

1 **The place-cell representation of volumetric** 2 **space in rats**

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12 **Supplementary information**

13 Supplementary data (15 figures, 1 table, 2 movies)

14 Supplementary methods (4 figures, 1 table, 2 movies)

15 All figures are available for download in high resolution format at: DOI: 10.5522/04/9977435

16 **Supplementary data**

17 *Movement patterns in the lattice mazes*

18 As reported in the main text, rats explored both lattice mazes fully but explored the
19 tilted lattice less ($\chi^2(1) = 19.4$, $p < .0001$, $\eta_p^2 = 0.34$, K-W) although the small number of tilted
20 maze sessions (N = 16) should be taken into consideration when interpreting this result
21 (Supplementary Fig. 1a-b). In both configurations there was a differential distribution of time
22 spent in the vertical dimension: rats spent a median of 1.63 times longer in the bottom half of
23 the maze in its aligned configuration (Z = 2.31, $p = .021$, U3 = 0.11, WSR) and 1.95 times in
24 the tilted configuration (Z = 1.46, $p = .14$, U3 = 0.25, WSR): these did not differ ($\chi^2(1) =$
25 0.095, $p = .76$, $\eta_p^2 = 0.007$, K-W, Supplementary Fig.1c&g). Rats also tended to remain close
26 to maze boundaries (median time outer half volume / inner half volume: arena: 2.60, Z =
27 3.18, $p = .001$, U3 = 0, lattice: 1.77, Z = 2.67, $p = .008$, U3 = 0, tilted: 1.73, Z = 1.46, $p =$
28 .144, U3 = 0.25, WSR tests) and to a similar extent in all three ($\chi^2(2) = 3.15$, $p = .21$, $\eta_p^2 =$
29 0.12, K-W, Supplementary Fig. 1d&g). This is interesting because the lattice mazes had no
30 solid boundaries, the boundaries were defined solely by the termination of maze struts.

31 In the arena animals unsurprisingly moved significantly slower along the Z-axis while
32 the X and Y axes did not differ (median speed in X,Y & Z: 0.081, 0.081 & 0.025 m/s, $\chi^2(2) =$
33 19.8, $p = .00005$, $\eta_p^2 = 0.51$, FT, post-hoc: X vs Z & Y vs Z, $p < .002$, X vs Y, $p > .99$,
34 Supplementary Fig. 1e). In the aligned lattice movements along the Z-axis were significantly
35 slower (median speed in X, Y & Z: 0.055, 0.054 & 0.032 m/s, $\chi^2(2) = 14.0$, $p = .0009$, $\eta_p^2 =$
36 0.52, FT, post-hoc: X vs Z & Y vs Z, $p < .02$, X vs Y, $p > .99$, Supplementary Fig. 1e). In the
37 tilted lattice animals moved slightly faster along the Z-axis (median speed in X,Y & Z: 0.041,
38 0.043 & 0.049 cm/s, $\chi^2(2) = 8.0$, $p = .018$, $\eta_p^2 = 0.67$, FT, post-hoc: X vs Y & Y vs Z, $p > .47$,
39 X vs Z, $p = .014$) but they moved at an equivalent speed along the (now rotated) maze axes,
40 which we labelled A, B and C (median speed in A,B & C: 0.042, 0.042 & 0.041 m/s, $\chi^2(2) =$

41 2.0, $p = .37$, $\eta_p^2 = 0.17$, FT, Supplementary Fig. 1e). The small number of tilted lattice
42 sessions ($N = 16$) should be taken into consideration when interpreting these results.

43 Animals spent significantly less time moving at slower speeds ($F(24,1225) = 126.0$, p
44 $< .0001$, $\eta_p^2 = 0.71$) but this did not differ significantly between environments ($F(2,1225) =$
45 0.2 , $p = .82$, $\eta_p^2 < 0.001$) nor was there a significant interaction between the two ($F(48,1225)$
46 $= 1.2$, $p = .17$, $\eta_p^2 = 0.045$, Univariate ANOVA comparing effects of speed and environment
47 on dwell time, Supplementary Fig. 1f).

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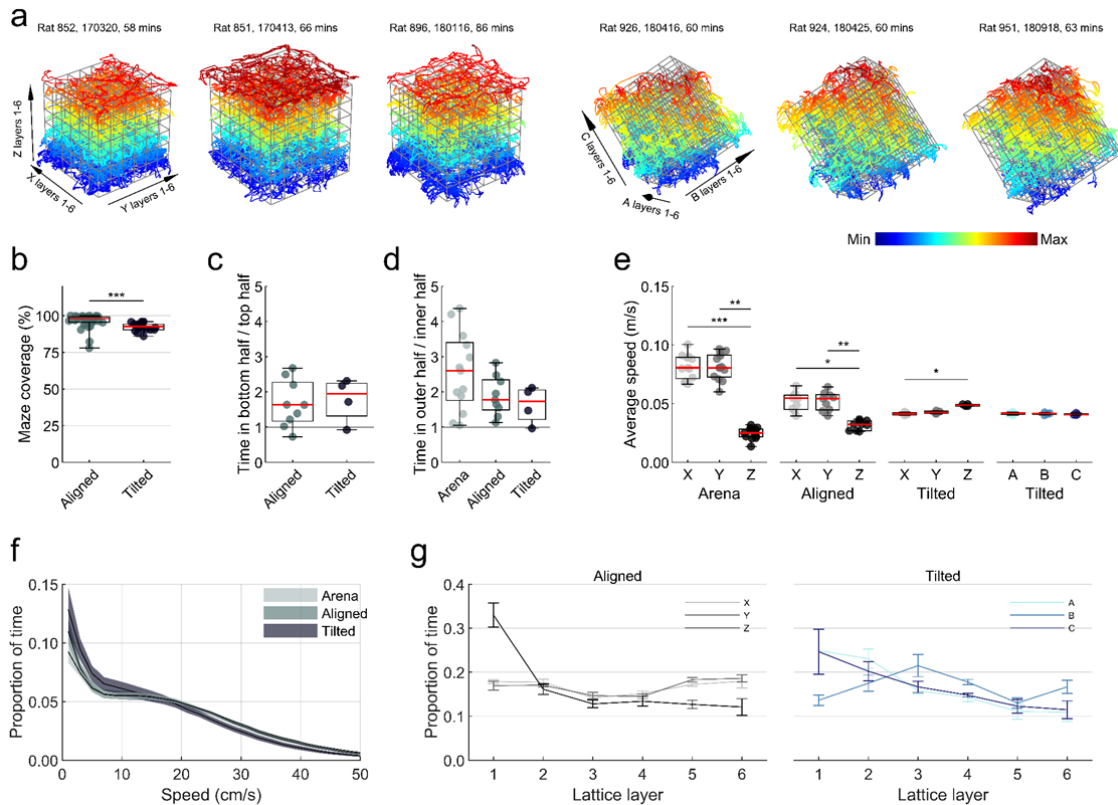
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Supplementary Fig. 1. Movement patterns in the lattice mazes. **a** Position tracking from 3 representative example aligned lattice sessions (left) and 3 tilted lattice sessions (right). Color denotes vertical height. Animals explored the full extent of the lattice, although some would not explore the very top level of the aligned lattice (first example) and some would spend more time in the bottom level (third example). Often animals would not explore the topmost vertex of the tilted lattice (fourth example). **b** Markers represent sessions. The percentage coverage (i.e. visited lattice nodes) in each maze. **c** Markers represent animals. The average ratio of time spent in the bottom half of each lattice compared to the top. **d** Markers represent animals. The average ratio of time spent in the outer half of each lattice compared to the inner half. **e** Markers represent animals. The average speed at which rats moved along each maze axis. **f** The mean and SEM proportion of time animals spent moving at various running speeds, averaged across sessions. **g** Left; aligned lattice, right; tilted lattice. Time spent in each lattice layer (see **a**), averaged across animals. Closer inspection of the distribution of dwell time in the mazes reveals that animals spent more time in the outer layers of the lattice mazes, especially in the bottom layer of the aligned lattice and the layers of the tilted lattice closest to the floor. Source data are provided as a Source Data file.

72 *Summary statistics of units and place cells*

73 **Supplementary Table 1**

Summary statistics of units and place cells recorded in all 3 environments

Maze	Rat	Sessions		Cells		Place cells		Place fields		Fields per Cell	Field elongation		Spatial information	
		n	% of total	n	% of total	n	% of total	n	% of total		μ	s.d.	μ	s.d.
Arena	750	1	2.0	11	1.0	1	0.2	1	0.1	1.00	1.27	0.00	1.10	1.46
	770	2	4.0	25	2.4	21	3.3	23	3.3	1.10	2.01	0.70	1.16	0.76
	775	4	8.0	96	9.0	60	9.6	50	7.2	0.83	1.89	0.83	1.48	1.36
	850	2	4.0	24	2.3	6	1.0	9	1.3	1.50	1.72	0.64	1.38	1.44
	851	5	10.0	62	5.8	16	2.6	24	3.5	1.50	1.69	0.46	1.38	1.24
	852	14	28.0	186	17.5	75	11.9	85	12.3	1.13	1.72	0.49	1.60	1.41
	853	3	6.0	45	4.2	20	3.2	17	2.5	0.85	1.84	0.66	1.30	1.11
	894	3	6.0	67	6.3	37	5.9	37	5.3	1.00	1.56	0.45	0.88	0.72
	896	5	10.0	196	18.4	163	26.0	179	25.8	1.10	1.85	0.59	1.40	1.01
	923	2	4.0	9	0.9	3	0.5	4	0.6	1.33	1.88	0.80	0.58	0.66
	924	2	4.0	5	0.5	3	0.5	3	0.4	1.00	1.40	0.23	1.16	0.71
	926	9	18.0	295	27.7	192	30.6	219	31.6	1.14	1.85	0.66	1.55	1.34
	951	5	10.0	43	4.0	31	4.9	42	6.1	1.35	1.83	0.67	0.98	0.62
Total	13	50	-	1064	-	628	-	693	-	-	-	-	-	-
Aligned	750	1	2.9	11	1.5	3	0.6	2	0.3	0.67	1.70	0.31	1.03	1.19
	770	2	5.9	25	3.5	18	4.2	26	4.3	1.44	1.64	0.39	1.88	2.39
	775	4	11.8	96	13.5	60	13.9	73	12.1	1.22	1.60	0.43	1.75	1.82
	850	2	5.9	24	3.4	17	3.9	26	4.3	1.53	1.81	0.61	1.48	1.04
	851	5	14.7	62	8.7	22	5.1	27	4.5	1.23	1.66	0.77	1.26	1.35
	852	14	41.2	186	26.1	80	18.5	100	16.6	1.25	1.61	0.44	1.47	1.32
	853	3	8.8	45	6.3	18	4.2	28	4.6	1.56	1.65	0.43	1.92	1.62
	894	3	8.8	67	9.4	40	9.3	63	10.4	1.57	1.75	0.59	0.73	0.70
	896	5	14.7	196	27.5	174	40.3	259	42.9	1.49	1.71	0.49	1.31	0.97
Total	9	34	-	712	-	432	-	604	-	-	-	-	-	-
Tilted	923	2	12.5	9	2.6	4	1.48	10	2.5	2.50	1.75	0.36	1.25	1.03
	924	2	12.5	5	1.4	3	1.11	2	0.5	0.67	1.39	0.14	1.43	0.90
	926	9	56.3	295	83.8	232	85.6	335	84.2	1.44	1.69	0.51	1.40	1.17
	951	5	31.3	43	12.2	32	11.8	51	12.8	1.59	1.68	0.50	1.14	0.76
Total	4	16	-	352	-	271	-	398	-	-	-	-	-	-

