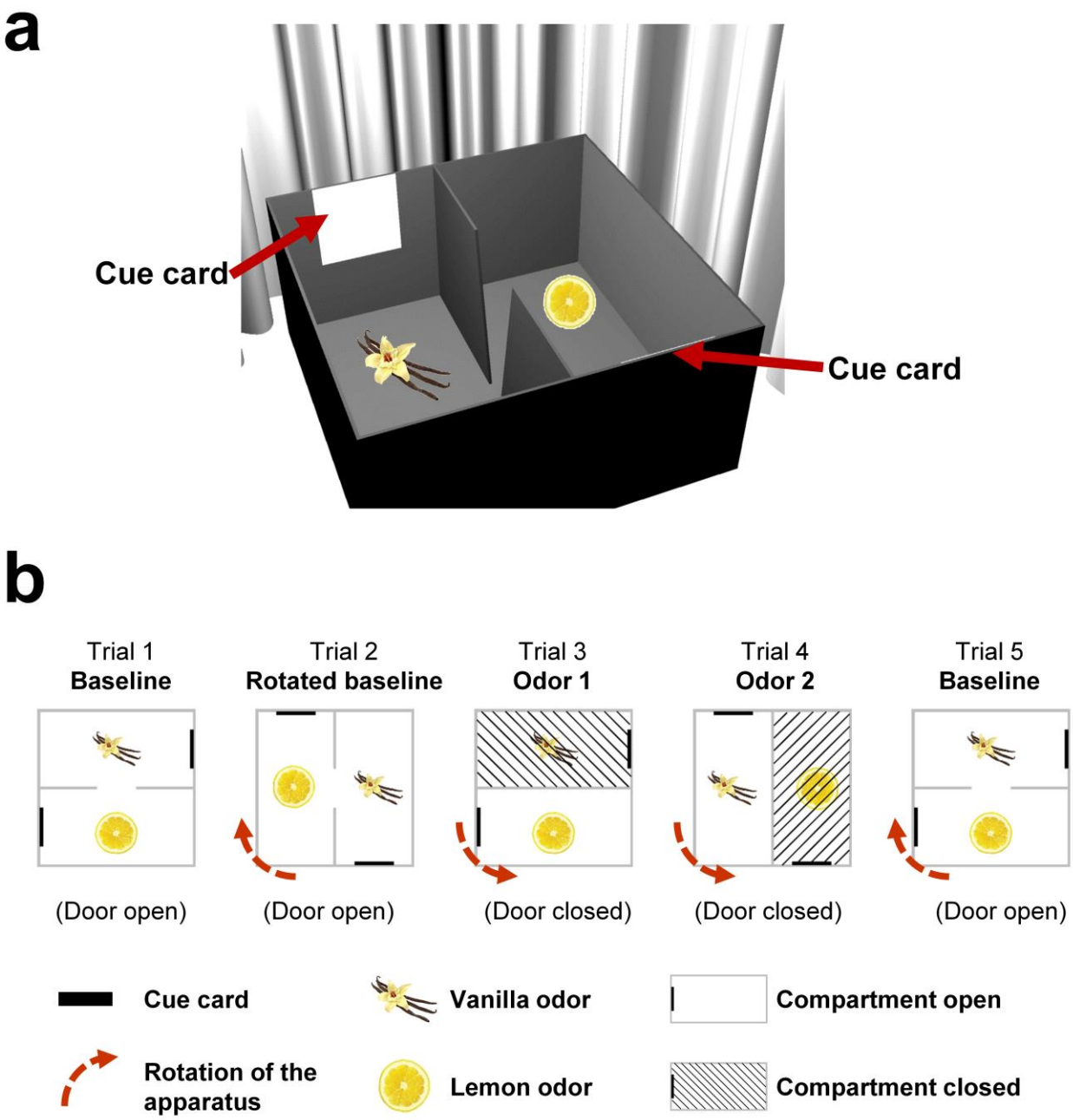
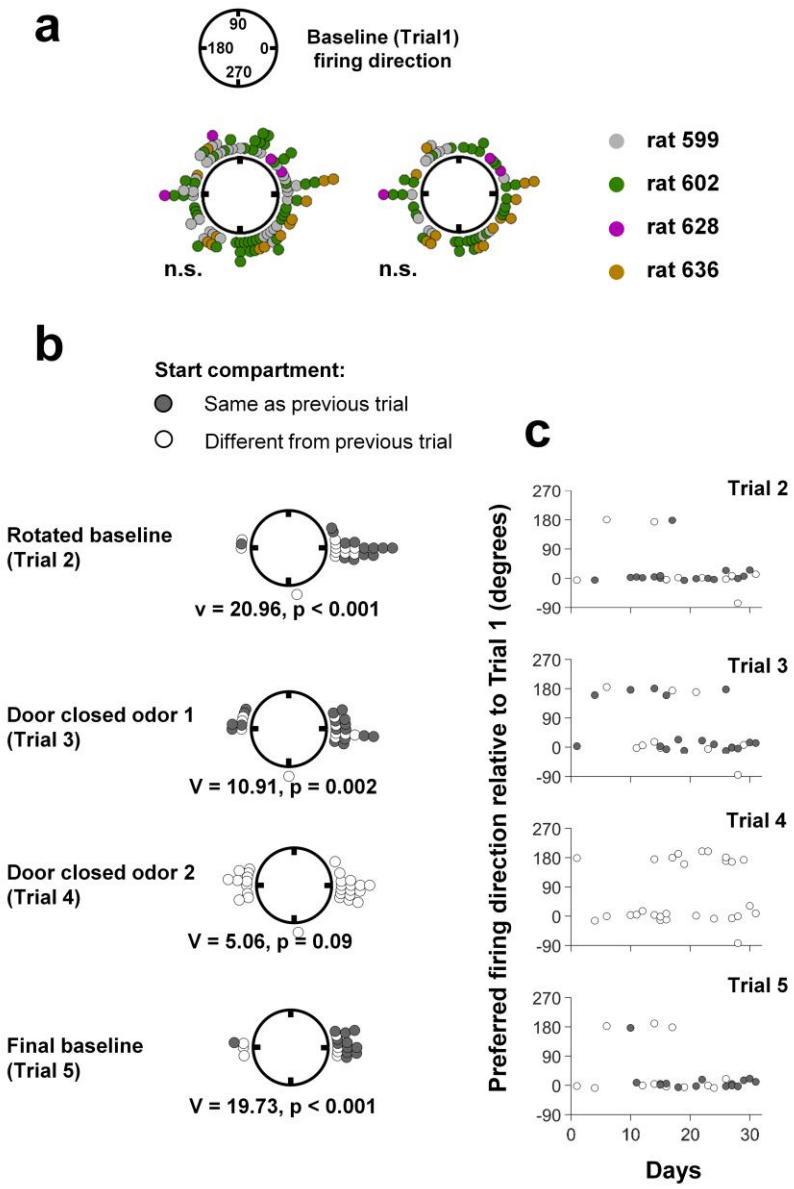


Supplementary Figure 1

Experimental setup and recording protocol.

a, Schematic of the two-compartment apparatus, with one compartment scented with lemon and one with vanilla. Note that the global two-compartment reference frame has 180-degree rotational symmetry so without the odors, a rat randomly placed in one or other compartment would not know which way it was facing. With the odors, symmetry is broken, so consistent firing of a HD cell across repeated trials indicates use of odor to inform the directional signal.

