these symmetries (respond differently in the environments), this indicates that these cues are used by the network, and suggests how (for orientation, positioning etc). The emerging picture so far is as follows. First, the cells distinguish identically shaped and oriented environments using context cues such as odor (Anderson and Jeffery, 2003): essentially breaking a translational symmetry, where odor is the dimension of translation. By contrast, they do not readily do this using path integration (movement-tracking; Spiers et al., 2015; Grieves et al., 2016). They do, however, use path integration to break rotational symmetry, enabling them to, for example, distinguish one end of a rectangular compartment from the other (Taube and Burton, 1995; Jeffery et al., 1997). They also readily use local (mainly visual) cues such as landmarks, to do this (Muller and Kubie, 1987). This rotational symmetry-breaking is thought to be mediated primarily by head direction (HD) cells (Taube et al., 1990; Harland et al., 2017).

HD cells are central to the organization of these firing patterns, so we recently asked the question of how the HD system itself solves this same problem of resolving environmental symmetries. We found to our surprise that a subset of cells in rat dysgranular retrosplenial cortex (dRSC), which outwardly resemble classic HD cells, are responsive to local but *not* global rotational symmetry-breaking cues (Jacob et al., 2017; Zhang et al., 2022). This was found by recording in the '2-box', which comprises two connected rectangular compartments in which the visual layouts are 180 degrees reversed from each other. This arrangement created a twofold global rotational symmetry for the whole apparatus. Additionally, if the door and cue cards are ignored there is also a translational symmetry and a local onefold rotational symmetry, arising from the rectangular geometry (Figure 1). We found that as the animal crossed from one box to another through the doorway in the central dividing wall, some cells ignored the global symmetry-breaking cues and rotated their directional firing to remain aligned with the local visual layouts. We proposed that these layout-following 'multidirectional' cells represent a link in the chain between local visual scene-processing and global head-direction orientation (Page and Jeffery, 2018). These experiments also revealed that ordinary HD cells can break the symmetry of the two-compartment layout by

using the odors in the two compartments, even though odor is not in and of itself a precise directional cue.

The question arises as to whether all this information is passed to place cells. Thus, the present study used this same 2-box environment with its multiple symmetries to investigate the spatial organization of place fields. Given our finding that the retrosplenial HD signal is not unitary, but rather has cells with differential sensitivity to the global vs. local directional cues, we wondered what place cells would do. Would they (see Figure 1):

(i) Repeat their fields between the two compartments, showing translational invariance, and sensitivity to global but not local rotational symmetry-breaking

(ii) Change their firing (remap), showing sensitivity to either translational or rotational symmetry-breaking cues (or both), or

(iii) Rotate, showing sensitivity to local rotational symmetry-breaking cues (the visual layout) but insensitivity to both the translational *and* the global rotational symmetry-breaking cues.

Furthermore, would their responses be global (across the whole population) or partial (affecting some cells only)?

Figure 1 near here

We found here that only (i) and (ii) held true; place cells repeated or remapped their fields but never rotated them. In terms of complete vs. partial responding, place cells showed complete (ensemble) responses to global rotational symmetry-breaking cues, but partial responses to the local cues. Furthermore, sometimes they collectively and spontaneously broke the global symmetry independently of the odor cues, indicating more than one way to signal global orientation.

This behavior is not readily explained by current models of place cells orientation and positioning. We therefore propose a new model, adapted from an existing ‘contextual gating’ model, that could explain these findings and map them to known circuit properties. It proposes that context plays three different roles in this setting, by organizing linkages between directional, positional and viewpoint signals that enable symmetry-breaking.

Materials and methods

Subjects

Five Lister Hooded rats were used for place cell recordings. All animals except one were naïve to the experimental apparatus prior to any recordings. The remaining animal had been exposed to the experimental apparatus as part of a behavioral experiment before entering the current experiment.

The animals were housed individually and maintained on a 12hr/12hr light/dark cycle. A week after surgery and throughout the whole experimental period, all animals were food restricted and their body weight maintained at 90% of their free-feeding body weight. All procedures were done in accordance with the restrictions and provisions listed in the Animals (Scientific Procedures) Act (United Kingdom, 1986) and European Communities Council Directive of November 24,1986 (86/609/EEC)).

Microdrive implantation

Axona microdrives (Axona Ltd, UK) containing four drivable bundles of tetrodes were prepared for implantation in rats. Each tetrode was made from four 25µm polyimide coated 90% platinum-10% iridium wires (California Fine Wire, Grover Beach, CA) twisted together and threaded through a 21G stainless steel cannula (Cooper's Needle Works Ltd, UK). Gold plating was used to reduce impedance to within the range of 180-300 kΩ (with a target impedance of 200 kΩ).

Standard procedures were used for stereotaxic implantation of microdrives into the cortex overlying the hippocampus (AP: -4.0, ML: ± 2.5). Carpofen (0.1ml of 10% v/v per 100g animal weight; Pfizer Ltd, UK) was given pre-surgically while Metacam was given post-surgically to reduce pain and inflammation.

Experimental apparatus

The animals were screened in a separate room distinct from the recording room. The screening room contained a square open box environment (120cm x 120cm) lifted off the floors by four stools, one in each corner. Strips of polypropylene sheeting were mounted onto the box wall to discourage the animals from climbing on or over the edge. Distal cues were available in the screening room.

The recording room contained the recording environment (2-box) surrounded by black floor-ceiling curtains, thus depriving animals of distal visual cues. The 2-box was constructed from

wood, painted black and lined with polypropylene sheets for the walls, and vinyl sheet for the floor. A white cue card (A4-sized; 21cm x 29.7cm) was mounted on one short end of each compartment, ‘North’ for one and ‘South’ for the other. The floors were scented at the start of every trial by wiping the vinyl flooring with a diluted (~50%) solution of either vanilla or lemon odors (Dr Oetker, Germany). Four halogen lamps were mounted onto the ceiling near each corner of the environment to provide symmetrical lighting, and white noise was played on a portable speaker mounted on the ceiling to mask extra-maze directional auditory cues. During recordings, the room lights were switched off and the halogen lights switched on.

Recordings and experimental protocol

A week following surgery, the animals began screening for place cells in the screening room. For screening and recording, the connector on the microdrive was connected to a headstage that in turn was connected via a thin flexible cable to the dacqUSB pre-amplifier and recording system (Axona Ltd, UK). The signals acquired from the tetrodes were digitized (48 kHz), amplified 10000-20000 times and high-pass filtered to detect neuronal spikes. Meanwhile, local field potential signals were amplified 2000-3000 times and low-pass filtered. The positions of the animals were tracked via a single LED on the headstage via a ceiling-mounted camera. If place cells were observed, the animals were transferred to the recording room in a black transport box after which the recording protocol commenced.

Rats were always started in the same compartment. They were not actively disoriented before the first trial, but were mildly disoriented in a gently rotated dark box between trials. Rice pops (Waitrose, UK) were thrown into the box to encourage the animal to explore while neural recordings were conducted. In two animals (R590 and R609), vanilla flavored rice was thrown into the vanilla compartment and non-flavored rice was used in the lemon compartment, to maximize the possibility for context discrimination. A single recording session consisted of five trials in sequence (Figure 2A): (i) baseline trial, (ii) rotation trial, (iii)-(iv) two single compartment trials, and (v) a second baseline trial. The rotation trial (90º or 180º) was to ensure that the cells were using the box cues and not uncontrolled distal cues, and the second baseline trial was to check the stability of the place cells. All two-compartment trials ranged from 15 to 25 min while the single compartment trials lasted for 10 min. In between trials, the animals were removed from the environment, placed back into black transport box and disoriented. The environment was cleaned of any urine or feces, the odors reapplied, and the box rotated to the appropriate configuration. At the end of a recording session, the screw on the microdrive was turned one-eighth of a turn (~25µm) to find new cells for the next recording session.