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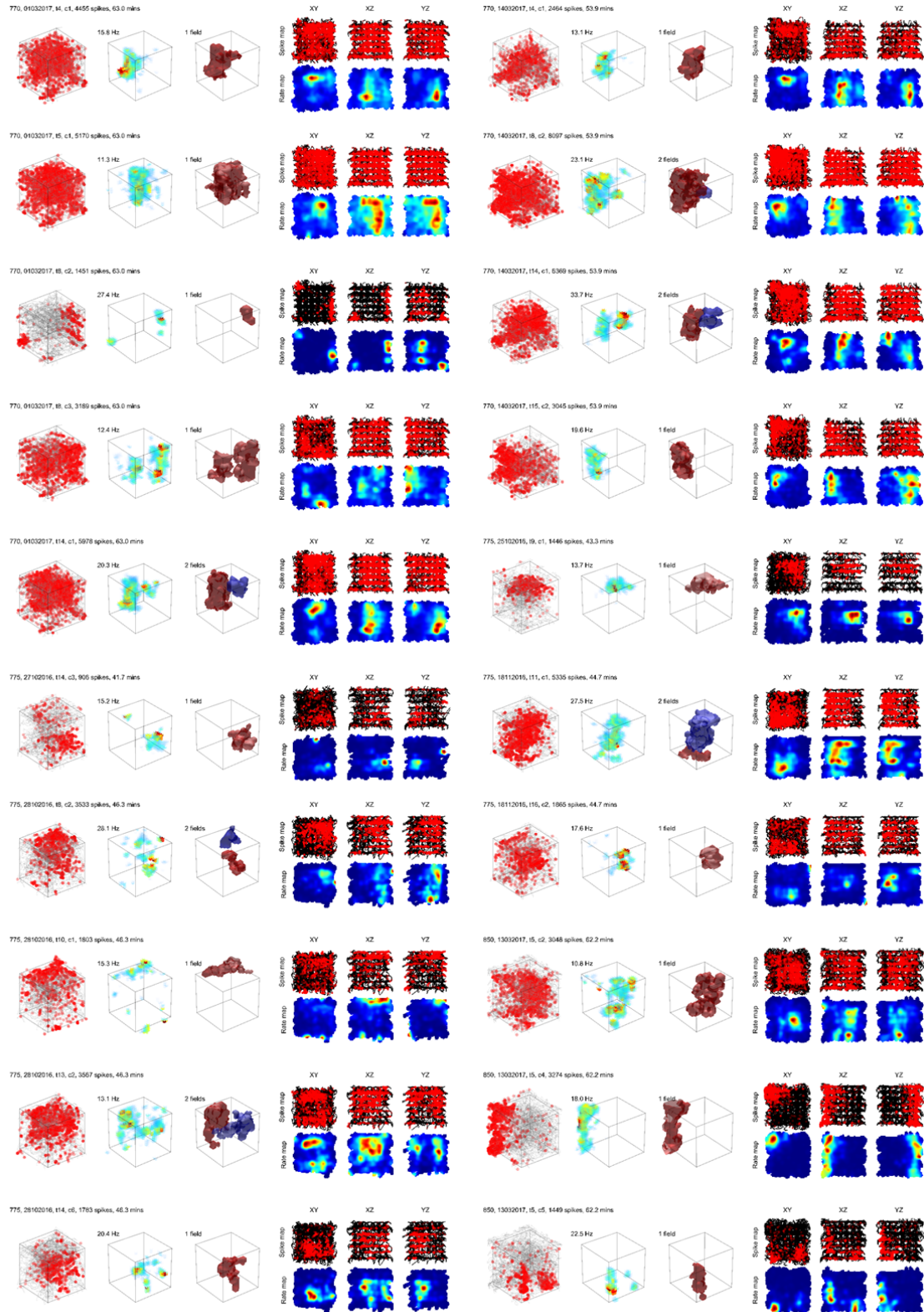
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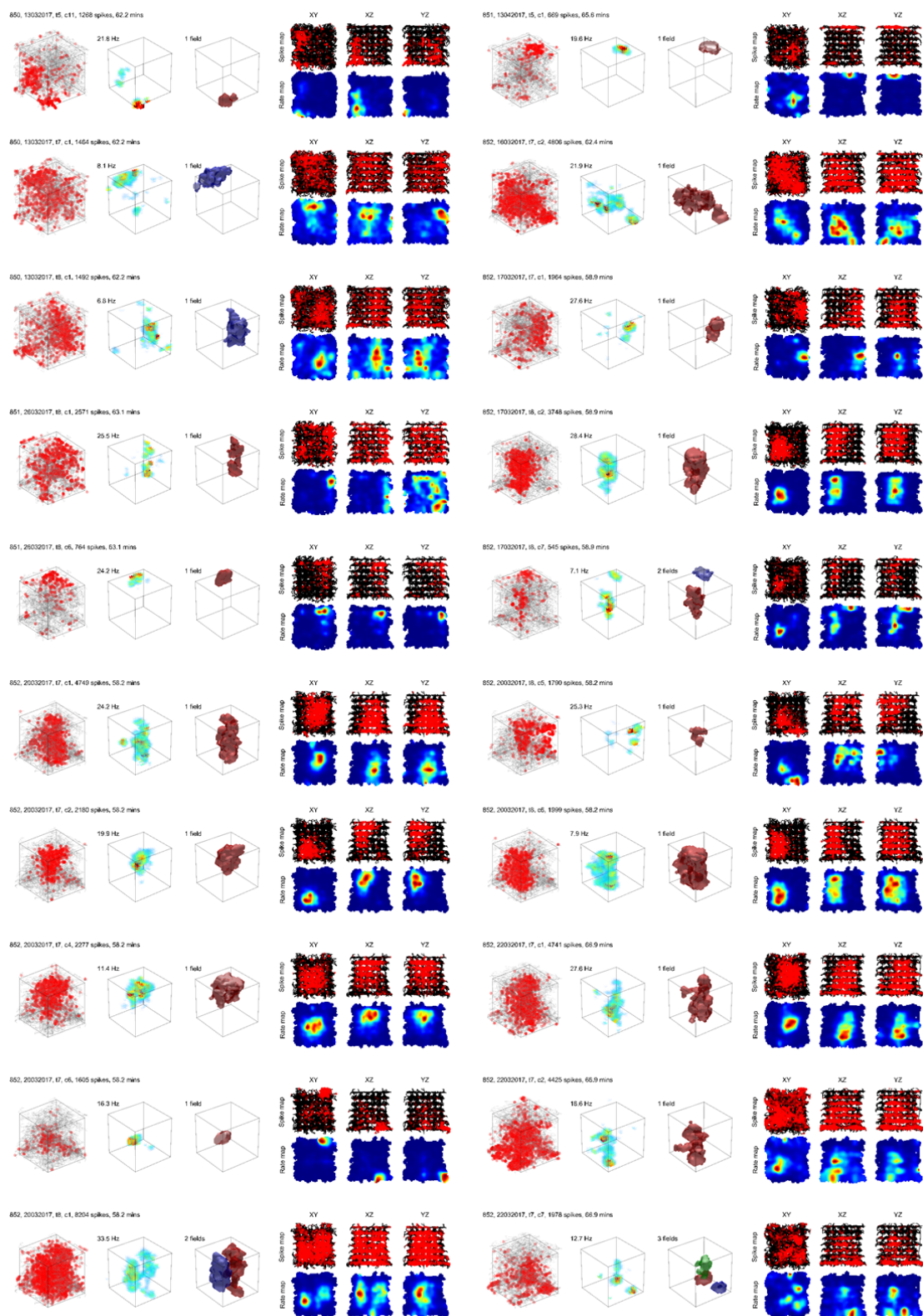
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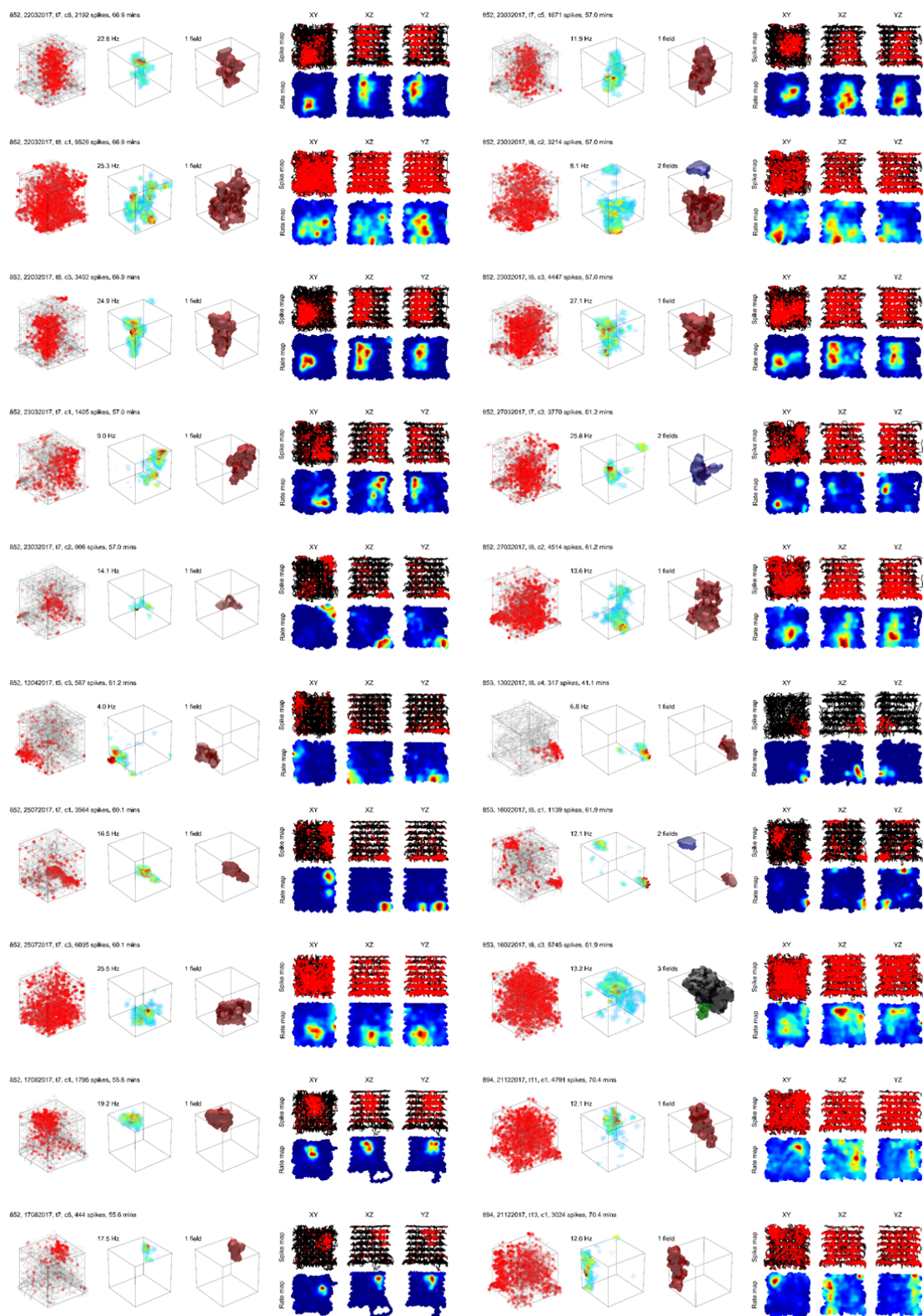
79 *Example place cells, aligned lattice*





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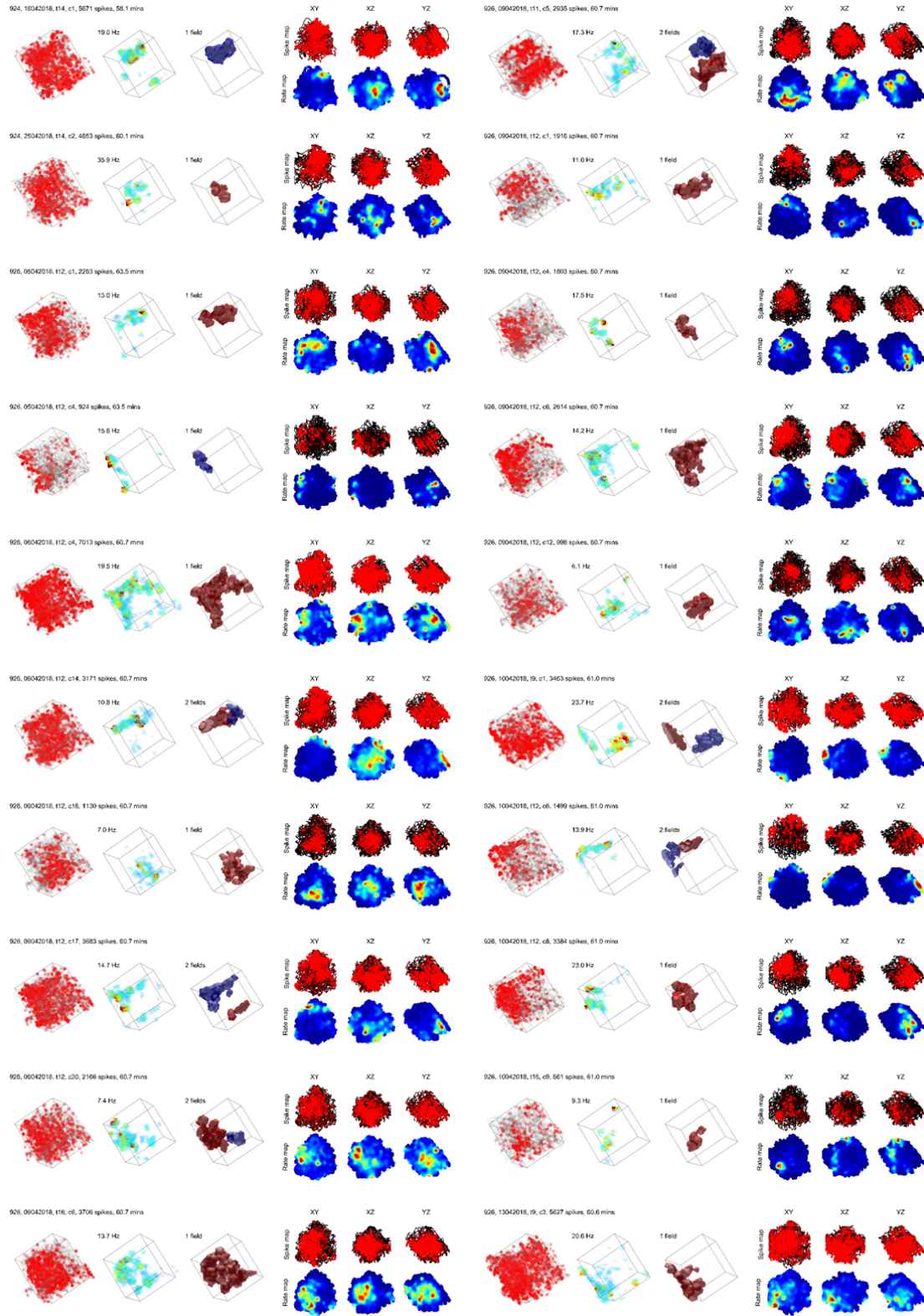