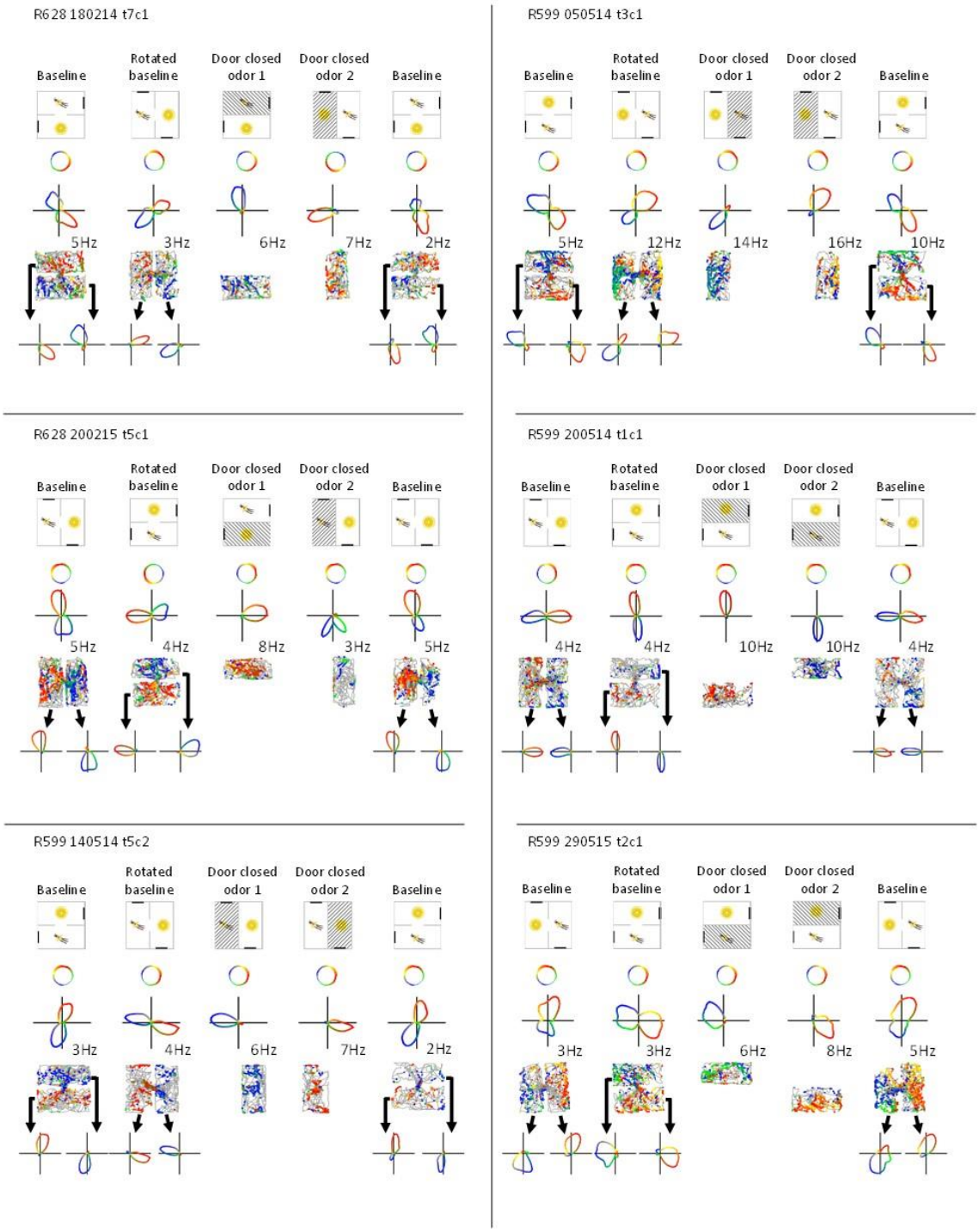
b, Protocol for recording trials. The entire apparatus was rotated within the curtained-off arena between trials so as to remove the influence of uncontrolled distal cues. In trials 3 and 4, the rat was randomly placed in one or other compartment, and the door closed to confine the rat to that compartment.



Supplementary Figure 2

Directional firing of RSC head direction cells.