Data were analyzed as follows.

Behavior analysis

To examine for any bias in the animals' preference for either compartment, a preference index was calculated as follows:

$$Preference\ Index = \frac{T_V - T_L}{T_V + T_L}$$

where T_V and T_L are time the animals spent in the vanilla and lemon compartments, respectively. PI values > 0 indicate a preference for the vanilla compartment while a PI < 0 indicate a preference for the lemon compartment. A value of 0 will indicate no preference for either compartment. The preferences for odors were only examined for two-compartment trials.

Spike sorting

After recording, neuronal spikes were sorted using graphical cluster-cutting software Tint (Axona, UK) with the help of clustering algorithm KlustaKwik 3.0 (Kadir et al., 2014). Recording data obtained across trials in the same session were loaded into Tint together for clustering. The resulting clusters were manually refined by examining their waveforms and cross-correlograms, and merging them if they occupied similar spaces in the cluster space and did not spike within the 2 ms refractory period. The sorted spikes were further analyzed offline in Matlab (MathWorks).

Place cell identification

Data from potential single units were preprocessed and analyzed in Matlab 2019a/b. First, positional data and spikes were speed-filtered. Spikes from epochs with an instantaneous speed < 5 cm/s were removed to exclude non-local spiking (replay events). Positional data and spikes with instantaneous speed > 100cm/s were considered to be tracking errors and removed.

To ensure good unit isolation, any units with more than 0.2% of spikes falling within an inter-spikes interval < 2ms and mean firing rate < 0.1Hz in both baseline trials were excluded. Units were considered to be putative pyramidal cells if the waveform peak to trough time > 250 µs and their mean firing rate was between 0.1 and 5 Hz in both baseline trials.

After identification of putative pyramidal cells, rate maps were constructed to examine for spatial firing. Rate maps were generated in the same manner as described in Leutgeb et al.,

(2005), but with slightly different parameters. Positional data were divided into 3x3 cm bins, and spikes falling within each bin were accumulated accordingly into spike maps, and position points similarly as dwell maps. Positions and spikes were smoothed separately with a Gaussian kernel (smoothing factor = 5 cm) centered on the bin. Any bins > 9 cm distant from the current bin were given a weight of 0. Bins in which the animals spent less than 0.1s were designated as unvisited. The smoothed rate maps were then generated by dividing the smoothed spike maps by the smoothed dwell maps.

Spatial firing was then examined using the smoothed rate maps. A place field was defined as region of at least 16 contiguous pixels with firing rates > 40% of the peak firing rate and peak firing of > 1Hz. Putative pyramidal cells with fields that had summed pixels > 50% of the dwell map pixels were considered ‘messy’ and excluded from further analysis.

To further remove cells with non-spatial firing, we calculated the spatial information content of each cell (Skaggs et al., 1992) and compared that to a shuffled null distribution. The shuffled null distribution is generated by circularly time shifting the spikes by a random time interval between 20s and the duration of the session minus 20s, for each round of the shuffle. This maintains the temporal relationship of the spikes but dissociates the spikes from the animal’s position. For each round of the shuffle, we generated a rate map based on the new spike positions and calculated the spatial information content. This process was repeated 100x for each cell, and the cell was considered to be spatially modulated (i.e. having a place field) if the cell’s spatial information content > 95th percentile of the shuffled null distribution.

For visualization purposes, spikes were plotted on positional data for each trial to create a spike plot. Paths of the animals in the lemon compartment were plotted orange, and in the vanilla compartment in brown. Spikes of neurons from the corresponding time point were overlaid as red dots.

Null distributions

To determine the chance level for which a place field is in a specific location between two correlation comparisons, null distributions were generated. To do this, randomly selected pairs of rate maps from the entire pool were correlated, and this procedure repeated 10,000 times. The 95th percentile of the distribution of the resulting values was used as a threshold for the probability a place cell could have a place field in the exact same location by chance. Correlation values above threshold indicate similar rate maps while correlation values at or below threshold suggest dissimilar rate maps and/or global remapping.

Rate maps correlation

To examine how similar or different spatial firing was across trials or compartments, Pearson's correlations on the smoothed rate maps were carried out between two trials or two compartments respectively. Before correlation, all rate maps were rotated to the appropriate orientation (either same orientation or 180º rotation depending on the question to be answered). If the rate maps were found to be of different sizes (due to unvisited regions), the smaller rate map was padded to the size of the larger rate map with NaN in all dimensions. The equivalent spatial bins between the two rate maps were then correlated on a bin-by-bin basis. The threshold correlation value for what constituted a similar spatial map depends on the null distributions generated for each type of comparison. For the entire 2-box taken as a whole, the null distribution threshold was 0.37, while for each sub-compartment the threshold was 0.46.

Duplicate cell detection/removal

To avoid over- or under-estimating the proportion of place cells that could display local or global encoding, place cells that were recorded over days were identified systematically using information about the waveform and the place fields. This method assumed that place cells in the two-compartment context box are stable and do not remap over days.

Place cells that displayed similar spatial firing were first identified by correlating the rate maps of all cells recorded from the same tetrode between consecutive recording sessions. Cell pairs with spatial correlation > 0.375 were then examined for similarities in waveforms using Tolias distances as a measure for waveform similarities (Tolias et al., 2007; Duvelle et al., 2019). Specifically, Tolias distances can be described using two separate metrics – Tolias distance 1 and Tolias distance 2 respectively. Tolias distance 1 captures the differences in waveform shapes and can be calculated by scaling one of the two waveforms to be compared by a factor α. After finding the best α fit for each channel, Tolias distance 1 can then be calculated by looking at the normalized Euclidean distance between the two waveforms. Tolias distance 2 examines the differences in amplitudes across the four channels. If the same cell was recorded over multiple days with minimal changes in waveforms, both Tolias distances will return small values.

We next sought to determine an objective threshold that would indicate if the two waveforms are similar or different. To do so, we fitted a linear discriminant analysis classifier using the Tolias distances calculated from experimentally defined *same cells* or *different cells*. *Same*

cells were defined as the Tolias distances obtained by comparing the waveforms of cells determined to be the same (via spike sorting) across the two different two-compartment trials (baseline 1, rotation and baseline 2) within a recording session. Meanwhile, *different cells* were defined as the Tolias distances obtained by comparing the waveforms of cells recorded in the very first and the very last session of an animals. Separate linear discriminant analysis classifiers were generated for each animal under the assumption that each animal (and each microdrive) has its own set of electrical noise during recording.

Tolias distances were then calculated for cells recorded on the same tetrode on consecutive sessions, and the posterior probability of cell pairs having similar waveform ('*same cell*') predicted from the trained classifiers. Spatially similar cell pairs were considered to be the same if the trained classifier predicted a posterior probability > 0.5. Occasionally, two cells recorded in the first session mapped to a single cell in the next session ('convergence'), or alternatively a single cell in the first session mapped to two different cells in the subsequent session ('divergence'). In these cases, If the difference in the posterior probability between the two cell pairs exceeded 0.1, the cell pair with the higher posterior probability was considered to be the same cell. Alternatively, if the difference in the posterior probability was less than 0.1, the cell pair with the higher spatial correlation was considered to be the same cell.

Using this method, 104 cells were found to have been recorded in multiple sessions (2 sessions n = 70; 3-8 sessions n = 34). We removed these cells from every session except the first. In addition, we removed place cells that did not exhibit cue control (R < 0.37 between rotation trials) or were unstable (R < 0.37 across two baseline trials).