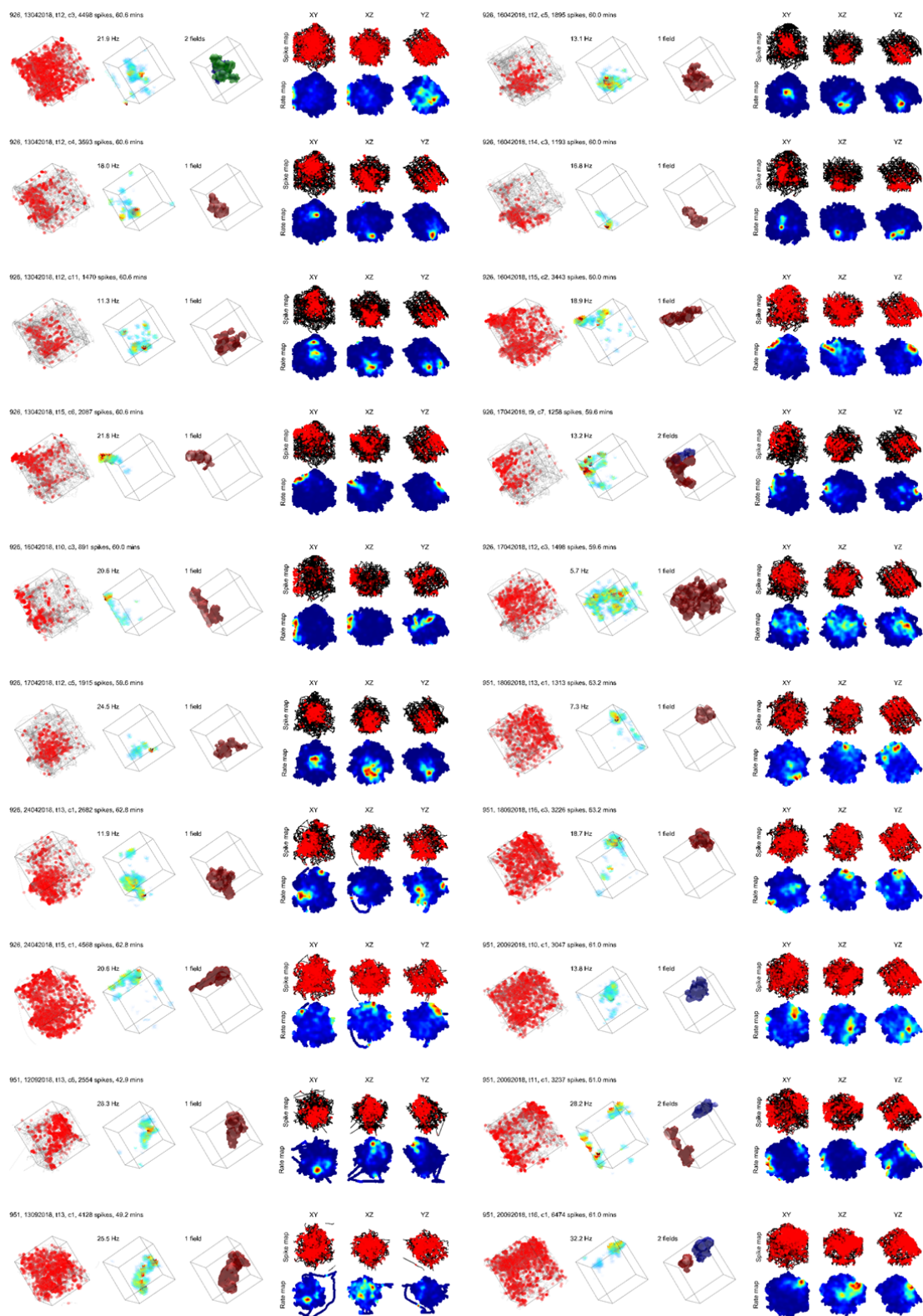
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Supplementary Fig. 2: Preceding 4 pages; activity of representative place cells recorded in the aligned lattice maze. For rotating plots see Supplementary Movie 1. These cells were chosen to reflect the varied firing patterns observed in the lattice maze, across as many sessions and animals as possible, but were also chosen for their simpler firing patterns which are easier to visualize here in 2D. Two cells are shown per row. Leftmost plots show the animal's path (grey lines) and the cell's spikes are represented by red markers. A volumetric firing rate map of the data is shown to the right of the spike plot. High firing rates are represented by hot colors, low firing rates are represented by cold colors. Voxels with low firing rates are more transparent. Additionally, unvisited voxels and voxels containing firing rate values <10% of the map maximum are completely transparent. To the right of the firing rate map is a volumetric outline of detected place fields, represented by colored polygons. Different colors represent different fields. The smaller plots to the right of this show the data when projected onto the cardinal planes. The top row shows the projected spike and position data, the bottom row shows the firing rate map produced using these projected data. The rat number, recording date, tetrode and cluster of each cell is shown above the spike and position plot.





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117 **Supplementary Fig. 3:** Preceding 2 pages; activity of representative place cells in the tilted lattice
118 maze, as in Supplementary Fig. 2. For rotating plots see Supplementary Movie 2.
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123 *Recording stability*

124 We compared the firing activity between the first and second arena sessions as a
125 measure of recording stability (Supplementary Fig. 4a, Supp. Methods: *Recording stability*).
126 When data were projected onto the XY axis, correlations between arena sessions were
127 significantly higher than would be expected by chance (blue and red areas respectively,
128 Supplementary Fig. 4b). On top of this, the median observed correlation exceeded the 95th
129 percentile of the shuffle distribution (blue and red vertical lines respectively, Supplementary
130 Fig. 4b). The same effects were observed when data were projected onto the XZ plane and
131 when correlating whole, volumetric firing rate maps (Supplementary Fig. 4b). However,
132 although YZ projections were significantly higher than chance, their median did not exceed
133 the 95th percentile.

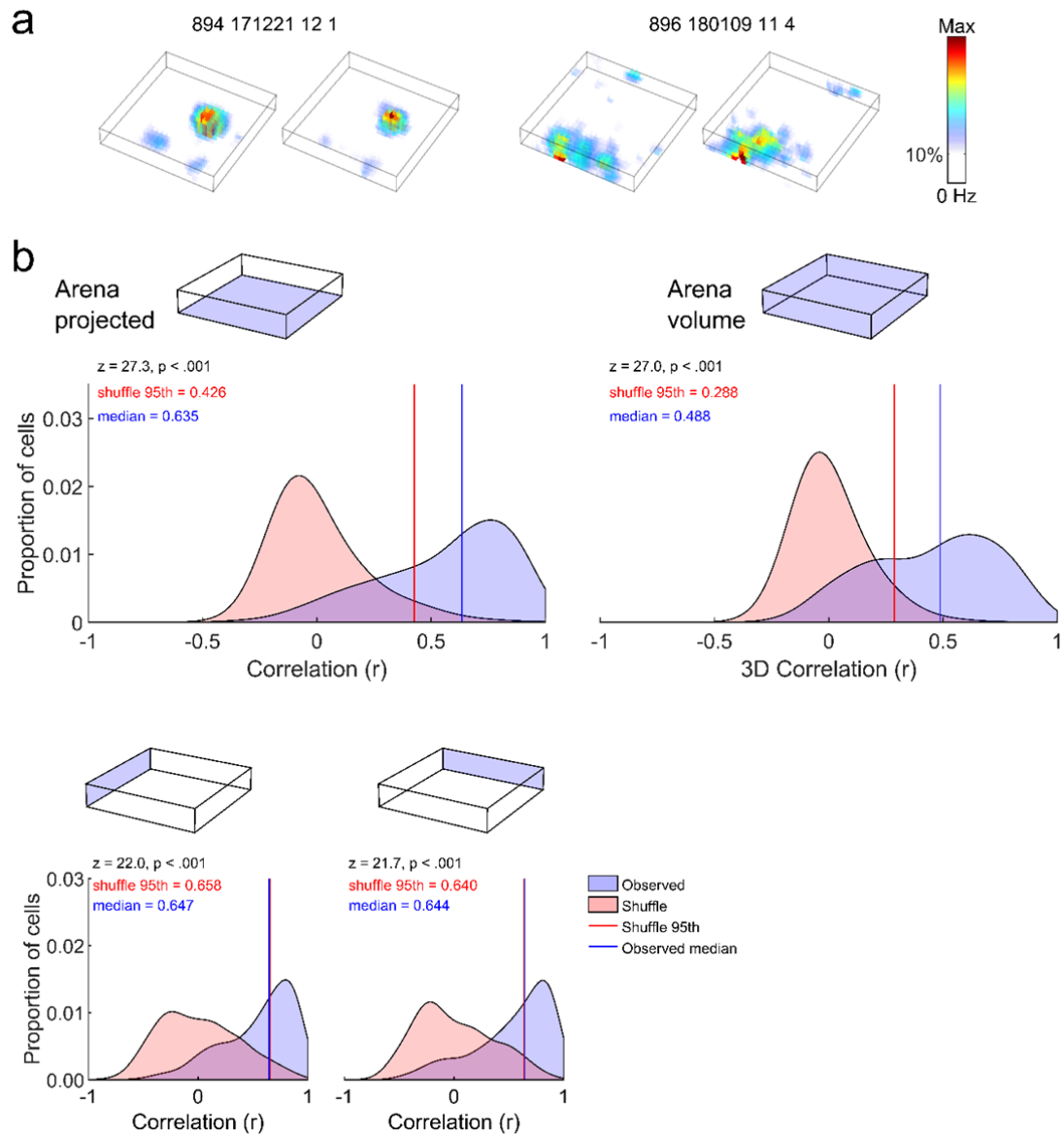
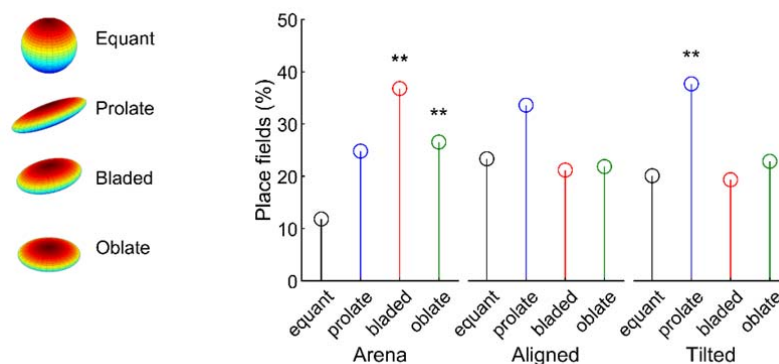


Figure S4: Stability of recording between first and second arena. **a** Consecutive arena volumetric ratemaps for two example cells. **b** Schematics indicate the type of comparison, either showing the plane of projection or highlighting the whole volume. Plots give the distribution of observed intra-trial correlation scores between the first and second open field sessions (blue shaded area) and between 1000 randomly shuffled cells (red shaded area). Black text gives the result of a right-tailed Wilcoxon rank sum test comparing the distributions. Red lines denote the 95th percentile rank position in the shuffled distribution, blue lines denote the median position in the observed distribution. The top left plot shows the result of this analysis when comparing the 1st and 2nd arena after projecting all data onto the XY plane. Smaller plots below this show the same when data are projected onto the XZ and YZ planes. Top right plot shows the result of this analysis when whole volumetric firing rate maps are compared without projection. Source data are provided as a Source Data file.