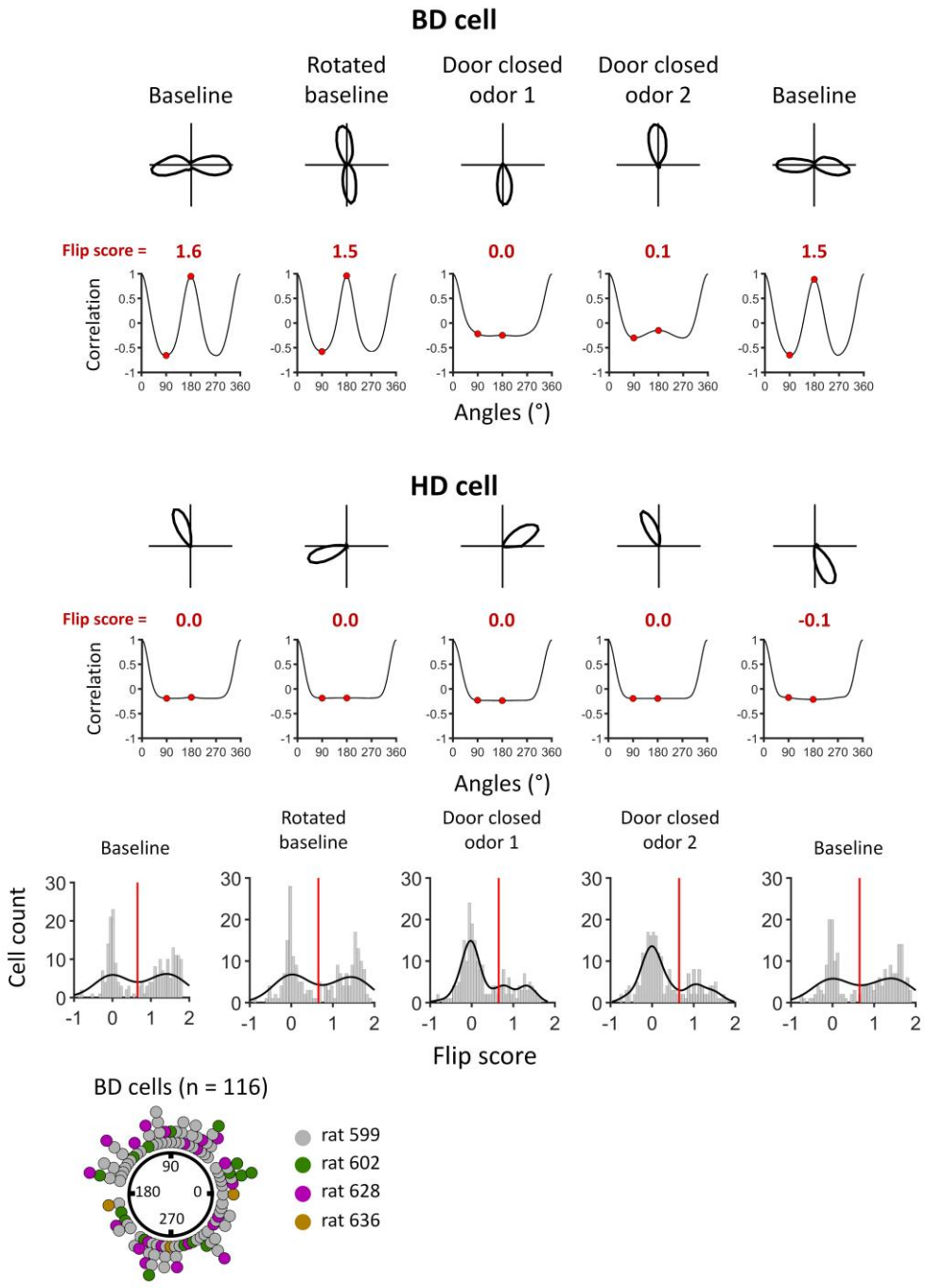
a, Firing directions in baseline Trial 1 were homogeneous (determined by a Rayleigh test) , both for the whole population of RSC HD neurons (left; n = 96; z = 0.63; p = 0.54) or for the subset recorded in random-start conditions (right; n = 59; z = 1.12; p = 0.33). **b and c**, Test for use of olfactory context cues. Because HD cells tend to rotate coherently, values for co-recorded cells were averaged to provide an ensemble value. Ensembles and trials were, however, treated as independent data points. Data are for trials 2-5 for the 28 random-start ensembles and shaded according to whether start compartment changed between trials. All firing directions are referred to that in the lemon compartment in Trial 1, with firing directions in the remaining trials expressed as a deviation from this. Data are plotted relative to the expected firing direction based on how the cell fired in baseline trial 1 (expected = zero degrees; directions shown by the compass rose). **b,** polar plots showing the data collapsed across time; statistics indicate deviation from bimodality (= successful context discrimination). **c,** The same data as in **b** but linearized and plotted as a function of recording day. There was significant preference for the original firing direction in some trials, but also significant ambiguity (Supplementary Table 2). Therefore, the HD system can use odor cues to break environmental symmetry, but this effect is weak.



Supplementary Figure 3

Polar plots from six BD cells.

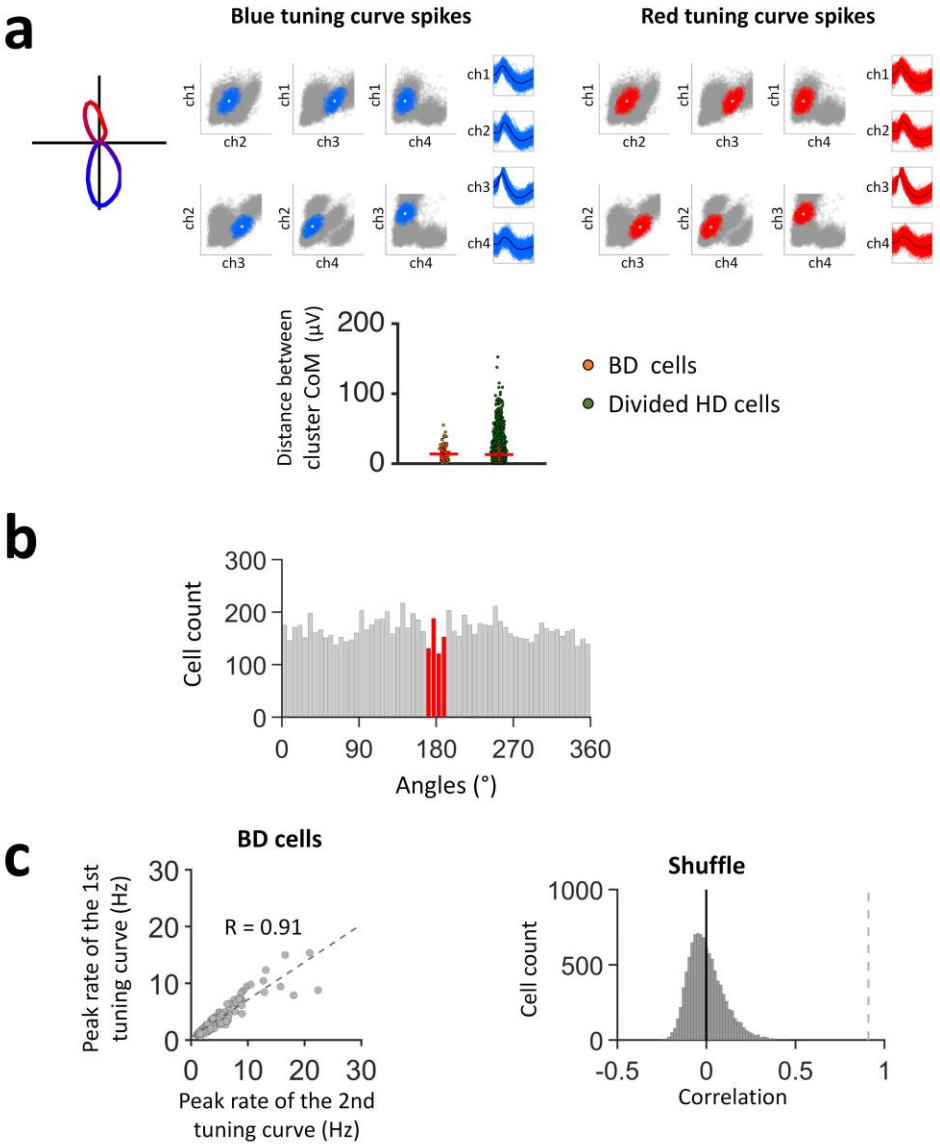
Note that the cells showed bi-directional firing, with two tuning curves at 180 degrees, illustrated as in Fig. 1. Note that when firing is analysed for only one compartment the tuning curves become uni-directional, indicating directional firing that is compartment-specific and reverses between the compartments. The directionally color-coded spatial spike plots (fourth row of each panel) confirm the compartment-specific distribution of directionality



Supplementary Figure 4

Calculation of the bimodality of directional firing (the 'flip score').