Histology

Results

The protocol for the experiment is shown in Figure 2A. All tetrodes were situated in the CA1 area of the hippocampus (Figure 2B). Five rats were recorded for 37 sessions of five trials yielding 437 unique place cells in the final analysis.

Figure 2 near here

Comparison of behavioral dwell time and place cell activity between compartments

First, in order to rule out potential effects of the odors, which may affect how place cells represent the environment (Mamad et al., 2017), we examined the preference of the rats for spending time in one compartment vs. the other and expressed it as a preference index (PI; see materials and methods, behavior analysis). We found a slight preference across all animals for the vanilla compartment (Figure 3A; one sample t-test $t(110) = 3.59$, $p < 0.001$). This declined over trials: a repeated-measures ANOVA found a main effect of trials [$F(2,34) = 6.55$, $p = 0.002$] with a significantly greater preference on the first trial than subsequently (baseline 1 vs rotation trials: $t(36) = 3.08$, $p = 0.004$; baseline 1 vs baseline 2 trials: $t(36) = 3.33$, $p = 0.002$) while the latter two trials did not differ significantly ($t(36) = 0.50$, $p = 0.62$). Thus, the vanilla preference slowly diminished over the trials such that by the rotation trial, the animals displayed no preference and explored both compartments equally.

Figure 3 near here

We next compared the properties of CA1 place cells between the two boxes in Baseline trial 1, when this preference was the most apparent. Place cell activity did not differ between the boxes (Figure 3B) either in the number of active cells ($X^2(1, N = 322) = 0.075$, $p = 0.78$) or the number of place fields ($X^2(1, N = 670) = 0.59$, $p = 0.44$). There were also no differences in firing statistics (Figure 3C; Area: *t* test $t(668) = -0.22$, $p = 0.83$; Peak firing rate: $t(668) = 0.14$, $p = 0.89$; Mean firing rate: $t(668) = 0.037$, $p = 0.97$). Thus, overall there was little to distinguish the representations of the two boxes other than firing field locations, as analyzed below.

Some place cells repeated their fields, most remapped and none rotated independently

We then looked at the spatial pattern of place cell firing in the two boxes, correlating the rate maps on a pixel-by-pixel basis under either translational or rotational transformation, or between the same box in different trials. The remapping correlation threshold was set to 0.46 based on the 95th percentile of a shuffle (see Methods), and we evaluated the firing patterns based on the three predictions outlined in Figure 1. Note that sometimes the whole firing pattern rotated 180 degrees independently of the box (examined in more detail below) and so we corrected for this before comparing the firing patterns, as described in the Methods.

A small but substantial proportion of place cells (~13.5%) repeated place fields between the boxes (see Figure 4 for an example), with average translational correlations of 0.66 ± 0.02

and rotational correlations of -0.12 ± 0.02. [comparing correlations for same-box, translational comparison and rotational comparison]. This proportion was significantly greater than chance ($X^2(1, N = 437) = 65.53$, $p < 0.001$), and suggests spatial encoding independent of the local visual environment. The number of place cells with repeating place fields did not appear to change as a function of experience, and they could be recorded throughout the recording sessions.

Figures 4 and 5 near here

The majority of place cells (83%) had fields that remapped between the boxes (Figure 5A and B), with a low correlation for both translational and rotational comparisons (translational correlation of 0.041 ± 0.009 and a rotational correlation of -0.004 ± 0.009), but a high correlation for repeated trials in the same box (0.68 ± 0.009), which is greater than the 2-box remapping threshold of 0.37, indicating stable recording and stable encoding.

A small population of cells (~3.2%) had rotated place fields, but this was not significantly different from the chance level of 5% (based on the 95th percentile threshold from the null distribution; $X^2(1, 437) = 3.06$, $p = 0.08$). Visual inspection showed these to be spurious correlations from cells having very few spikes in one of the boxes.

We examined whether place cells generated their box-specific firing patterns even in single boxes when the central door was closed. To do so, we aligned the boxes according to the visual scene and then correlated the immediately preceding rotation-trial maps with those from the single-box closed-door trials. If place cells can distinguish the compartments during the single-compartment trials based purely on odors, same-odor maps should have significantly higher correlations than different-odor maps. This was indeed the case (Figure 5 C; Two-tailed Wilcoxon signed rank test, Z = 17.46, $p < 0.001$). This indicates that place cells can use odor to recognize the single compartment.

Place cells sometimes rotated their fields en bloc independently of odor

As mentioned earlier, when we examined the place cell maps within a recording session, we saw that sometimes place cells rotated their entire 2-box firing patterns 180° relative to the odors from one trial to the next (Figure 6A). We call this phenomenon ‘odor-switching’.

Figure 6 near here

To detect odor-switching systematically, we correlated every pair of rate maps for the two baseline trials, and baseline 1 with the rotation trial, in either the same orientation or with a 180° rotation: we then compared the result to a 95th percentile threshold determined using a null distribution. When these two sets of correlations were plotted against each other (Figure 6B) then most data points, as expected, fell within the lower right quadrant, meaning there was a high correlation for the unrotated maps and low correlation when one of the maps was rotated 180 degrees. However a smaller but distinct cluster showed the reverse, occupying the upper left quadrant. Cells that showed this effect co-occurred, and never co-occurred with those that did not, indicating rotation of the whole ensemble. This effect was more evident at the beginning of the session and had disappeared by the last trial, suggesting that the system had consolidated the relationship between odor and direction (see Discussion).

Discussion (1500)

In order to investigate how place cells resolve environmental ambiguities, we recorded the cells in a two-compartment environment, the '2-box', which has several symmetries that are broken with experimenter-controlled cues. Global rotational symmetry is broken with odor, while local rotational symmetry is broken with the within-box layout (the cue card and the doorway). The experiment was motivated by the recent finding that head direction (HD) cells in dysgranular retrosplenial cortex (dRSC) show two types of responding in this environment, with some cells sensitive to the local and some to the global cues (Jacob et al., 2017). We wanted to see if place cells, connected to RSC, would show this mixed pattern, expressing information about the ambiguous cues that could be useful for cognitive mapping.

We looked for three possible response patterns (Figure 1), but found only two of them. Specifically, we found that some place cells repeated their firing fields between the boxes, while some altered their firing altogether (remapped). However, we saw virtually no cells that rotated their firing to follow the rotated layouts. It thus seems that the directional ambiguity seen in RSC has been resolved by the time information reaches the place cells. Importantly, an additional observation was that although the orientation of the firing-fields ensemble generally matched that of the odor cues, sometimes the whole pattern dissociated from them and rotated *en bloc*. And finally, we observed that some types of symmetry-breaking were complete (affected all the cells together) and some partial.

A couple of things were surprising about the above observations. First, we had expected to see partial rotational remapping, whereby some cells might rotate their fields to follow the rotated environment cues. This is because the directionally ambiguous signal found in dRSC

could potentially have found its way into the place cell system via the RSC-HPC interconnections; and also because a previous experiment dissociating directional and local cues did find that a subset of CA1 cells would follow the local cues (Knierim, 2002). Our observation is, by contrast, more similar to that of Fuhs et al. (2005) who found remapping but no rotation (albeit in a small sample from only two rats). It seems that no trace of the directional ambiguity remains in the place cell map.

The other surprising observation from the present study was that the place field ensemble could dissociate from the odor cues without remapping (Figure 6). The reason this is surprising is that the cells definitely could detect the odor cues, because the odors usually oriented the ensemble firing pattern, including in the single compartment trials. We had thus assumed that the remapping cells were also using the odors to distinguish the compartments and position their fields appropriately, since odors have been previously shown to influence such positioning (Anderson and Jeffery, 2003). However, that the ensemble would sometimes decouple from the odors and rotate *en bloc* suggests that odor-induced remapping was not at play here (or at least not always).