Zingg shape categorisation

Place fields took on different shapes in the mazes, most fields were elongated in the lattice mazes while they exhibited a flattened shape in the arena (Supplementary Fig. 5). Significance was determined by a random shuffle of all place field axis lengths (Supp. Methods: *Zingg shape categorization*). In the arena more fields were bladed and oblate than would be expected by chance (36.8% and 26.6%, shuffle 99th percentiles: 22.1% and 24.8%). In the lattice maze fields were most often prolate (33.6%) but no field type exceeded chance. In the tilted lattice more fields were prolate than would be expected by chance (37.7%, shuffle 99th percentile: 35.0%).

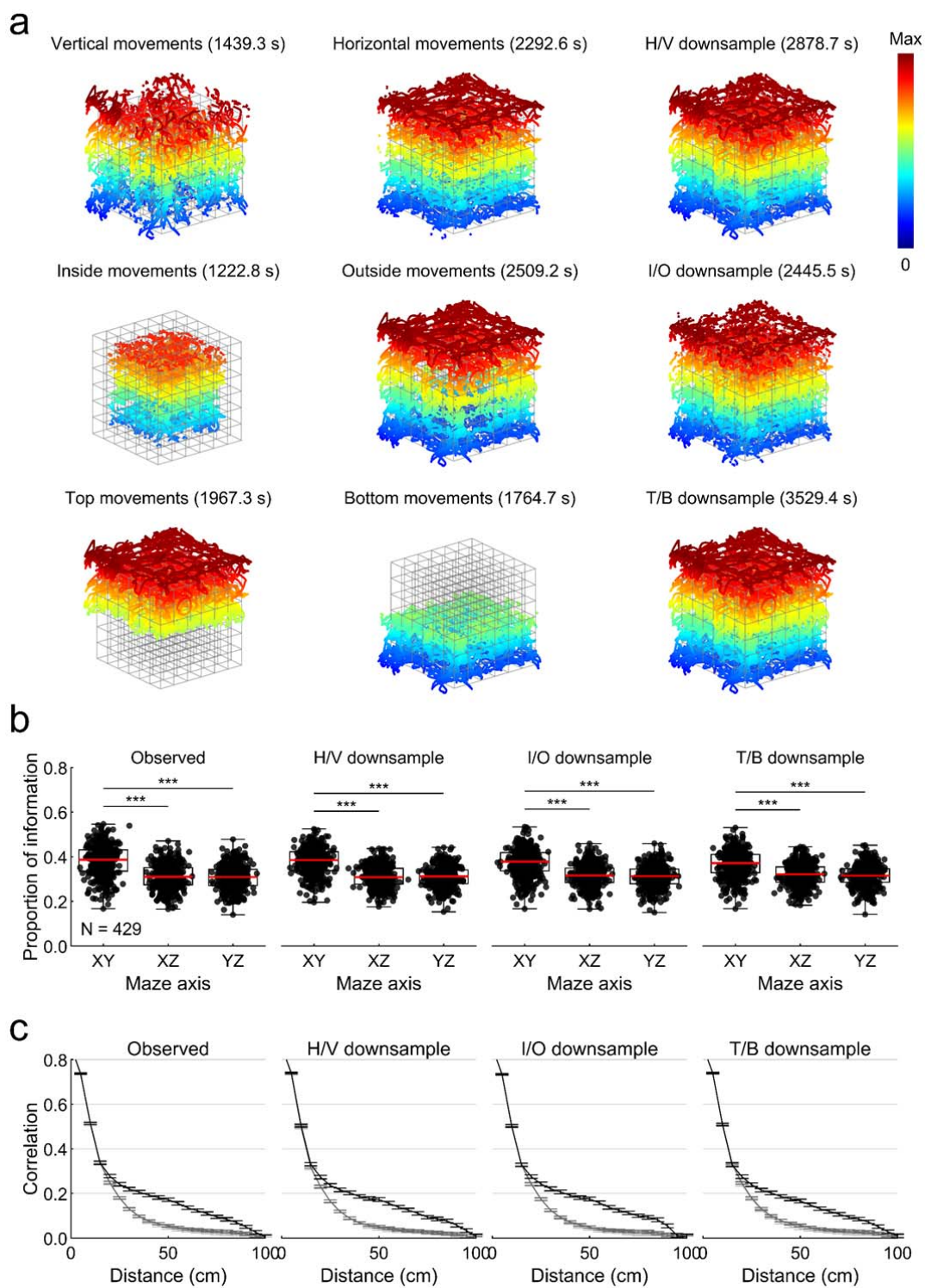


Supplementary Fig. 5: Zingg shape classification of place fields in each maze environment. Equant fields are equidimensional, prolate fields resemble a cigar, oblate fields resemble a pancake and bladed fields are different along every dimension. Observed values were compared to a shuffle, asterisks indicate the value exceeded the 99th percentile of the shuffle. Source data are provided as a Source Data file.

163 *Trajectory downsampling*

164 To test whether the animals' biased movements in the aligned lattice affected our
165 spatial information and correlational analyses we performed a downsampling procedure to
166 correct for these effects (Supplementary Fig. 6a, Supp. Methods: *Trajectory downsampling*).
167 As reported in the main text, the spatial information exhibited by place cells in the aligned
168 lattice differed along the X, Y and Z axes with horizontal slices along the Z-axis
169 demonstrating the greatest spatial information (Fig. 8). In the downsampled data this effect
170 was preserved (Supplementary Fig. 6b). As reported in the main text, autocorrelation values
171 were higher at longer distances in the aligned lattice along the Z-axis. This effect was also
172 preserved after downsampling (Supplementary Fig. 6c).

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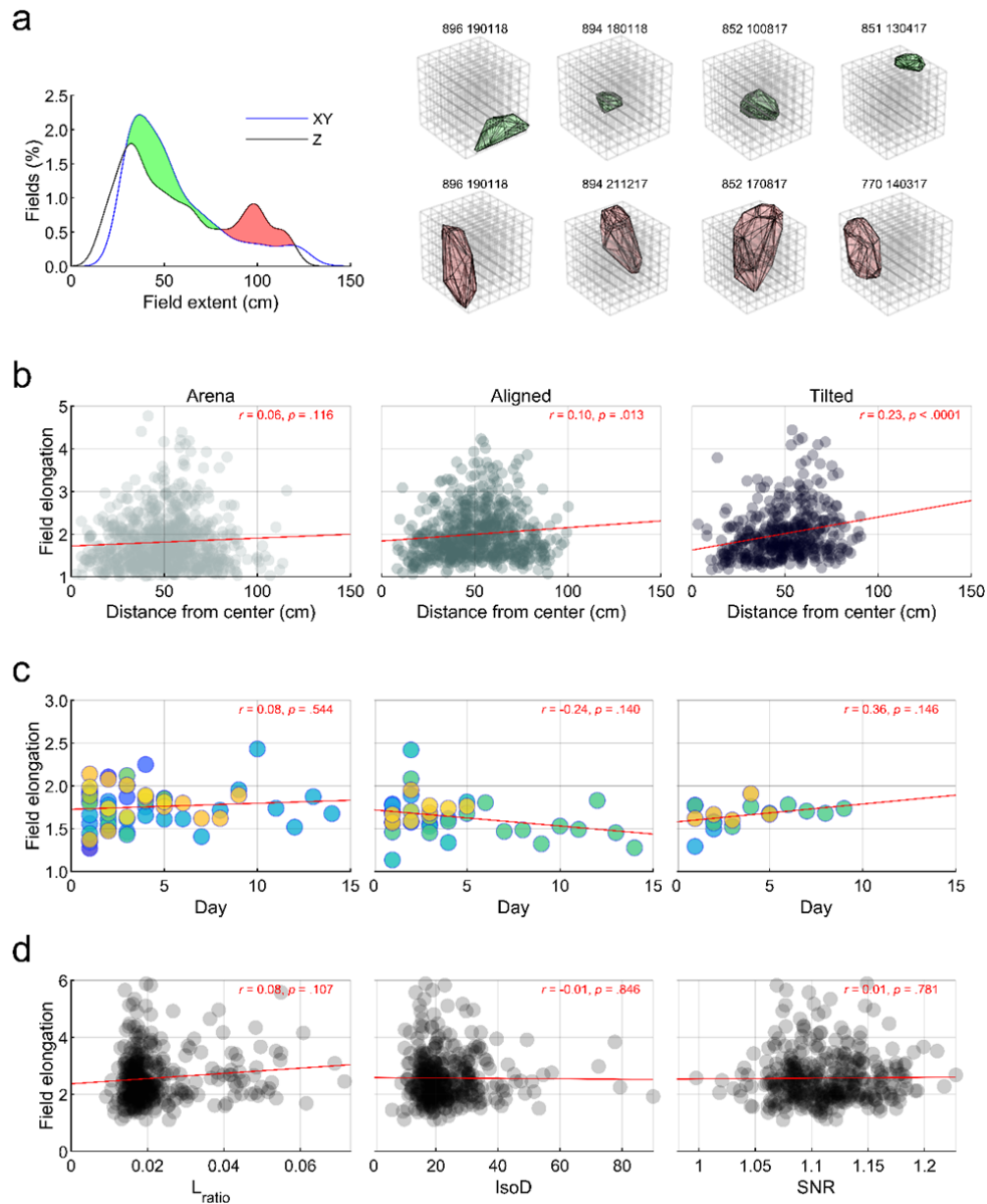


Supplementary Fig. 6: The effect of downsampling trajectories on self-similarity and spatial information. **a** example trajectory of a rat recorded in the aligned lattice, filtered to include different subsets of data. Top row; the trajectory filtered to include only the periods where he was moving vertically (left), moving horizontally (middle), or a combination of these so that 50% of the data are horizontal and vertical movements (right). Middle row; same, but the data are filtered to include only data within the inner 50% volume of the lattice, the outer 50% volume or an equal combination of these. Bottom row, the same but for the top 50% of the lattice and the bottom 50% of the lattice. **b** The same spatial information analysis reported in the main text carried out on observed/unfiltered data (left) and on the 50% combinations (right plots). **c** The same autocorrelation analysis reported in the main text carried out on observed/unfiltered data (left) and on the 50% combinations (right plots). In all cases the effects remain the same in all forms of downsampled data. Source data are provided as a Source Data file.

187 *Field elongation*

188 In the aligned lattice the lengths of fields along X and Y were unimodal (deviation
189 from unimodality: $p = .44$, $p = .41$ respectively) but the height of fields along the Z axis
190 deviated from a unimodal distribution (deviation from unimodality: $p < .001$) and instead
191 formed a bimodal one (deviation from bimodality: $p = .46$, all tests bootstrap modality tests).
192 The left-hand peak of this bimodal distribution contains many horizontally elongated fields,
193 while the smaller right-hand peak contains many vertically elongated fields, as would be
194 expected (Supplementary Fig. 7a). There was no significant deviation from modality for any
195 axis in the tilted lattice ($p > .4$ in all cases).

196 Place field elongation in the lattice mazes was weakly but significantly positively
197 correlated with field centroid distance from maze center (Supplementary Fig. 7b). There was
198 no significant relationship between field elongation and experience in the mazes, although
199 our animals were pre-exposed to small lattice mazes for weeks before recording
200 (Supplementary Fig. 7c, Methods: *Animals*). There was also no relationship between cluster
201 quality measures and place field elongation (L_{ratio} : $r = 0.084$, $p = .11$; Iso-D: $r = -0.010$, $p =$
202 $.85$; RPVs: $r = -0.100$, $p = .06$; spike amplitude: $r = -0.046$, $p = .38$; SNR: $r = -0.015$, $p = .781$,
203 pairwise Spearman's correlations, Supplementary Fig. 7d).