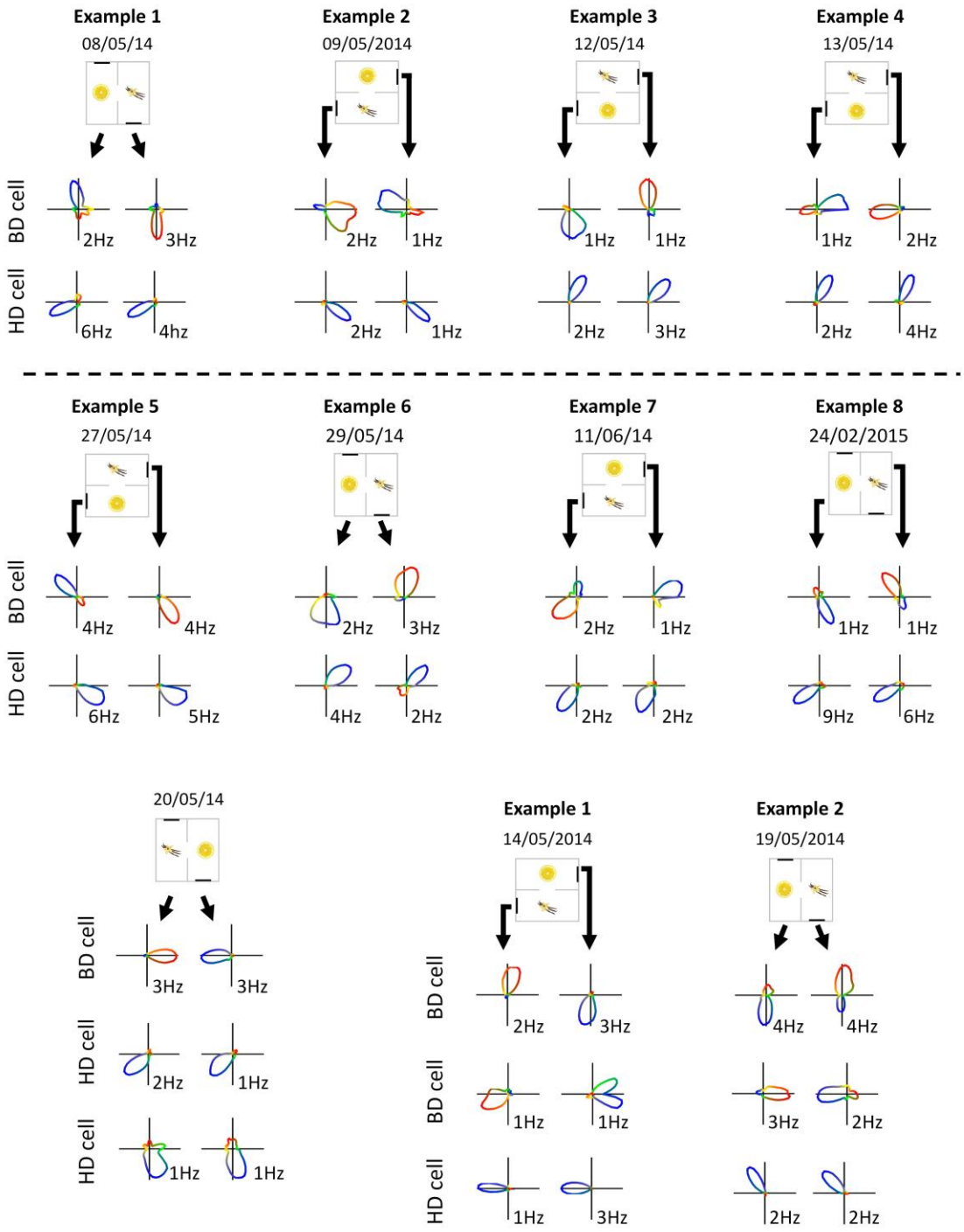
a, Computation of the flip score for a BD (top) and a classic HD cell (bottom) across five recording trials. The flip score is the difference of correlation values at 180° and 90° extracted from the circular autocorrelogram. **b**, Distribution of flip score values of BD and HD cells, showing the bimodality and the index. The black line shows the kernel density estimation of distributions and the red line is set at the natural splitting of flip score (value equal to 0.6). **c**, Polar distribution of firing direction of all 116 BD cells after using the double-angle procedure (see Methods). Each dot corresponds to a BD cell firing direction and is color-coded according to rat (n = 4). There was no significant clustering of firing direction (Rayleigh test, p = 0.31).



Supplementary Figure 5

Ruling out that bi-directional tuning curves might have been recording/analysis artefact.