How, then, did the remapping cells distinguish the compartments in order to remap, rather than rotate? The answer might lie with the only other symmetry-breaking information available, which is the relationship of the visual scene, arising from the layout, to global direction. If we suppose that in the lemon compartment the cue card is ‘North’ and the doorway ‘East’, then in the vanilla compartment the cue card is ‘South’ and the doorway ‘West’. Cells could thus, in principle, distinguish the compartments if they could associate these scenes with the global directional signal. This notion is unpacked, below.

Boundary vectors and context gating

An association between global direction and local environmental cues was earlier proposed by Burgess and colleagues, to explain why place fields ‘follow’ environment walls when these are moved relative to each other (O’Keefe and Burgess, 1996; Hartley et al., 2000; Barry et al., 2006). In their ‘boundary vector’ (BV) model (Figure 7A), each place cell is driven by inputs from some (not all) of the walls located at given allocentric directions. The BV model can explain the behavior of the cells that repeated their fields, because there are two ‘East’ and two ‘West’ walls in the 2-box.

Figure 7 near here

The BV model does not explain all the results in the 2-box, however, because it predicts that every place cell should repeat its fields (except at the doorway) and thus does not account for the remapping cells. To explain remapping, such as when environment odor or color is changed, we have previously extended the BV model to include contextual cues (Jeffery et al., 2004; Hayman and Jeffery, 2008; Figure 7B). In our 'contextual gating' (CG) model, the subset of BVs that drives a place cell is selected (gated) by the extant context cues. The CG model can thus explain the remapping cells in the 2-box. The co-occurrence of remapping and repeating cells can further be explained by assuming that repeating cells receive un-gated BV inputs while the remapping cells receive context-gated ones.

However, the CG model does not explain the results of our experiment, in which we saw occasional decoupling of the place field ensemble from the odor context, because when this happens the BVs driving the remapping place cells have dissociated from their contexts, so how are these cells able to fire in their usual locations?

We come back to this issue in a moment. First, however, we consider the issue of how head direction is determined in the 2-box, given that this environment lacks a global polarizing visual cue.

Environmental determination of the HD signal

The BV model and its CG variant assume the presence of a unitary global directional signal. It is notable that, as mentioned earlier, we never saw cells rotate to follow the environment layout cues independently of the ensemble, so we assume (as most models do) that a unitary HD signal is passed to all the place cells together. In most experiments, directional information is provided to HD cells by a single polarizing cue such as a cue card on the wall of the recording chamber, but in our 2-box there are two such cards, in opposing directions. We broke this twofold structural symmetry by scenting the boxes with different odors: we know that HD cells are able to use this sometimes, based on direct observation in RSC HD cells (Jacob et al., 2017) and also inferred here from the ensemble behavior of our place cells. But how does odor orient the HD signal, given odor's inherent non-directionality? Also: given this role of odor in orienting the signal, how is it that the signal sometimes dissociates from the odors?

In fact, there *is* actually directional information provided by the odors, since the lemon and vanilla compartments are spatially separated and thus could in principle define an axis across the space. However the place cell patterns persisted even in the single compartments, so this seems unlikely (though not impossible). An alternative explanation,

which we prefer, is that odor can drive the head direction signal by forming an association with the visual scene perceived at a given facing direction (Figure 7C). Thus, if (for example) the ‘North’ HD cells are active when the rat is in the lemon box and facing the cue card, then the combination of lemon odor plus cue card scene could associate with this subset of cells by Hebbian learning, such that in future, that combination of odor and visual scene could reactivate those HD cells. With the ‘South’ HD cells, although the visual scene is the same, the presence of the other (vanilla) odor forms a distinguishable configuration that prevents confusion.

Another way of thinking about this is to formulate it in the standard ring attractor framework within which theoretical discussions of HD cells are usually situated. By this view, the two identical cue card scenes drive the HD ring attractor at two different places180 degrees apart, North and South; the odors break this ambiguity by selectively associating the cue card scene inputs to the ‘North’ or ‘South’ cells, as appropriate for that box, adding additional drive to one or other of the ring attractor locations and thus breaking the activation symmetry. This function for the odor cues is analogous to the gating role proposed for context in the CG model of place cell positioning.

Three roles for context

By the above proposition, context has two separable roles: one in which it associates with visual scenes to enable retrieval of heading direction (gating the ring attractor, as just described), and one in which it interacts with the BV inputs and controls which ones drive the place cells (gating the BVs, as described earlier). This can explain how it is that the place field ensemble can act coherently in the directional domain (all cells orient together) but incoherently in the spatial domain (yielding both repeating and remapping cells). It does not, however, explain how the whole ensemble can decouple from the odor cues and rotate, because according to the foregoing, odor is the source of the context signal that organizes both the HD cells *and* the BVs.

We can explain this decoupling, however, if we propose a third role for context, which is to organize formation of a linkage between visual scene and HD cells (Figure 7D), again by Hebbian coactivation. We assume that during learning, simultaneous presence of a visual scene, an HD cell signal and the context cues causes formation of a three-way connection such that activation of two of the elements can subsequently retrieve the third, again by a process of pattern completion. This is shown by the blue arrows in Figure 7E. The consequence is that after learning, if odor inputs are weak or absent, the co-occurrence of a

visual scene plus previously associated HD activity can regenerate the context signal and gate the BVs appropriately.

This provides an explanation for why the whole ensemble sometimes rotates independently of the odor cues and yet the place cells retain their usual relative firing locations, as shown on the right in Figure 7E. Suppose the odor cue inputs occasionally drop out: in this case the HD ring attractor is driven in two places, and presumably randomly settles on one or other orientation by chance. Having done this, the learned association of visual scene with HD orientation reactivates the context signal, substituting for the odor cues, and then drives the BVs in the usual way. Thus, all the place fields are correctly positioned relative to each other.

Anatomical locations of the interactions

We can speculate about the possible location of these processes based on the known anatomy and physiology of the region. Visual scene information from visual cortex may possibly pass through a scene-selective region such as parahippocampal cortex. Olfactory or other context information comes via the peripheral senses to a multimodal sensory area, which could be retrosplenial cortex or even hippocampus. Head direction information comes from an interaction of cortical and subcortical brain regions that combine self-motion information with static environmental information.

Where do these signals converge and interact? We propose that retrosplenial cortex is the most likely site. The Jacob et al (2017) experiment in the 2-box found cells that respond to visual layout independently of context (the so-called ‘bidirectional cells’), as well as directional cells that are context-sensitive (the classic HD cells). RSC receives context either directly from hippocampus or indirectly via the subiculum (see Vann et al., 2009; Mitchell et al., 2018 for review) and receive scene information from the visual cortex directly (van Groen and Wyss, 1992; Groen and Wyss, 2003) or from as-yet-unidentified scene-selective regions of cortex. We propose here that this information can set the HD signal. This differs from previous formulation of HD cells in which that signal produces a unidirectional influence on place cells, and suggests that the construction of HD signals is, like many other signals in the brain, the product of feed-back as well as feed-forward inputs. Recordings from these areas as the cues are manipulated will be required to confirm the existence and determine the formation mechanism of these configural signals.