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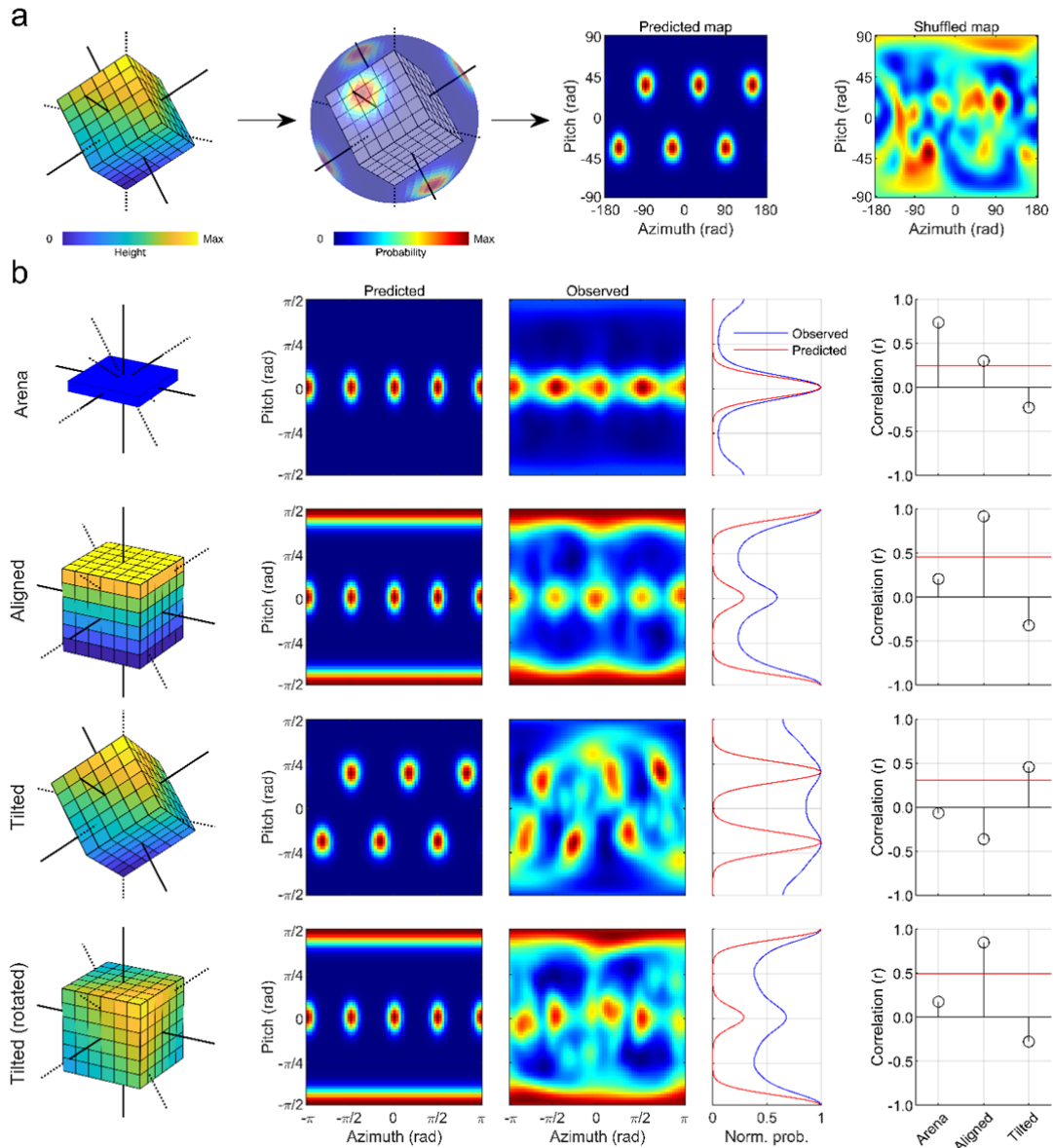
205 **Supplementary Fig. 7: Place field elongation, continued from Fig. 6. a** Two distributions are shown,
 206 the average of the X & Y distributions in Fig. 6 (blue line) and the Z distribution. The regions between
 207 these curves correspond roughly to fields that are short and horizontally elongated or long and
 208 vertically elongated (green and red shading respectively). Examples of fields satisfying these criteria
 209 can be seen on the right (green area top row, red area bottom row). **b** Scatter graphs showing the
 210 relationship between field position in the mazes and field elongation. In both lattice mazes place field
 211 elongation increases further from the maze center (i.e. closer to the boundaries). Red lines represent
 212 the least squares line of best fit, red text outlines the result of a pairwise Pearson correlation on each
 213 data set. **c** Markers represent sessions, columns are as above. The relationship between place field
 214 elongation and time in the mazes. Text gives the result of independent pairwise Spearman's
 215 correlations. Different colors correspond to different rats. **d** Markers represent fields, the relationship
 216 between place field elongation and three cluster quality measures. Red text outlines the result of a
 217 pairwise Spearman correlation on each data set. Source data are provided as a Source Data file.
 218

219 *Field orientation*

220 For more information on this analysis see Supplementary Methods: *Field orientation*
221 *and size* and Supplementary Fig. 8a. For each maze, the observed field orientation density
222 map best correlates with the prediction made for that maze based on the maze axes (arena
223 correlation with predicted arena, aligned and tilted maps: 0.74, 0.30 & -0.23 respectively,
224 chance 99th percentile: 0.38; aligned correlation with predicted arena, aligned and tilted
225 maps: 0.21, 0.92 & -0.32 respectively, chance 99th percentile: 0.41; tilted correlation with
226 predicted arena, aligned and tilted maps: -0.07, -0.36 & 0.46 respectively, chance 99th
227 percentile: 0.4; tilted (rotated) correlation with predicted arena, aligned and tilted maps: 0.17,
228 0.85 & -0.28 respectively, chance 99th percentile: 0.44). These effects can be seen in
229 Supplementary Fig. 8b.

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233 **Supplementary Fig. 8:** Place field orientation in three dimensions. **a** Diagram demonstrating how
 234 place field orientations can be predicted from maze axes. The axes of the diagonal lattice (left) are
 235 projected onto a unit sphere and the Von-Mises Fisher density of these points are mapped (middle),
 236 this map can be projected cylindrically to form a 2D map (right). Shuffled maps can be generated in
 237 the same way from random spherical points (far right). **b** From top to bottom, rows show data for the
 238 arena, aligned lattice, tilted lattice and tilted lattice after rotating its data to mimic the aligned position.
 239 The first column shows a schematic of the maze. The second column shows the predicted probability
 240 of fields aligning with every possible three-dimensional orientation if they are parallel to the mazes
 241 axes (the walls, boundaries or lattice bars) in a 2D projection. The third column shows the observed
 242 pattern of results in each case, also as a 2D projection. The fourth column shows the summed,
 243 normalized probability of observing fields at each pitch angle in both the predicted and observed data.
 244 The last column shows the result of correlating the observed field density map with each possible
 245 prediction. Red lines show the upper 99th percentile of a shuffle distribution (Supp. Methods: *Field*
 246 *orientation and size*).

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248 *Autocorrelation, spatial information and binary morphology*

249 In the main text we report the result of an autocorrelation analysis where the central
250 regions of the autocorrelation maps are extracted along each axis by interpolation. We found
251 similar results when taking the median of central portions extracted from these
252 autocorrelations. See Supp. Methods: *Autocorrelation and spatial information* for the
253 distinction between these. Utilizing this approach we found that place cell firing rate maps in
254 the aligned lattice were significantly more self-similar along the Z dimension (aligned X, Y &
255 Z median autocorrelation: 0.05, 0.06 & 0.14; $\chi^2(2) = 115.5$, $p < .0001$, FT; X vs Y, $p = 0.97$,
256 X vs Z and Y vs Z, $p < .0001$). In the tilted lattice there was a small but significant difference
257 between the X and Y axes (tilted X, Y & Z median autocorrelation: 0.02, 0.04 & 0.04; $\chi^2(2) =$
258 6.9, $p = .032$, FT; X vs Y, $p = 0.026$, X vs Z and Y vs Z, $p > .50$) although none of the axes
259 reached a correlation nearly as high as the Z axis in the aligned lattice ($\chi^2(3) = 271.6$, $p <$
260 $.0001$, K-W; all comparisons to aligned Z, $p < .0001$). There were no differences between the
261 A, B and C axes (tilted A, B & C median autocorrelation: 0.05, 0.06 & 0.06; $\chi^2(2) = 5.1$, $p =$
262 $.079$, FT). These effects can be seen in **Error! Reference source not found.9c**.

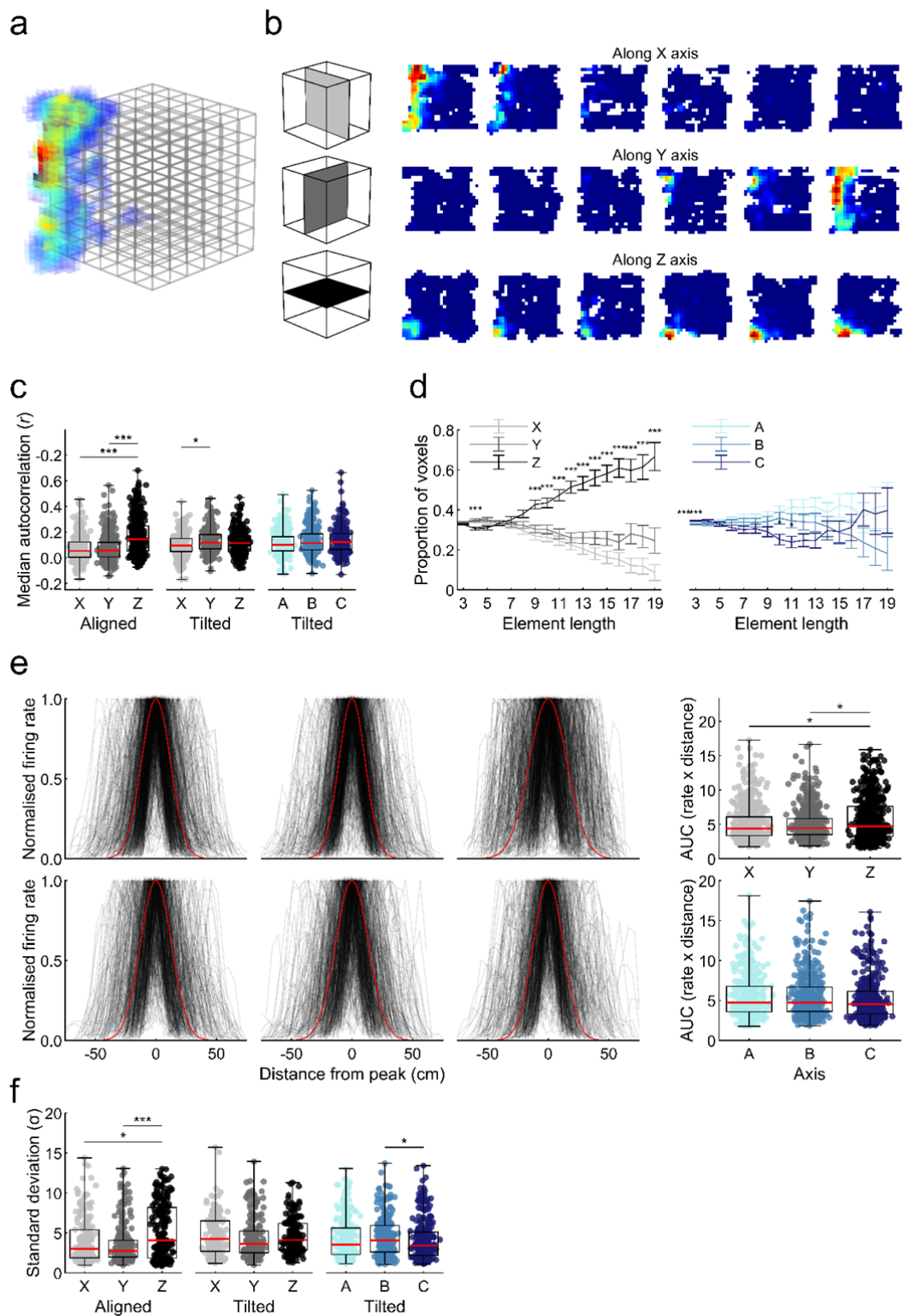
263 In addition we also employed a binary morphological approach, which utilizes
264 thresholded ratemaps (Supp. Methods: *Binary morphology*). This method concentrates on
265 the connectivity of neighboring voxels rather than firing rate or correlation. Through this
266 approach we found that aligned lattice connectivity (see Supplementary Fig. 19 for a
267 graphical explanation) was near 33% for all three axes up to a distance of 7 voxels, meaning
268 that voxels were equally connected to contiguous neighbors 7 voxels away along all three
269 axes. However, after this point the connectivity begins to diverge significantly (main effect of
270 axis: $F(2,9382) = 328.3$, $p < .0001$, $\eta_p^2 = 0.063$, interaction between axis and distance:
271 $F(35,9382) = 17.6$, $p < .0001$, $\eta_p^2 = 0.061$, repeated measures ANOVA comparing effects of
272 dimension and voxel distance on voxel proportion). Post-hoc tests confirm that each
273 dimension differs from every other, with the Z-axis demonstrating higher connectivity at

274 longer distances (X, Y & Z, mean proportion: 0.245, 0.297 & 0.457; all comparisons, $p <$
275 .0001, pairwise comparisons with Bonferroni correction).