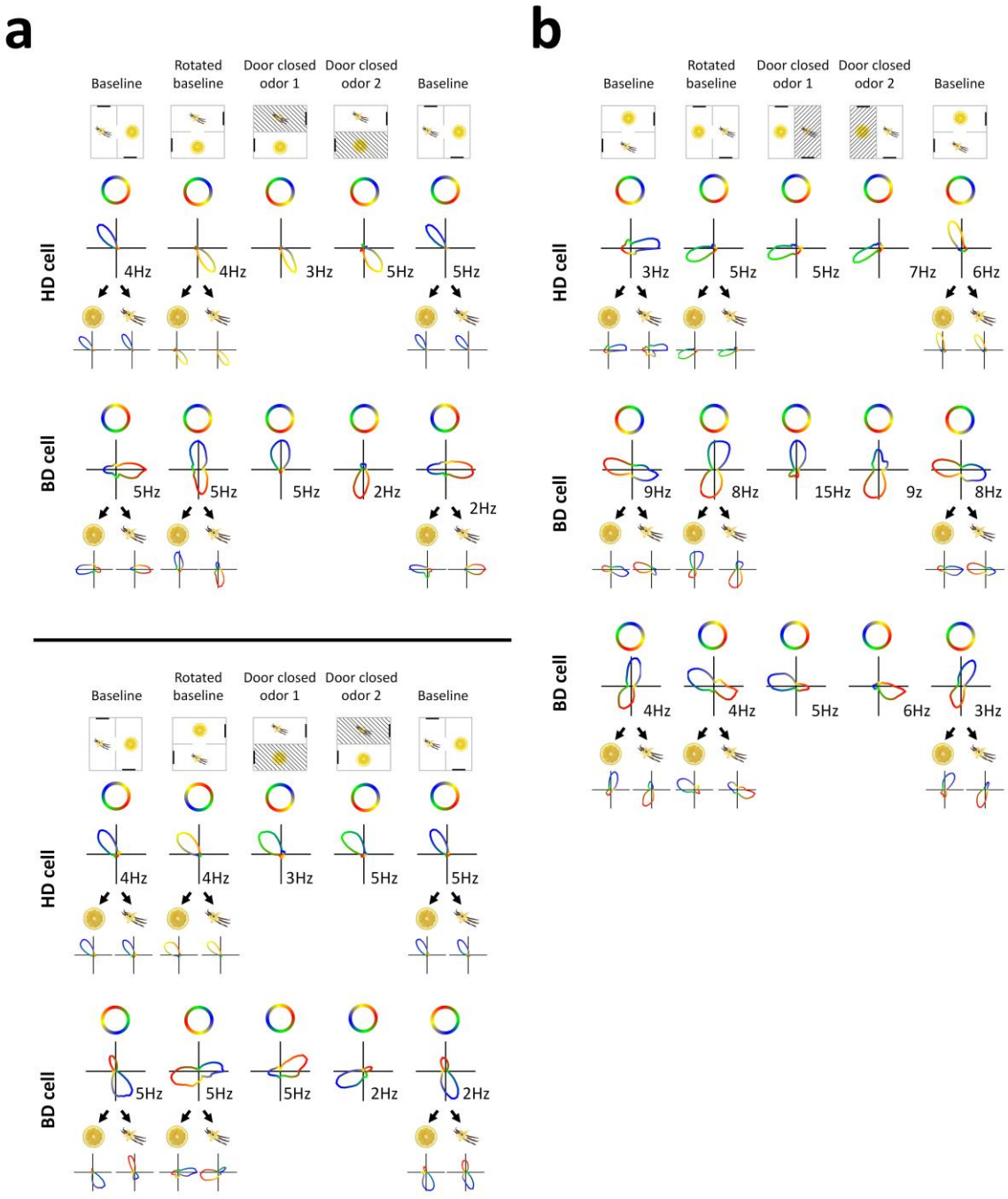
a, BD cells were not two cells because centers of mass (CoM) of clusters from the individual tuning curves were no further apart in cluster space than for HD cells randomly divided in two. The Euclidean distance in µV between the centers of mass (CoM) of BD clusters was compared with the distance generated by a control procedure, in which 96 HD cell clusters were arbitrarily divided in two 400 times. The distances of the resulting sub-clusters were then averaged. Mean (+/- s.d.) CoM distance (uV) between the two sub-clusters of BD cells was 13.79 +/- 10.35 and for the randomized data it was 12.98 +/- 10.25; these did not differ [$t(38513) = 0.85$, $p = 0.40$]. **b**, The angles between tuning curves of randomly selected pairs of HD cells were near 180 degrees apart only 4% of the time (red bars) whereas 100% of the 116 double-tuning curve cells in our data had angular separations that fell within this range. ($\chi2(1) = 185$, $p < 0.0001$). **c** Peak firing rates for the individual tuning curves of the BD cells were highly correlated (left; $R = 0.91$) whereas for randomly selected cell-pairs the firing rates showed a low correlation (right; $R = 0.00 \pm 0.01$), which the value for the data (dotted line) far exceeded ($z = 12.04$, $p < 0.00001$). And finally, apparent bi-directionality due to confusion of the large vs small headstage lights (used to determine head direction) can be ruled out because HD and BD cells were co-recorded (Fig. 2, Supp. Fig. 6).



Supplementary Figure 6

Co-recording of BD and HD cells reveals dissociation between tuning curve directions in different compartments.

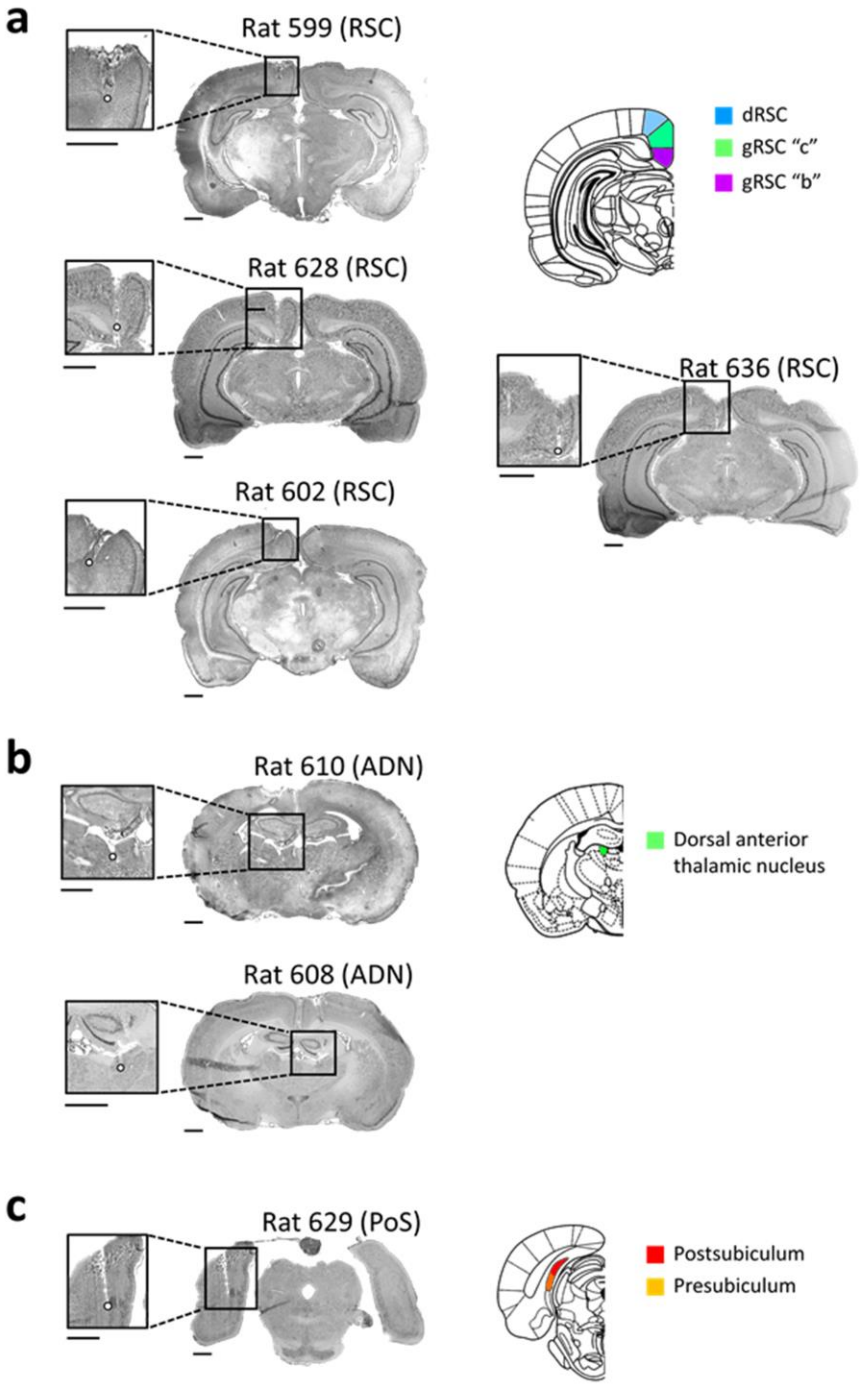
a, Eight examples, from six different days and three different animals, of simultaneous recordings of BD and HD cells. **b**, Example of one BD cell simultaneously recorded with two HD cells. **c**, Two examples of two BD cells with two different firing directions simultaneously recorded with one HD cell. In all these examples, note that the BD cell tuning curves reverse between compartments while the HD cell tuning curves do not.