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Figure legends

Figure 1 Multiple symmetries in the two-compartment context box. Left: The two-compartment context box comprises two visually identical compartments, painted grey and scented with either lemon or vanilla. The local symmetry of each sub-compartment is broken by the asymmetrically placed visual cues (cue card, plus the doorway). The global symmetry is broken by the relative positions of the two odors. Right: Three types of symmetry-breaking that could be detected by place cells (see text). (i) The place fields (ovals) could be identical in both compartments but both located at the same end, which is a translational symmetry. This would mean they do not use local odor cues nor local visual asymmetry, but are oriented by the global odor cues.(and hence follow the global environment orientation when the whole environment is rotated). (ii) The cells might remap between compartments. Here it is not possible to determine whether symmetry is locally broken by the odors (inducing contextual remapping) or by the directional cues, in which the system detects that the cue cards have different relationships to the global directional orientation. (iii) The cells might generate fields in the same local location in the two sub-compartments, ignoring both local odor context and the global odor-referenced directional cues.

Figure 2 recording protocol in the two-compartment context box. (A) The experimental protocol showing the 5-trial sequence (door was closed for trials 3 and 4). (B) Recording locations, showing an example photomicrograph, and a schematic of the sites for the 5 animals.

Figure 3 (A) Preference index of all trials (grey) and also averaged across trials for individual animals (colored bars showing mean, s.e. and inter-quartile range) showing mild preference for the vanilla compartment. (B) Comparison of the number of active cells (left) and place fields (right) in the two boxes showing no preference of the cells for encoding one vs. the other box. (C) Place field firing statistics did not differ between the two compartments.

Figure 4 Examples of a repeating cell. Plots of five trials. Note that the firing fields are located at the same end of the box in a global reference frame even though both the odors and the local visual layout are different.

Figure 5 Global place encoding in the two-compartment context box. *(*A) A cell that distinguished the compartments (remapped) by firing in only one of them, maintaining a constant within-compartment position. (B) A cell that remapped by shifting its firing location depending on the box identity. This cells thus receives two sets of positional inputs, which are selected (gated) by the odor context. (C) Quantification of remapping across the odors vs. repeated trials in the same odor (rotate trial compared with first single-compartment trial).

Bars and lines show mean, s.e. and interquartile range; dots show individual cells. The low mean correlation for the different-odor comparison shows significant discrimination of the compartments based on their odor and/or relative location.

Figure 6 Odor switching. (A) Firing pattern of two example cells in which the firing location within a given box reversed its relationship to the box odors. (B) Correlations between rate maps in the first trial vs. the second trial, made either with the maps in their canonical orientations (unrotated maps) or with one of the maps reversed 180 degrees (rotated maps). For the majority of fields (grey circles) the correlations were high in the unrotated condition and low in the rotated condition, meaning that the fields maintained the original relationship to the box configuration. For some fields (colored circles) the relationship reversed 180 degrees in the second trial such that the correlation was low in the unrotated condition and high in the rotated condition. These fields are color-coded by session; note that the rotated fields generally belonged to the same session, meaning that the rotation was an ensemble behavior.

Figure 7 The logic of the configural encoding of sensory inputs driving place cells. (A) The boundary vector (BV) model of Burgess and colleagues (Barry et al., 2006). Separate signals about facing direction, boundaries and the distance from the boundaries combine to form boundary vectors (signals conveying distance to boundaries located at a given direction). When two or more orthogonal boundary vectors meet, they uniquely specify a location in the environment. (B) The context gating model of Jeffery et al. (Jeffery et al., 2004; Hayman and Jeffery, 2008). This extends the BV model by proposing that contextual cues control which boundary vectors can drive a place cell. (C) The configural combining of context and viewpoint to recover heading direction. In Context 1 (e.g., the lemon box in the 2-box) then the rat sees Viewpoint 1 when ‘North’ HD cells are active, and Viewpoint 2 when ‘South’ are active (S). In Context 2 the reverse holds. This configural signal (viewpoint + direction) can then input to boundary vectors. (D) The configural encoding of viewpoint and direction to recover context. A direction signal, which may have come from the process in (C) or may alternatively have come from spontaneous jumping of the HD network to the opposing orientation, combines with the viewpoint. If ‘North’ (N) HD cells are active when the rat sees Viewpoint 1 then it must be in Context 1, etc. This information can then be used to gate the boundary vectors driving the place cells, retrieving Context 1 or Context 2 pattern as appropriate. (E) How the foregoing scheme explains how ensemble rotation of the place fields decouples the firing patterns from odor. The diagrams show hypothetical changes in information flows in the 2-box depending on whether or not the odor context cues are driving HD cells. Left: when they are, they combine configurally with viewpoint, enabling the HD

system to infer facing direction using just the environmental cues, and in turn driving the boundary vector cells. These odor cues simultaneously also gate the boundary vectors so that the place fields are appropriately positioned in the two boxes (with the gated cells remapping). This is the canonical place field alignment that remains consistent with the odor cues most of the time. A consequence of this process is that the viewpoint and HD signal, which have been coactive, become configurally combined with the context (dotted lines), such that they also can gate the boundary vectors. Right: Occasionally the odor cues are weak. The HD system, lacking odor context inputs, chooses randomly from the two attractor states linked to that viewpoint, meaning that sometimes it aligns inconsistently with respect to the odor. However it can combine with viewpoint cues to recover a context signal, as in (D). This can then gate boundary vectors.