276 In the tilted lattice connectivity was also near 33% for all three axes at shorter
277 distances but again this diverged significantly at longer distances (main effect of axis:
278 $F(2,5766) = 17.1$, $p < .0001$, $\eta_p^2 = 0.006$, interaction between axis and distance: $F(36, 5766)$
279 $= 2.2$, $p < .0001$, $\eta_p^2 = 0.014$, repeated measures ANOVA comparing effects of dimension
280 and voxel distance on voxel proportion) although this effect is accompanied by much smaller
281 effect sizes. Post-hoc tests confirmed that the A-axis demonstrated higher connectivity
282 overall, while the B and C axes did not differ (A, B & C, mean proportion: 0.376, 0.310 &
283 0.314; A vs B and A vs C, $p < .0001$, B vs C, $p > .99$, pairwise comparisons with Bonferroni
284 correction). However, in this maze all three axes remained much closer to equilibrium (33%)
285 than in the aligned lattice. The same pattern of results was also obtained using raw values
286 instead of proportions (data not shown). These effects can be seen in Supplementary Fig.
287 9d.

288 Lastly, to investigate this reduced vertical resolution at the level of individual place
289 fields we found the three orthogonal 1D Gaussians (parallel to the Cartesian axes) that best
290 fitted each place field. In the aligned lattice these Gaussians differed in terms of their
291 standard deviation (median X, Y & Z s.d.; 3.0, 2.7 & 4.1, $\chi^2(2) = 15.4$, $p = .0004$, $\eta_p^2 = .023$,
292 K-W) with the vertical Gaussian (parallel to the Z-axis) best described by a larger standard
293 deviation (X vs Z & Y vs Z, $p < .02$, X vs Y, $p > .92$). By contrast, fields in the tilted lattice
294 could be adequately described by three Gaussians with equivalent standard deviation
295 (median X, Y & Z s.d.; 4.2, 3.6 & 4.1, $\chi^2(2) = 5.0$, $p = .08$, $\eta_p^2 = .008$, K-W). However, the
296 same analysis repeated with Gaussians parallel to the axes of the tilted lattice revealed a
297 small but significant difference between the B and C axes, although this was accompanied
298 by a small effect size (Median A, B & C s.d.; 3.5, 4.1 & 3.4, $\chi^2(2) = 7.5$, $p = .023$, $\eta_p^2 = .009$,

299 K-W; A vs B & A vs C, $p > .23$, B vs C, $p = .02$). These effects can be seen in Supplementary
 300 Fig. 9f.

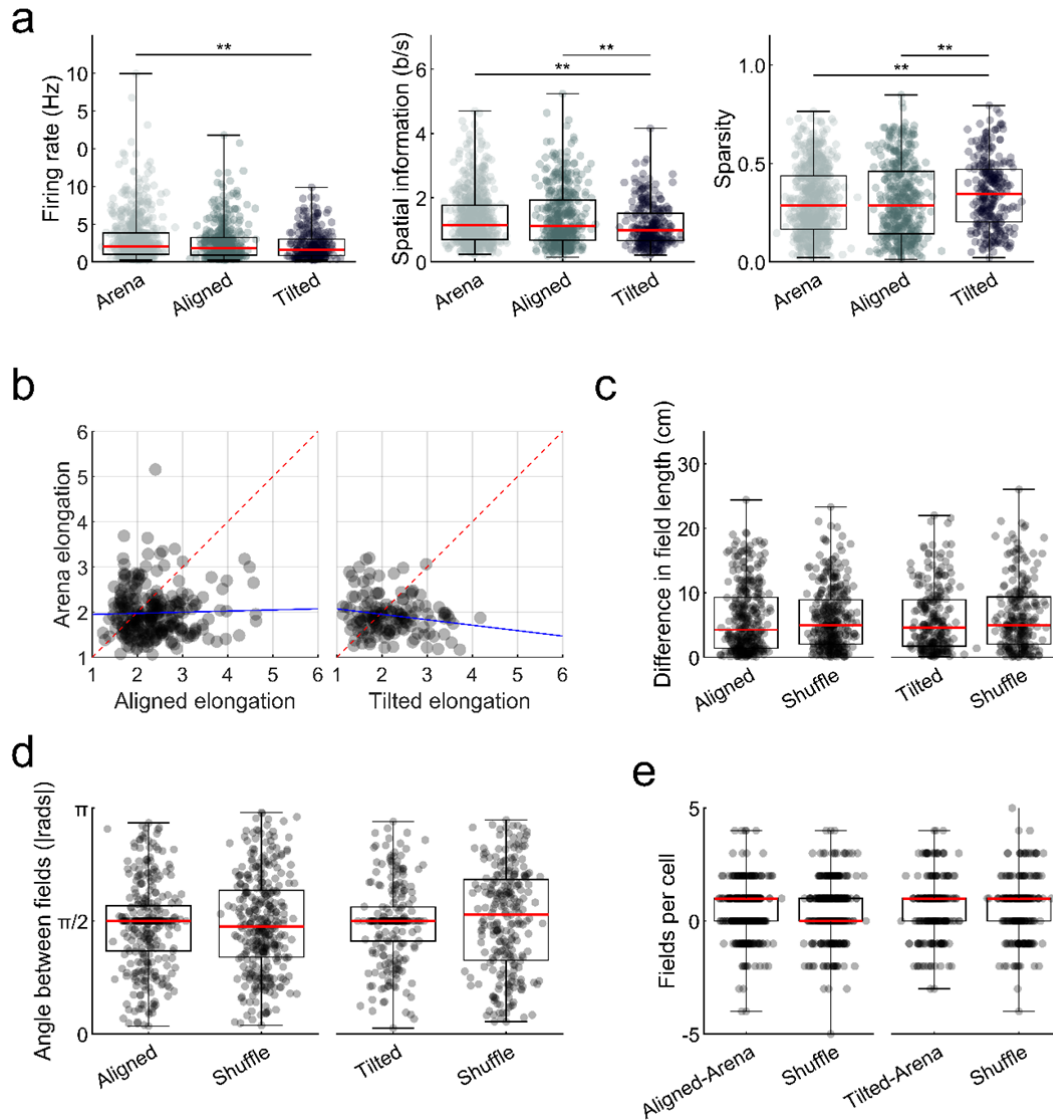


301

Supplementary Fig. 9: Spatial information in three dimensions. **a** An example ratemap of an elongated field recorded in the lattice maze. **b** The result of taking slices along each axis of the ratemap shown in **a**, so that each slice represents a layer of the lattice maze. **c** The median value in the central portion of each place cell's autocorrelogram (see Supplementary Fig. 20 for a description). Values are shown for when the portion is taken parallel to the X, Y and Z-axes. Place cells generally have a higher median autocorrelation along the Z-axis, suggesting that lattice maze ratemaps are more self-similar along this axis. **d** The results of binary morphological analysis on thresholded firing rate maps for the aligned lattice (left) and tilted lattice (right). This analysis tests how many neighboring voxels each voxel is connected to (where connected means sharing one face). In the aligned lattice, voxels are more highly connected along the Z-axis, in the tilted lattice the maze axes are equivalent. **e** Black semi-transparent lines represent the peak-normalized firing rate distribution of every place field recorded in the aligned lattice (top row) and tilted lattice (bottom row) when projected onto the X, Y & Z axes (for the aligned lattice) or the A, B & C axes (for the tilted lattice). Red lines represent the mean Gaussian fitted to each population - at a width of 3 standard deviations. Note the slightly wider shape to the Z axis projections in the aligned lattice. The right box plots show the area under the curve (AUC, Matlab function *trapz*) of these distributions. Note the significantly higher values for Z in the aligned lattice. **f** The standard deviation of orthogonal 1-dimensional Gaussians fitted to place fields in each lattice maze. Source data are provided as a Source Data file.

Comparison of firing properties between mazes

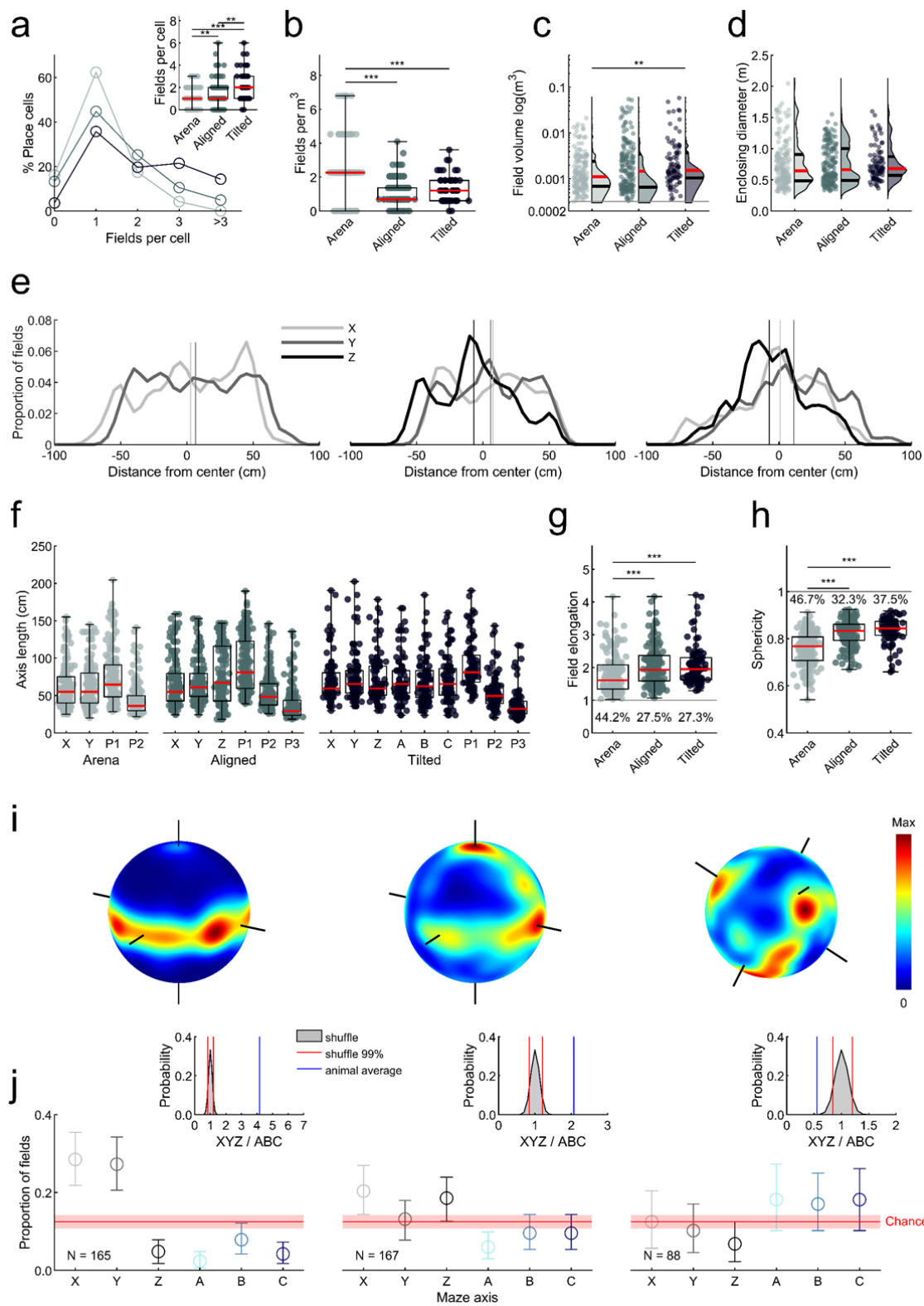
See Supp. Methods: *Comparing activity between mazes* for an explanation of the analyses used here. The firing rate of place cells was significantly lower in the tilted lattice compared to the arena ($\chi^2(2) = 10.2$, $p = .0062$, $\eta_p^2 = .008$, KW; arena vs aligned, $p = .16$, arena vs tilted, $p = .0067$, aligned vs tilted, $p = .56$, Supplementary Fig. 10a). Spatial information content calculated on volumetric firing rate maps was also significantly lower in the tilted lattice than the other mazes ($\chi^2(2) = 11.8$, $p = .0027$, $\eta_p^2 = .009$, KW; arena vs aligned, $p > .99$, arena vs tilted, $p = .0058$, aligned vs tilted, $p = .0048$, Supplementary Fig. 10a), related to this sparsity was significantly higher in the tilted lattice than the other mazes ($\chi^2(2) = 11.0$, $p = .0041$, $\eta_p^2 = .008$, KW; arena vs aligned, $p > .99$, arena vs tilted, $p = .0072$, aligned vs tilted, $p = .0080$, Supplementary Fig. 10a). There was no relationship between the average elongation of fields in the arena and aligned lattice ($r = 0.030$, $p = .64$, Pearson's pairwise correlation, Supplementary Fig. 10b) but there was a weak negative correlation between the elongation of fields in the arena and tilted lattice ($r = -0.177$, $p = .033$, Pearson's pairwise correlation, Supplementary Fig. 10b). In the lattice mazes the lengths of place fields belonging to the same cell were not more similar than would be expected by chance (aligned vs chance: $\chi^2(1) = 0.9$, $p = .35$, $\eta_p^2 = .001$, FT, tilted vs chance: $\chi^2(1) = 1.5$, $p = .22$, $\eta_p^2 = .003$, FT, Supplementary Fig. 10c) nor did fields belonging to the same cell share similar orientations at an above chance level (aligned vs chance: $\chi^2(1) = 0.0$, $p = .96$, $\eta_p^2 < .001$, FT, tilted vs chance: $\chi^2(1) = 1.5$, $p = .22$, $\eta_p^2 = .003$, FT, Supplementary Fig. 10d). The number of fields expressed by cells was also not more consistent between the arena and lattice mazes than would be expected by chance (aligned vs chance: $\chi^2(1) = 0.0$, $p = .85$, $\eta_p^2 < .001$, FT, tilted vs chance: $\chi^2(1) = 0.0$, $p > .99$, $\eta_p^2 < .001$ FT, Supplementary Fig. 10e).