Supplementary Figure 7

Disjunctive rotations of HD and BD cells further reveal dissociated directionality.

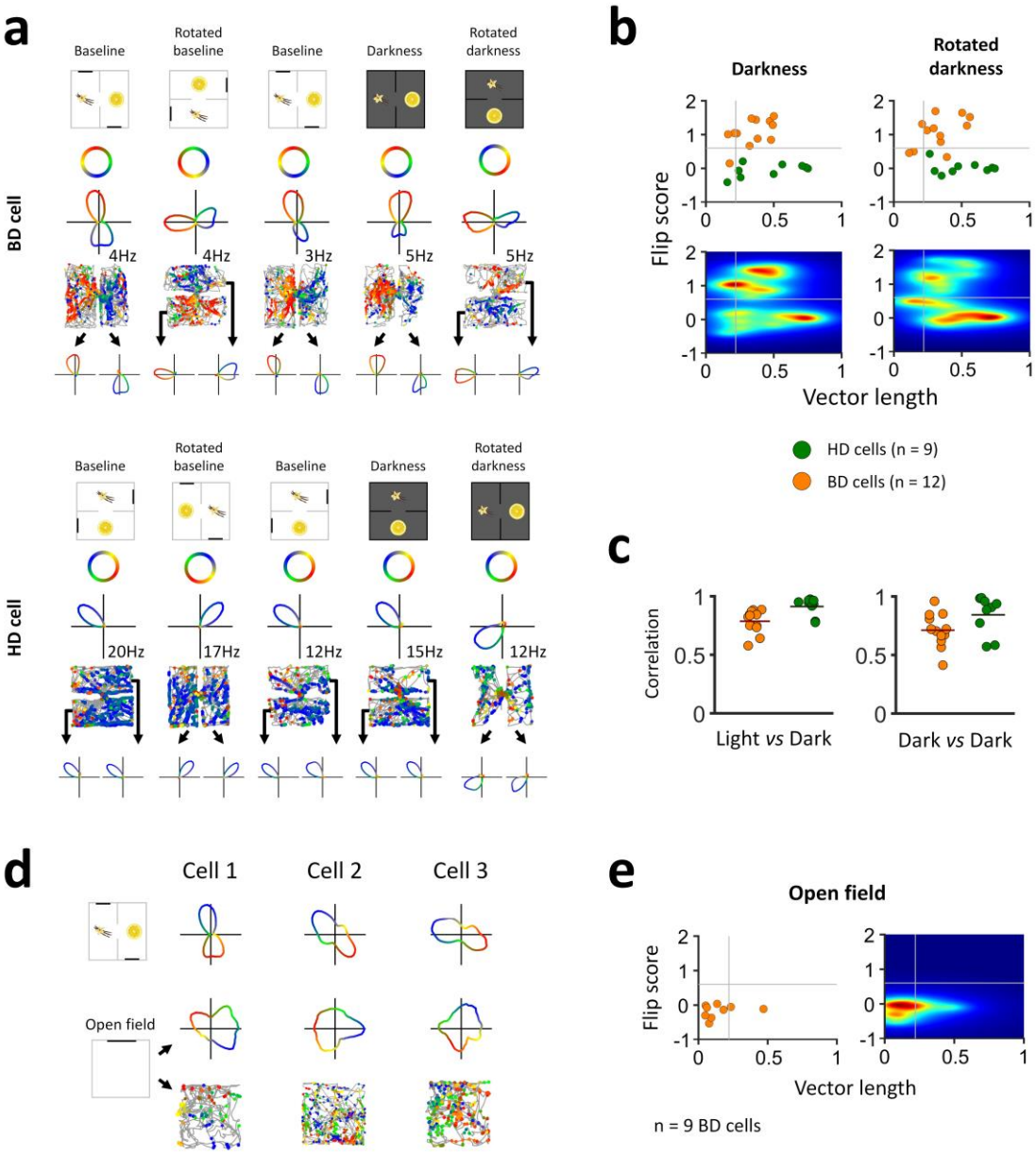
a, Simultaneous recordings of HD and BD cells from two recording sessions (top and bottom). **b**, Example of an HD cell simultaneously recorded with two BD cells. HD cells showed incoherent rotations of their firing directions relative to apparatus rotations, while BD cells follow these apparatus rotations. Comparing baseline with rotated baseline, all 116 BD cells followed rotation of the box, while only 75 of 96 HD cells did so; $\chi 2(1) = 28.165$, $p = 1.1 \times 10^{-7}$. Comparing trial 1 and trial 5 baselines, all 116 BD cells followed the box, while only 77 of 96 HD cells did so, $\chi 2(1) = 25{,}218$, $p = 5.1 \times 10^{-7}$. This demonstrates uncoupling within the unipolar attractor network, and suggests stronger control by visual cues over BD cells than HD cells.



Supplementary Figure 8

Histology.