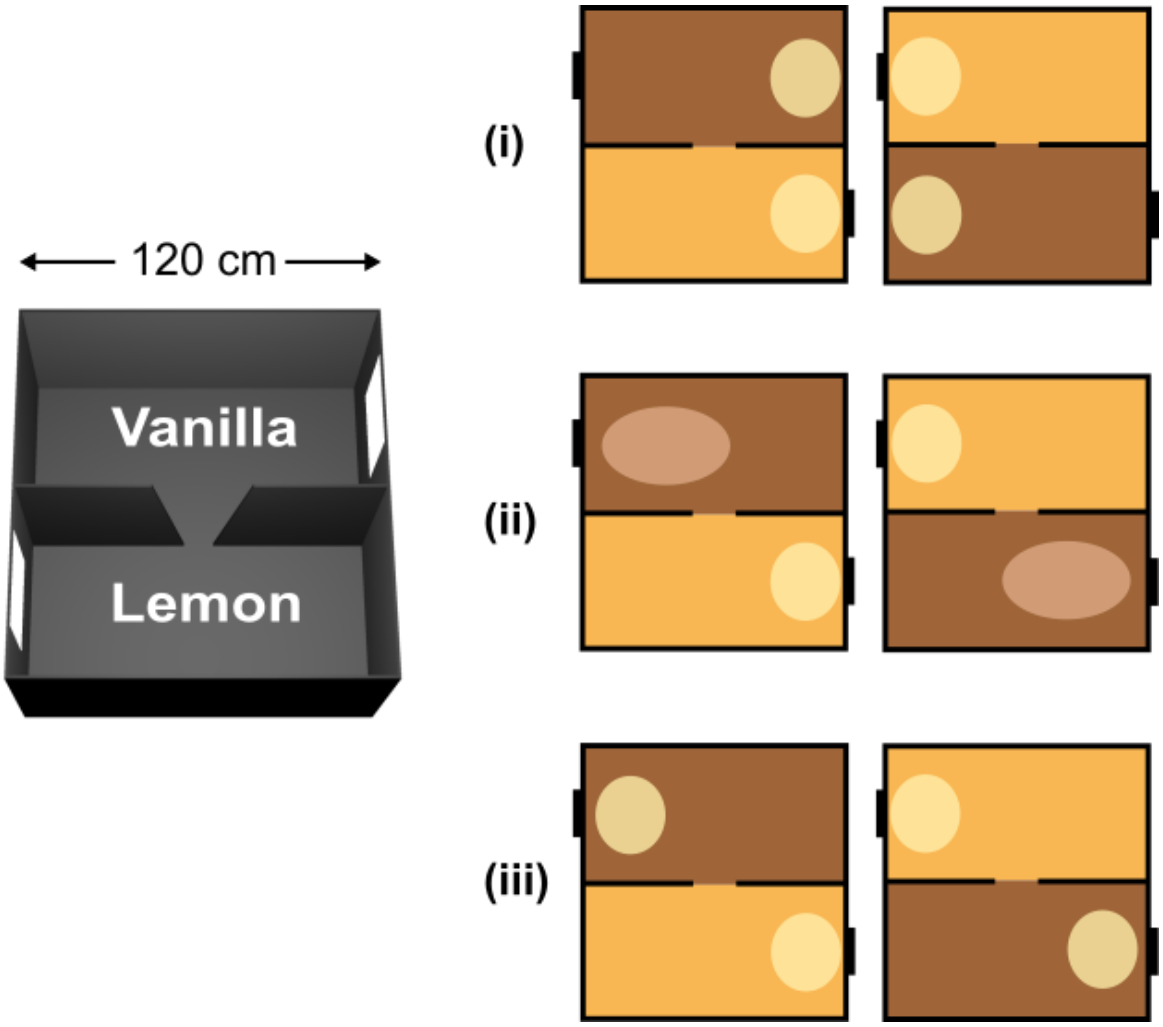


Figure 1

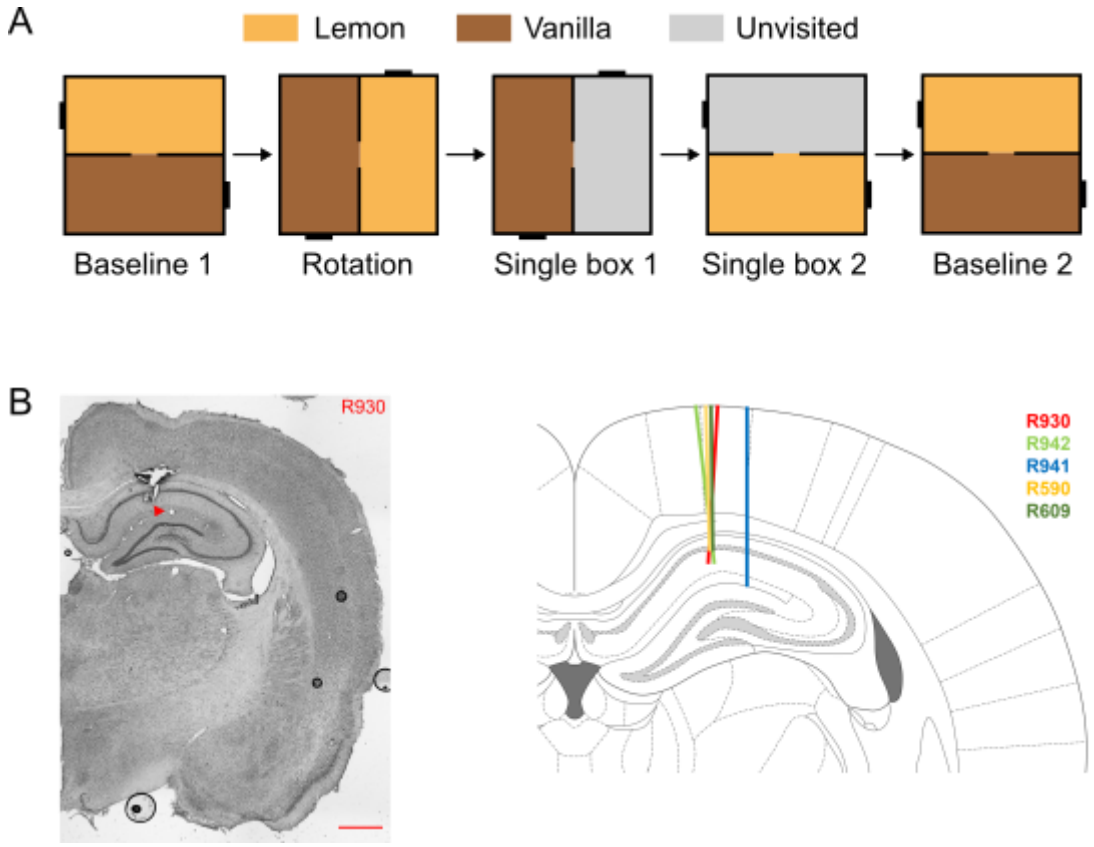

A
Lemon
Vanilla
Unvisited
Baseline 1
Rotation
Single box 1
Single box 2
Baseline 2
B
R930
R930
R942
R941
R590
R609