Supplementary Fig. 10: Comparison of various firing and field characteristics between mazes. **a** Firing rate, spatial information content and sparsity of place cells recorded in the three mazes. **b** Relationship between field elongation in the arena and lattice mazes. Markers represent average values for cells, dotted red line denotes a linear correspondence, blue line shows the relationship resulting from a linear least squares fit. **c** Markers represent cells. For cells with multiple place fields in the aligned or tilted lattice, the average pairwise difference in major axis length of these fields. **d** Markers represent cells. For cells with multiple place fields in the aligned or tilted lattice, the average angle between their major axes. **e** Markers represent cells. For all place cells in the aligned or tilted lattice, the number of fields expressed in the lattice minus the number of fields expressed in the arena. Source data are provided as a Source Data file.

357 *Subsampled dataset analysis*

358 To investigate if the effects we observed could be due to the overrepresentation of a
359 small population of cells due to repeated sampling we recomputed all of the main analyses
360 on a dataset containing only one session per rat (the session with the most place cells).
361 Using this subsampled dataset virtually every statistical effect reported in the main text was
362 replicated (Supplementary Fig. 11, Supplementary Table 2 & Supplementary Table 3)
363 suggesting that these effects cannot be due to repeated sampling. These analyses can also
364 be replicated using the provided data set and code.



Supplementary Fig. 11: Summary figure of main results when using only one session per rat (the session with the most cells). Source data are provided as a Source Data file. Markers in boxplots represent fields. Omnibus test results can be seen in Supplementary Table 2; post-hoc test results are displayed here: * = significant at the .05 level, ** = significant at the .01 level, *** = significant at the .001 level. **a** Number of place fields exhibited by place cells in each maze. Inset: same data in boxplot representation. **b** Number of fields per cubic meter exhibited by place cells in each maze. **c** Distribution of place field volumes observed in each maze. **d** Enclosing diameter of place fields in each maze. **e** The kernel smoothed distribution of place fields along the X, Y and Z dimensions of the arena, aligned lattice and tilted lattice respectively (Z is not shown for the arena). Vertical lines represent the median value of these distributions. **f** Cartesian axis and principal axis lengths of place fields in all three mazes (P1-3 are the principal, semi-major and semi-minor axes respectively). **g** Elongation index of all place fields in each maze. An index of 1 (grey line) would indicate a spherical field, higher values indicate elongation. The percentage of fields that are equally or more spherical than would be expected by chance is given by the text below each boxplot. **h** Same as **g** but for sphericity. **i** Three-dimensional heat plots of place field orientation for the three maze configurations. Note concentration around the three axes of the aligned and tilted mazes. **j** Graphs show proportion of total fields oriented roughly parallel to each possible maze axis. Circles give the observed proportion per axis, error bars represent 95% confidence intervals calculated through a bootstrapping procedure. Red lines show the 50th percentile of a shuffle distribution while shaded red areas denote the interval between the 2.5th and 97.5th percentiles. Inset plots show the result of a shuffle testing the probability of observing this ratio of total XYZ fields to total ABC fields by chance. Red lines denote the 1st and 99th percentile rank positions in the shuffled distribution of ratio values (grey area), blue line denotes the overall ratio value averaged across rats.

403 **Supplementary Table 2**

Same as Table 1 but for analyses using the subsampled dataset containing only one session per rat

Comparison	Test	Results	Fig.
Proportion of total time along X & Y, arena	FT	$\chi^2(1) = 0.08, p = .78, \eta_p^2 = 0.001$	-
Proportion of total time along X & Y, aligned		$\chi^2(1) = 0.11, p = .74, \eta_p^2 = 0.0041$	-
Proportion of total time along A, B & C, tilted		$\chi^2(2) = 1.50, p = .47, \eta_p^2 = 0.125$	-
Fields per cell, arena, aligned & tilted	KW	$\chi^2(2) = 34.30, p < .0001, \eta_p^2 = .095$	Supplementary Fig. 11a
Fields per m ³ , arena, aligned & tilted		$\chi^2(2) = 100.05, p < .0001, \eta_p^2 = .277$	Supplementary Fig. 11b
Field volume, arena, aligned & tilted		$\chi^2(2) = 8.70, p = .0129, \eta_p^2 = .020$	Supplementary Fig. 11c
Field diameter, arena, aligned & tilted		$\chi^2(2) = 1.8, p = .026, \eta_p^2 = .004$	Supplementary Fig. 11d
Field elongation, arena, aligned & tilted		$\chi^2(2) = 27.10, p < .0001, \eta_p^2 = .064$	Supplementary Fig. 11g
Field elongation arena	WSR (compare to 1)	$Z = 11.12, p < .0001, U3 = 0$	Supplementary Fig. 11g
Field elongation aligned		$Z = 11.20, p < .0001, U3 = 0$	Supplementary Fig. 11g
Field elongation tilted		$Z = 8.14, p < .0001, U3 = 0$	Supplementary Fig. 11g
Field sphericity, arena, aligned & tilted	KW	$\chi^2(2) = 100.80, p < .0001, \eta_p^2 = .239$	Supplementary Fig. 11h
Field sphericity arena	WSR (compare to 1)	$Z = -11.14, p < .0001, U3 = 1$	Supplementary Fig. 11h
Field sphericity aligned		$Z = -11.20, p < .0001, U3 = 1$	Supplementary Fig. 11h
Field sphericity tilted		$Z = -8.14, p < .0001, U3 = 1$	Supplementary Fig. 11h
Field length distributions, aligned	Multiple KS with Bonferroni	X vs Y: $z = 0.12, p > .50$ X vs Z: $z = 0.16, p = .048$ Y vs Z: $z = 0.16, p = .048$	-
Field length distributions, tilted		$p > .99$ in all cases	-
Autocorrelation aligned, X, Y & Z	FT	$\chi^2(2) = 44.10, p < .0001, \eta_p^2 = .105$ X vs Z & Y vs Z, $p < .0001, X$ vs Y, $p > .76$	-
Autocorrelation tilted, A, B & C		$\chi^2(2) = 3.0, p = .225, \eta_p^2 = .018$	-
Proportion of spatial information aligned, X, Y & Z		$\chi^2(2) = 44.30, p < .0001, \eta_p^2 = .10$ X vs Z & Y vs Z, $p < .0001, X$ vs Y, $p > .27$	-
Proportion of spatial information tilted, A, B & C		$\chi^2(2) = 2.8, p = .24, \eta_p^2 = .018$	-
Area under curve, aligned, X, Y & Z		$\chi^2(2) = 7.9, p = .019, \eta_p^2 = 0.015$ X vs Y, $p > .99, X$ vs Z, $p = .022, Y$ vs Z, $p = .11$	-
Area under curve, tilted, X, Y & Z axes		$\chi^2(2) = 1.2, p = .56, \eta_p^2 = 0.0044$	-
Area under curve, tilted, A, B & C axes		$\chi^2(2) = 0.2, p = .92, \eta_p^2 = 0.0006$	-

Test abbreviations and details can be found in Methods: Statistics

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408 **Supplementary Table 3**

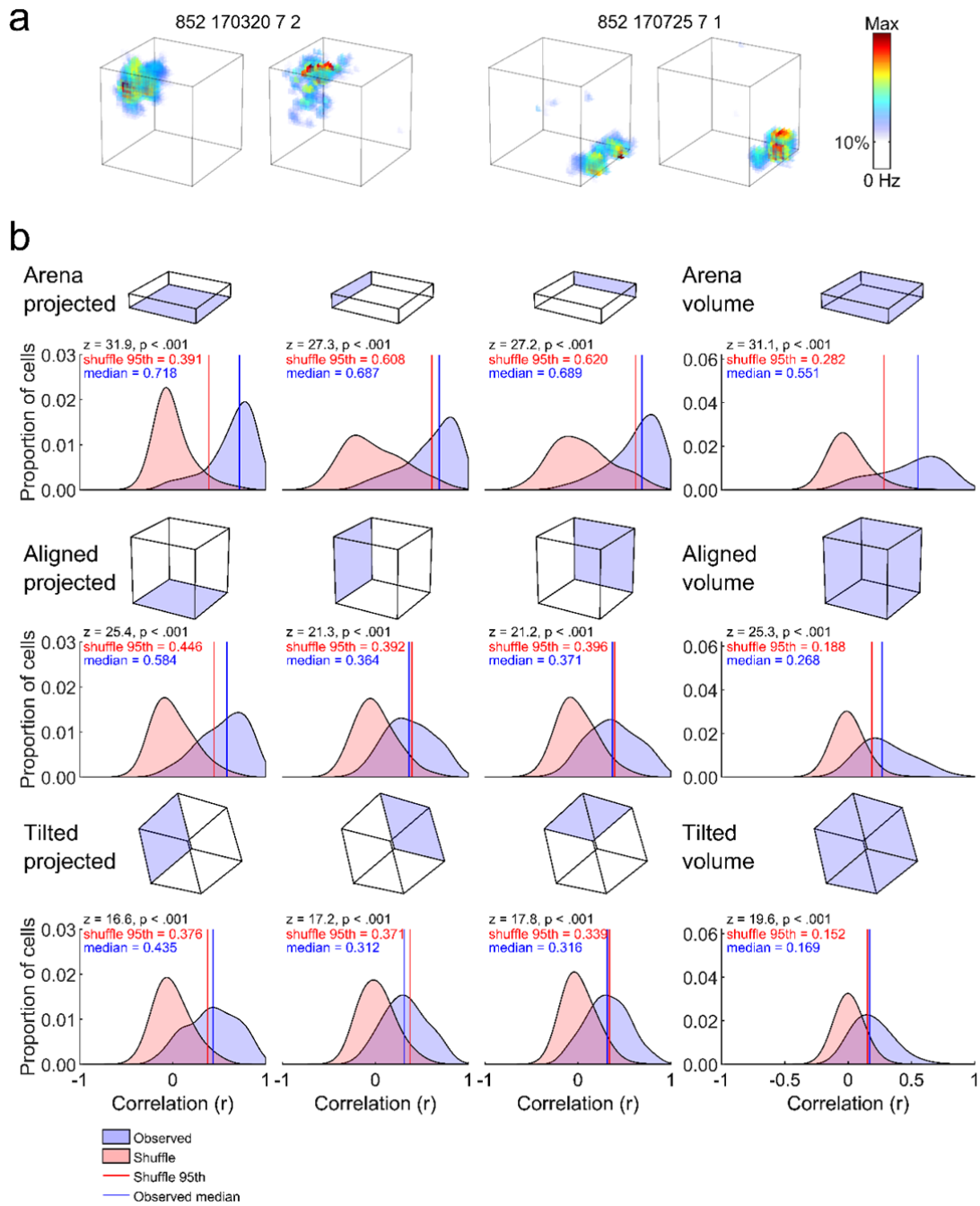
409

Same as Supplementary Table 1 but for the subsampled dataset containing only one session per rat