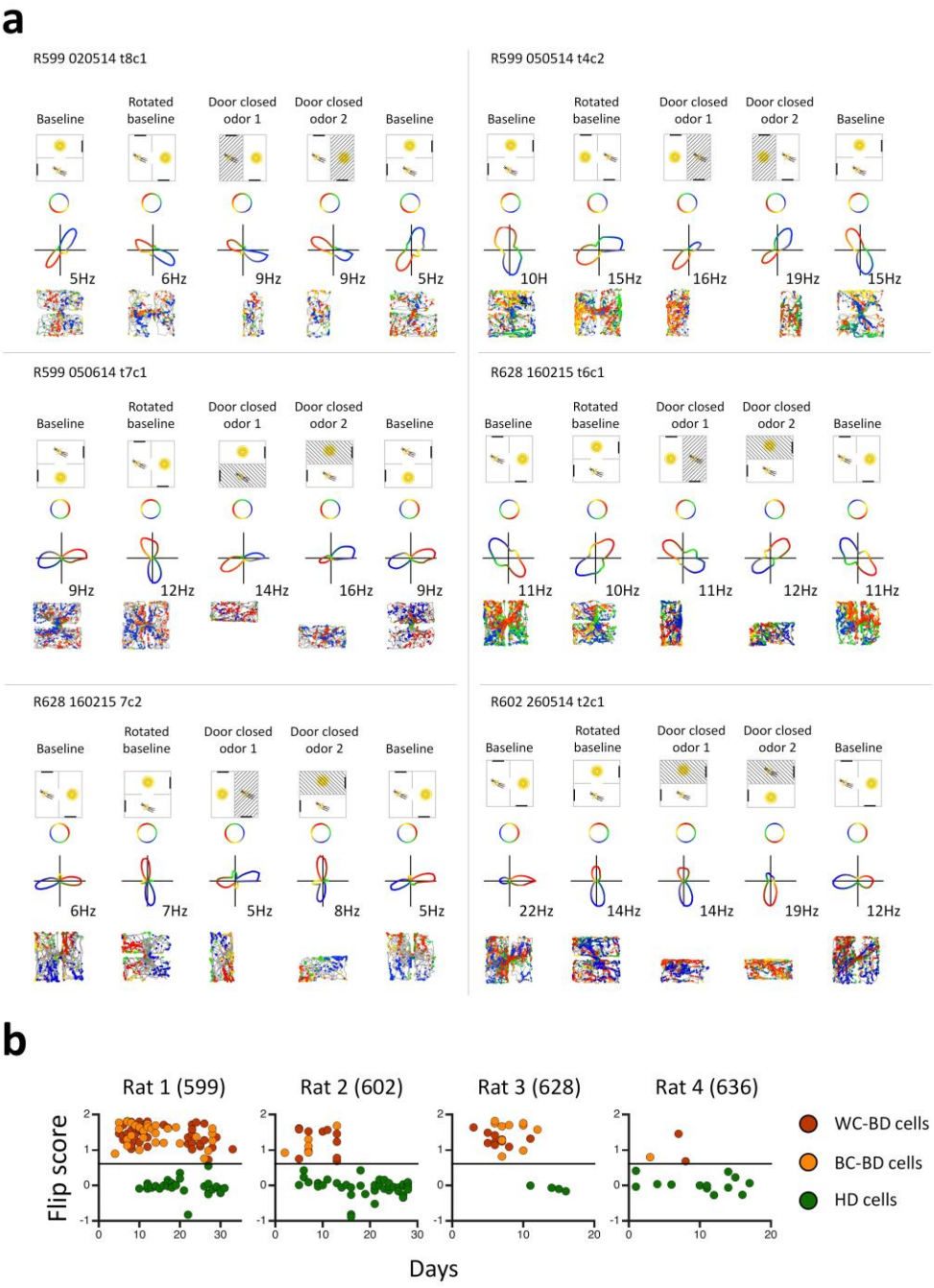
Coronal slices through the region of electrode implantation from (**a**) the retrosplenial animals (RSC), (**b**) the anterodorsal nucleus of the thalamus (ADN), and (**c**) postsubiculum (PoS). Main images are at low power and insets at high, with scale bars indicating 1 mm. The hollow dots show the deepest location at which directional neurons were found, estimated using the line drawings (right) adapted from Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 6th Edition. Amsterdam ; Boston: Academic Press/Elsevier; 2007.



Supplementary Figure 9

Preservation of firing direction reversal in the dark, but not in an open field.

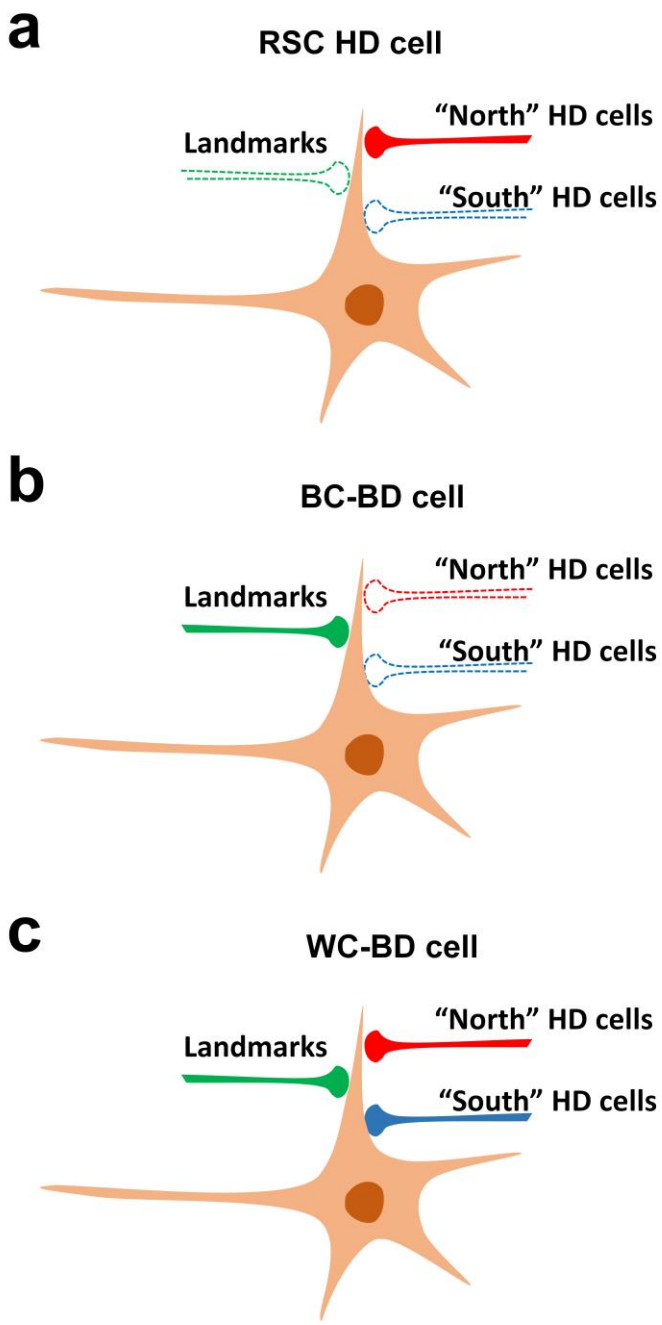
We turned off the lights and recorded RSC and PoS cells in light followed by two dark trials (n = 12 sessions), the first being a continuation of the last trial, then a second dark trial after removal, mild disorientation and a rotation of the environment. **a**, Examples of BD and HD cells recorded during two darkness trials, showing preserved directionality. **b**, Scatter and data density distributions showing a similar distribution between the two populations in darkness as in light (average bi-directionality or 'flip' score for BD cells in both 'darkness' and 'rotated darkness' trials = 1.06 ±0.40 for both). **c**, Correlation of firing between light-dark trials (baseline vs. dark) and dark-dark trials (dark vs. rotated dark): HD cells: light/dark = 0.91; dark/dark = 0.84 ; BD cells: light/dark = 0.78; dark/dark = 0.71. These high correlations indicate that the bi-directional firing is maintained without visual information. Both types of cells rotated coherently with the apparatus during the second dark trial, suggesting that directional firing was not due solely to visual cues. **d,** Examples of three BD cells recorded in an open field. No clear directional activity is apparent. **e**, Scatter and data density distribution showing that 9 BD cells lost both their directional and bi-directional pattern in an open arena (average flip score = -0.17 ± 0.18).