Figure 2

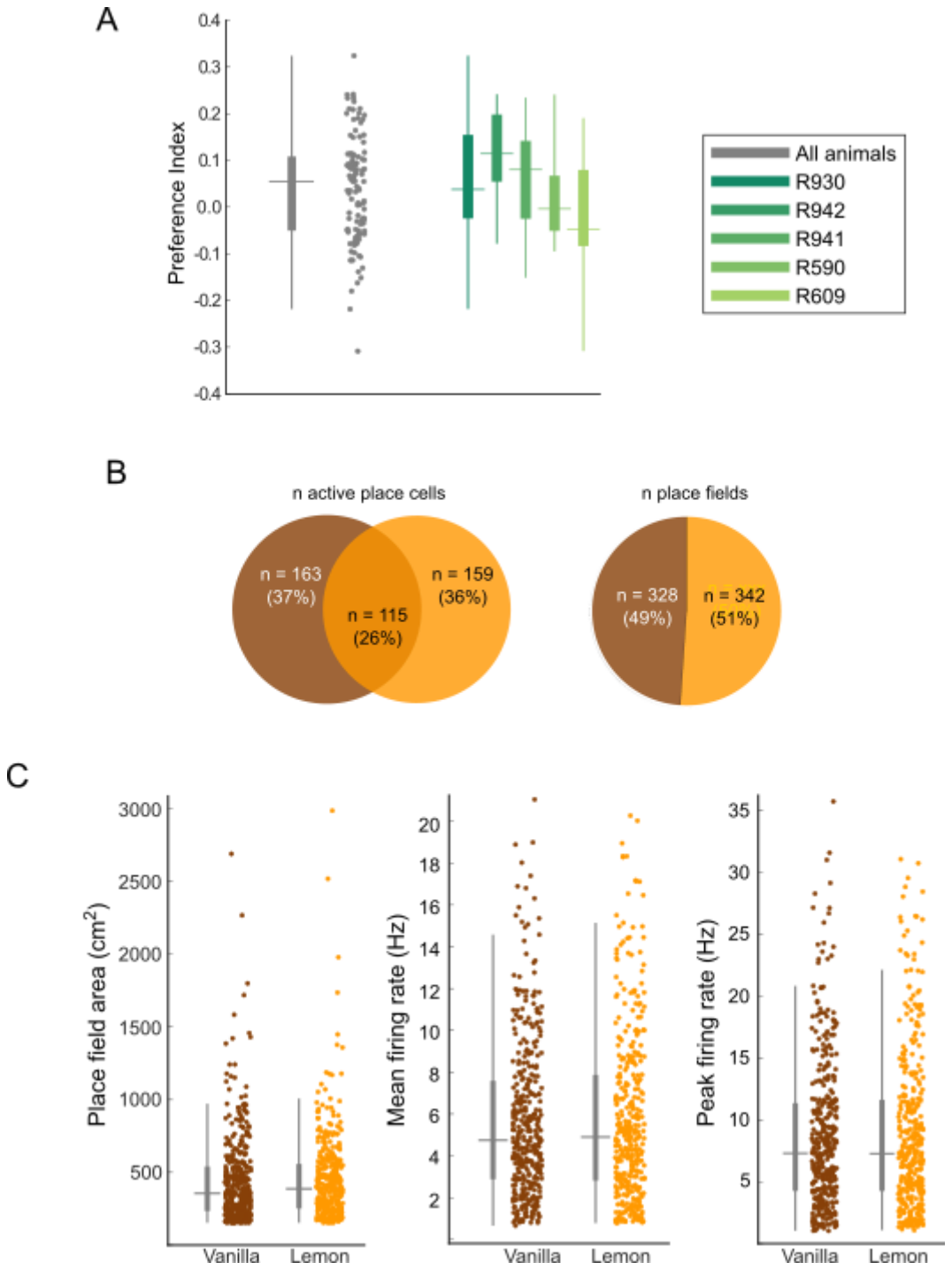

A
Preference Index
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
All animals
R930
R942
R941
R590
R609
B
n active place cells
n = 163
(37%)
n = 115
(26%)
n = 159
(36%)
n place fields
n = 328
(49%)
n = 342
(51%)
C
Place field area (cm²)
3000
2500
2000
1500
1000
500
Vanilla
Lemon
Mean firing rate (Hz)
20
18
16
14
12
10
8
6
4
2
Vanilla
Lemon
Peak firing rate (Hz)
35
30
25
20
15
10
5
Vanilla
Lemon

Figure 3

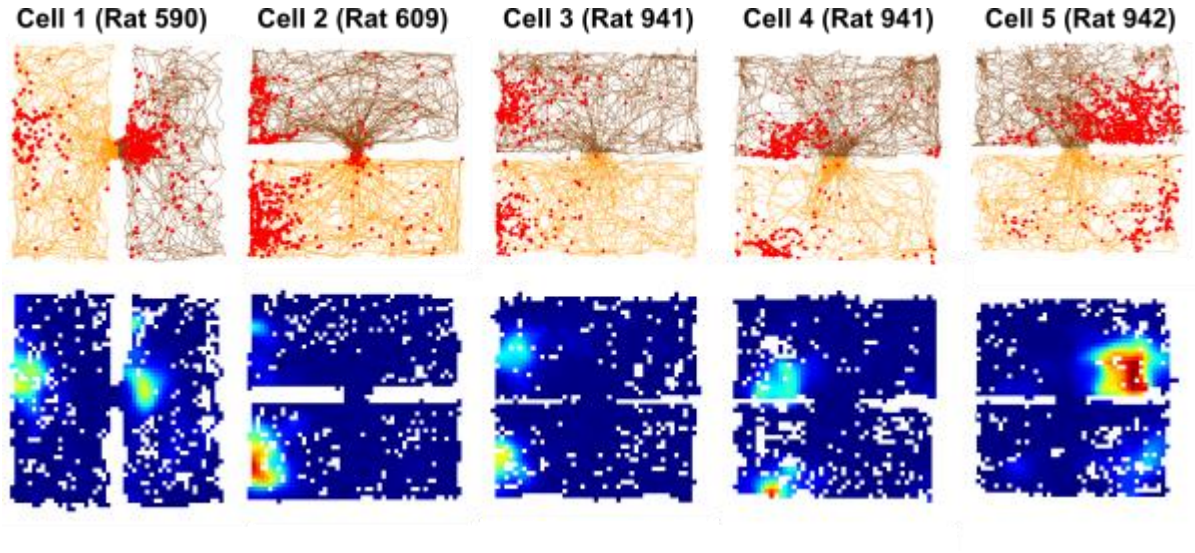
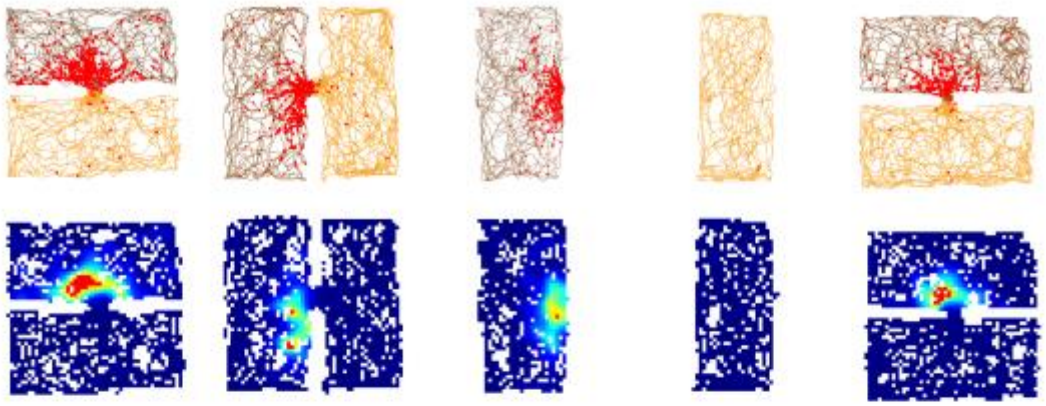

Cell 1 (Rat 590)
Cell 2 (Rat 609)
Cell 3 (Rat 941)
Cell 4 (Rat 941)
Cell 5 (Rat 942)

Figure 4

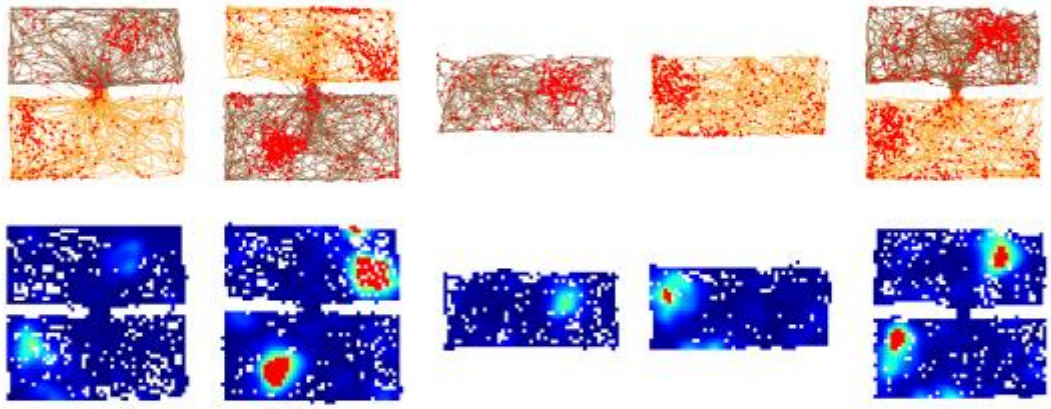
A

Active in one box only



B

Active in both boxes but in different places



C

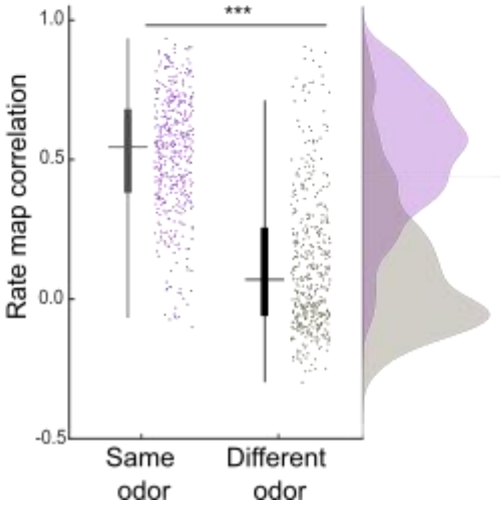
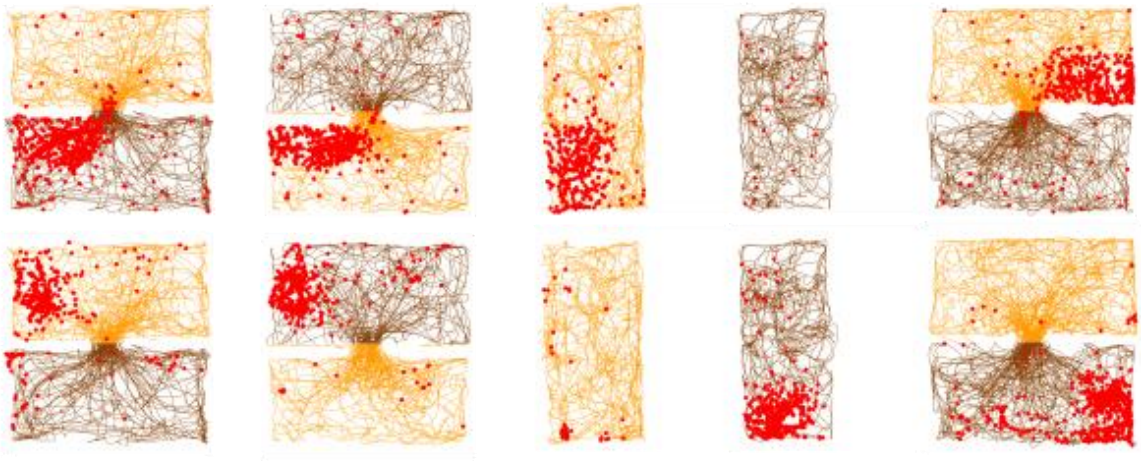


Figure 5

A



B

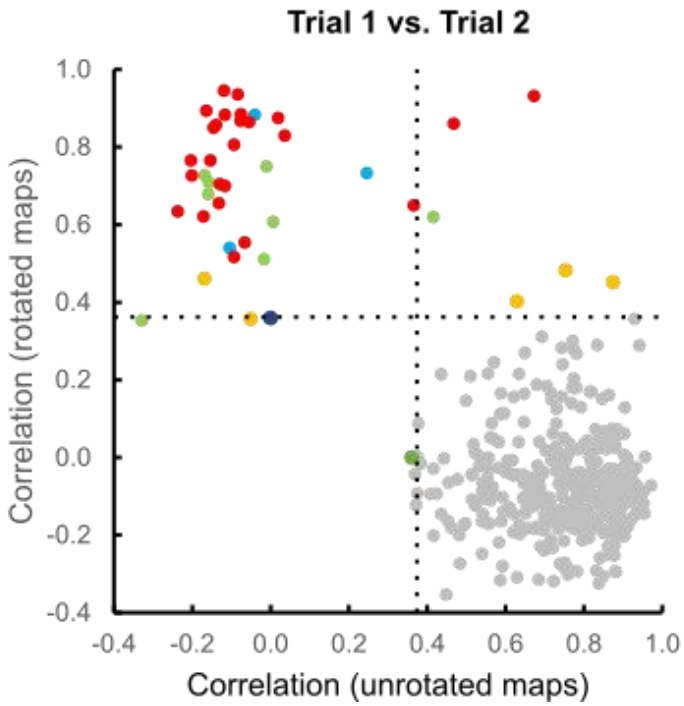

Trial 1 vs. Trial 2
Correlation (rotated maps)
Correlation (unrotated maps)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
1.0

Figure 6

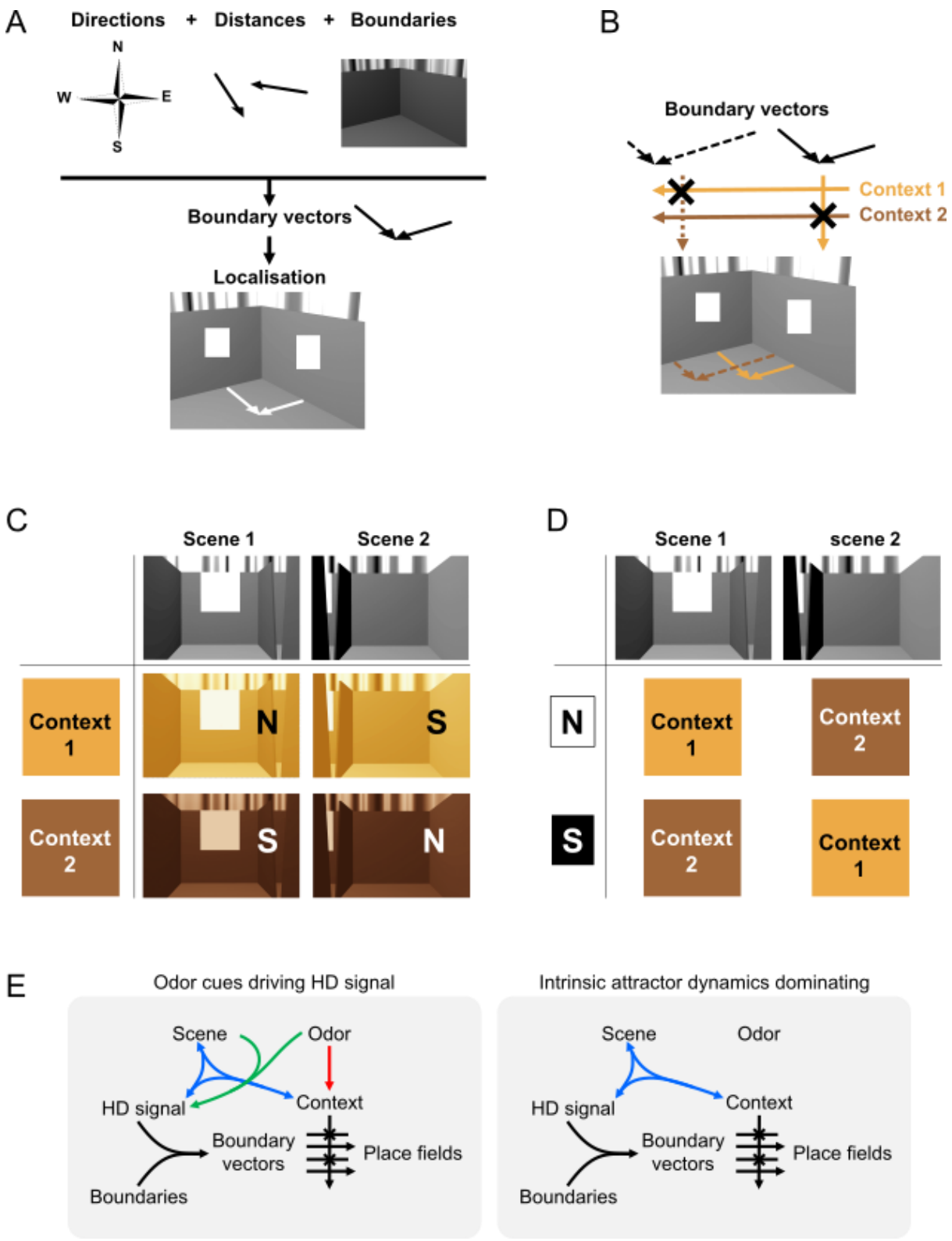

A
Directions + Distances + Boundaries
N
W
E
S
Boundary vectors
Localisation
B
Boundary vectors
Context 1
Context 2
C
Scene 1
Scene 2
Context 1
N
S
Context 2
S
N
D
Scene 1
scene 2
N
Context 1
Context 2
S
Context 2
Context 1
E
Odor cues driving HD signal
Scene
Odor
HD signal
Context
Boundary vectors
Place fields
Boundaries
Intrinsic attractor dynamics dominating
Scene
Odor
HD signal
Context
Boundary vectors
Place fields
Boundaries

Figure 7