Supplementary Figure 10

Within-compartment bi-directional activity.

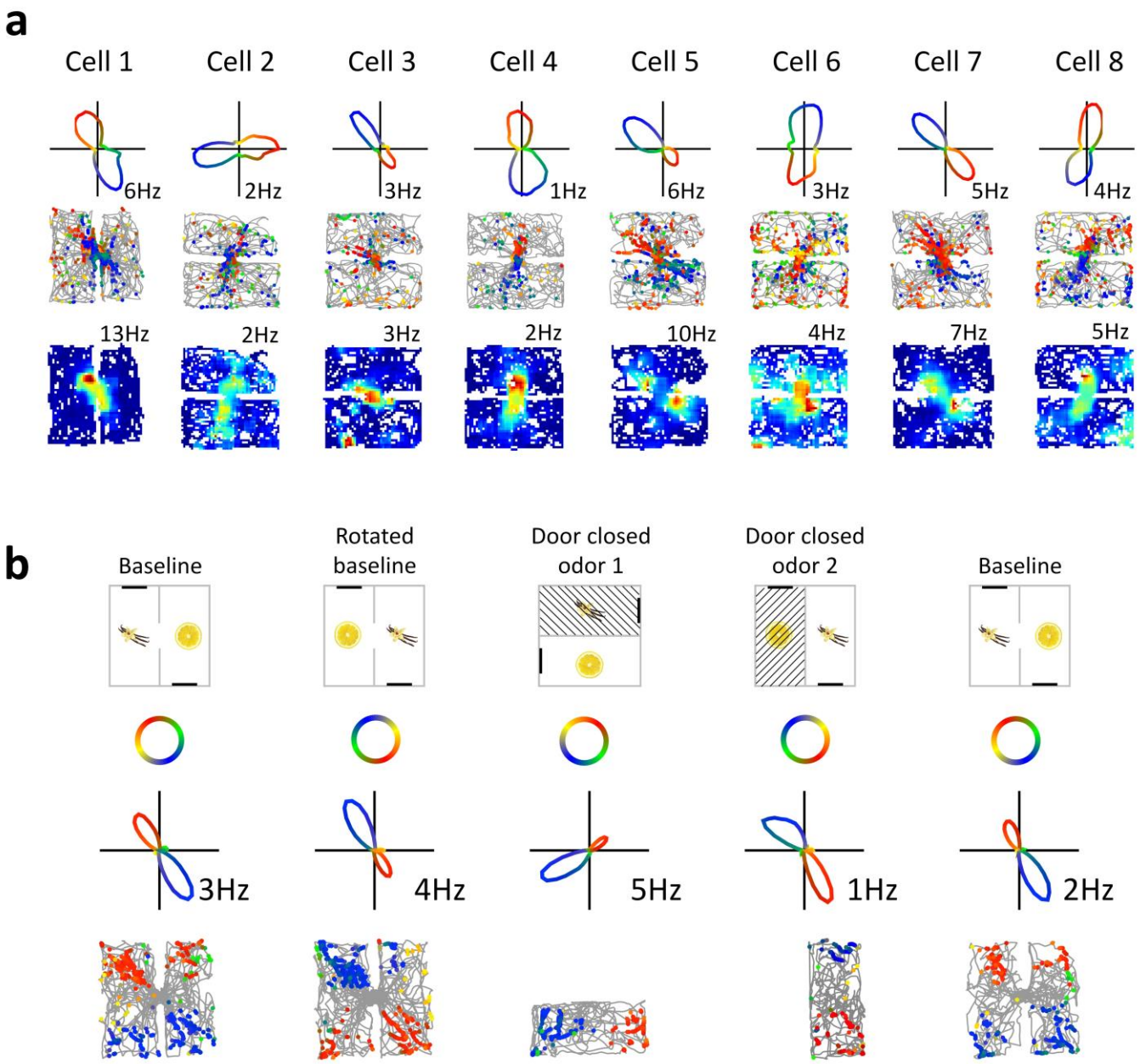
a, Polar plots from six within-compartment bi-directional (WC-BD) cells. Note that for door-closed odor 1 and 2 trials the tuning curves remained bi-directional, indicating directional firing that reversed within the compartments. The directionally color-coded spatial spike plots (fourth row of each panel) show that firing direction is not compartment-specific as it is with the between-compartment bi-directional (BC-BD) cells. **b,** Distribution of flip score as a function of recording days for BC-BD cells and WC-BD cells, and HD cells, in trial 1 for the four RSC implanted animals. The two types of bi-directional cells were both evident from the first recording day indicating that plasticity, if it occurs, must be rapid.



Supplementary Figure 11

Hypothesis concerning formation of within-compartment bi-directional firing patterns.

a, A RSC HD cell receives an input from the main HD network, and fires when the rat faces in just one direction. **b**, A BC-BD cell mainly receives inputs from the local landmark array, and fires when the rat faces in a direction defined by the array; hence it reverses firing direction between compartments. **c**, A WC-BD cell is driven by landmark inputs, and so initially fires "North" in one compartment and "South" in the other. This induces it to be co-active with "North" and South" HD cells, which acquire inputs by Hebbian co-activation. Now, the cell can fire when the rat faces in either direction. However, in one of the directions it also receives inputs from the landmarks, and so fires slightly more strongly.



Supplementary Figure 12

Spatial patterns of firing by BD neurons.

a, Eight BD cells showing spatial activity related to the doorway. **b**, Examples from a WC-BD cell revealing a spatial reversing pattern of the tuning curves within each compartment. Such examples were extremely rare, but suggest a spatial component in the inputs to RSC directional neurons.