Maze	Rat	Cells		Place cells		Place fields		Fields per Cell	Field elongation		Spatial information	
		n	% of total	n	% of total	n	% of total		μ	s.d.	μ	s.d.
Arena	750	10	3.7	1	0.6	1	0.6	1.00	1.27	-	1.15	-
	770	16	6.0	13	7.8	13	7.9	1.00	1.94	0.57	1.44	0.89
	775	32	11.9	20	12.0	13	7.9	0.65	1.87	0.82	1.87	1.06
	850	15	5.6	3	1.8	5	3.0	1.67	1.91	0.73	1.08	0.80
	851	18	6.7	3	1.8	3	1.8	1.00	1.85	0.36	2.09	1.94
	852	21	7.8	9	5.4	7	4.2	0.78	1.71	0.32	2.13	1.39
	853	13	4.9	5	3.0	3	1.8	0.60	1.61	0.26	1.10	0.35
	894	26	9.7	19	11.4	17	10.3	0.89	1.43	0.40	0.91	0.50
	896	56	20.9	45	27.0	47	28.5	1.04	1.85	0.58	1.50	0.85
	923	3	1.1	3	1.8	4	2.4	1.33	1.88	0.80	0.71	0.22
	924	2	0.8	2	1.2	2	1.2	1.00	1.37	0.31	0.93	0.97
	926	45	16.8	36	21.6	39	23.6	1.08	1.80	0.59	1.43	0.79
	951	11	4.1	8	4.8	11	6.7	1.38	1.99	0.82	0.91	0.21
Total	13	268	-	167	-	165	-	-	-	-	-	-
Aligned	750	10	4.8	3	2.1	2	1.2	0.67	2.02	0.51	1.97	1.23
	770	16	7.7	10	7.1	13	7.8	1.30	2.10	0.63	1.28	0.94
	775	32	15.5	20	14.2	17	10.2	0.85	1.90	0.48	2.16	1.10
	850	15	7.3	12	8.5	17	10.2	1.42	2.03	0.62	1.82	0.93
	851	18	8.7	8	5.7	8	4.8	1.00	2.19	0.78	1.94	1.06
	852	21	10.1	11	7.8	12	7.2	1.09	1.77	0.34	1.94	0.68
	853	13	6.3	8	5.7	11	6.6	1.38	2.03	0.47	1.43	0.74
	894	26	12.6	19	13.5	32	19.2	1.68	1.95	0.67	0.80	0.71
	896	56	27.1	50	35.5	55	32.9	1.10	2.18	0.62	1.59	0.93
Total	9	207	-	141	-	167	-	-	-	-	-	-
Tilted	923	3	4.9	3	5.7	9	10.2	3.00	2.10	0.49	0.45	0.22
	924	2	3.3	2	3.8	1	1.1	0.50	1.63	-	1.21	0.58
	926	45	73.8	40	75.5	62	70.5	1.55	2.11	0.68	1.14	0.63
	951	11	18.0	8	15.1	16	18.2	2.00	1.92	0.38	1.18	0.89
Total	4	61	-	53	-	88	-	-	-	-	-	-

410 *Field stability*

411 We compared the first and second half of each maze session to determine the
412 stability of place cell firing patterns (Supplementary Fig. 12a, Supp. Methods: *Field stability*).
413 These effects can be seen in Supplementary Fig. 12b. Similar effects were observed when
414 tilted lattice maze data were projected onto the Cartesian planes (XY projections: $z = 17.1$, p
415 $< .001$, shuffle 95th: 0.309, observed median: 0.274; XZ projections: $z = 17.4$, $p < .001$,
416 shuffle 95th: 0.323, observed median: 0.275; YZ projections: $z = 17.0$, $p < .001$, shuffle 95th:
417 0.279, observed median: 0.259; whole volume: $z = 20.6$, $p < .001$, shuffle 95th: 0.125,
418 observed median: 0.146, statistics refer to right-tailed Wilcoxon rank sum tests).



Supplementary Fig. 12: Stability of recording between first and second halves of each maze recording. **a** Ratemaps for two example cells from the first (left map) and second half (right map) of an aligned lattice recording. **b** Distributions of observed correlation scores between the first and second halves (blue shaded area) and between 1000 shuffled first and second halves (red shaded area). Black text gives the result of a right-tailed Wilcoxon rank sum test comparing the distributions. Red lines denote the 95th percentile rank position in the shuffled distribution, blue lines denote the median position in the observed distribution. The top row shows the result of this analysis when comparing the 1st and 2nd half of arena recordings after projecting all data onto the XY, XZ and YZ planes respectively; and when volumetric firing rate maps are compared without projection. The second row shows the same for the aligned lattice. The third row shows the same for the tilted lattice but here projections are made along the AB, AC and BC planes instead; values for the Cartesian planes are given in text. Source data are provided as a Source Data file.

Theta-speed relationships differed between the environments

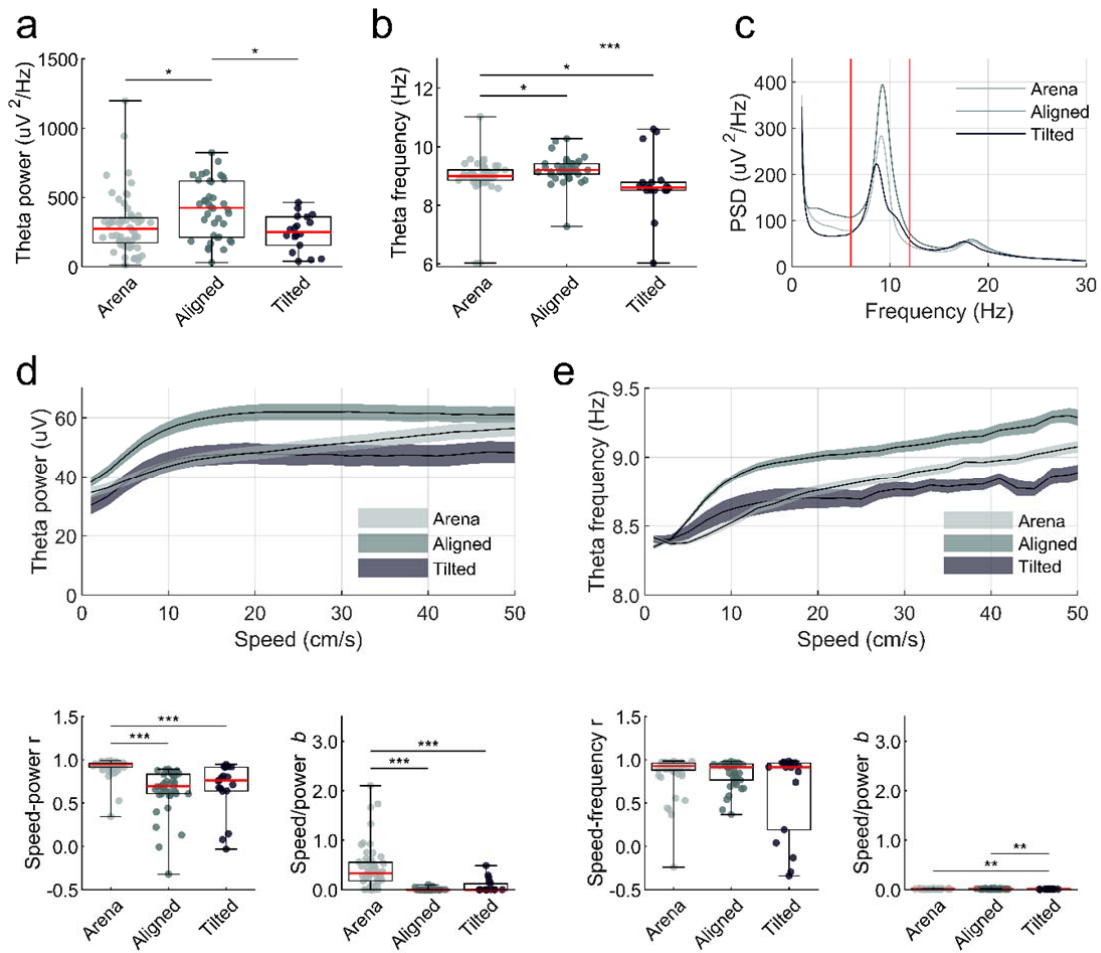
Theta power was significantly higher in the aligned configuration (Supplementary Fig. 13a&c; arena, aligned & tilted median power: 264, 421 & 252 $\mu V^2/Hz$; $\chi^2(2) = 9.9$, $p = .007$, $\eta_p^2 = 0.09$, KW; arena vs aligned: $p = .015$, arena vs tilted: $p > .99$, aligned vs tilted: $p = .036$), and increased with running speed in the arena but not the lattice (Supplementary Fig. 13d; speed/power correlations: arena, aligned & tilted median r : 0.94, 0.69 & 0.76; $\chi^2(2) = 64.4$, $p < .0001$, $\eta_p^2 = 0.57$, KW; arena vs aligned: $p < .0001$, arena vs tilted: $p < .0001$, aligned vs tilted: $p = .95$). Fitted b parameters (Supp. Methods: *Running speed analyses*) were significantly higher for the arena than the lattice mazes (arena, aligned & tilted median b : 0.34, 0.01 & 0.01; $\chi^2(2) = 62.8$, $p < .0001$, $\eta_p^2 = 0.55$, KW; arena vs aligned: $p < .0001$, arena vs tilted: $p < .0001$, aligned vs tilted: $p > .99$) indicating a linearly increasing relationship in the open field data but a downwardly curved or approximately flat one in the lattice data.

Theta frequency was significantly lower in the tilted configuration (Supplementary Fig. 13b-c, arena, aligned & tilted median frequency: 8.99, 9.21 & 8.61 Hz; $\chi^2(2) = 19.5$, $p < .0001$, $\eta_p^2 = 0.55$, KW; arena vs aligned: $p = .055$, arena vs tilted: $p = .015$, aligned vs tilted: $p < .0001$) but increased with running speed equally in all environments (Supplementary Fig. 13e; arena, aligned & tilted median r : 0.93, 0.91 & 0.91; $\chi^2(2) = 2.7$, $p = .26$, $\eta_p^2 = 0.02$, KW). Fitted b parameters were close to zero in all cases, with the aligned lattice values significantly lower than the other two mazes (arena, aligned & tilted median b : 0.01, 0.01 & 0.01; $\chi^2(2) = 12.5$, $p = .002$, $\eta_p^2 = 0.11$, KW; arena vs aligned: $p > .99$, arena vs tilted: $p = .0052$, aligned vs tilted: $p = .002$) indicating approximately flat relationships in all cases.

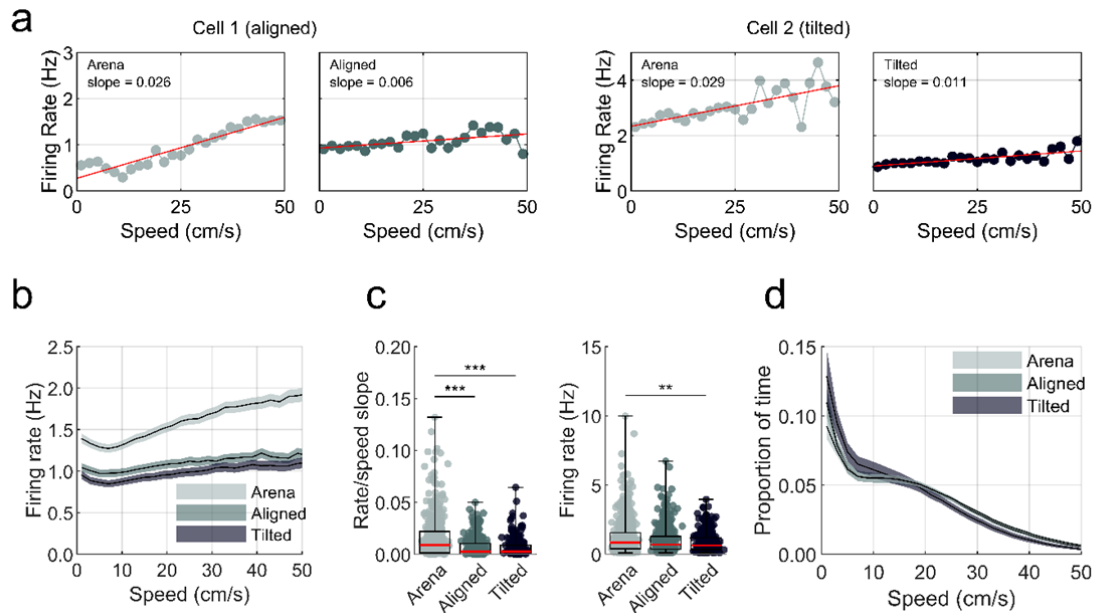
When comparing the three mazes firing rates were found to be modulated by running speed ($F(24,23072) = 12.8$, $p < .0001$, $\eta_p^2 = 0.013$) but this differed significantly between environments ($F(2,23072) = 1147.0$, $p < .0001$, $\eta_p^2 = 0.09$) with a significant interaction

between speed and environment ($F(48,23072) = 2.3, p < .0001, \eta_p^2 = 0.005$). Post-hoc tests confirmed that every maze differed significantly from every other maze ($p < .0001$ in all cases, Supplementary Fig. 14a-b).

Firing rates were generally lower in the lattice mazes but only the open field and tilted lattice differed significantly (arena, aligned & tilted median firing rates: 0.83, 0.73 & 0.64 Hz; $\chi^2(2) = 10.2, p < .0062, \eta_p^2 = 0.008$, KW; arena vs aligned: $p = .16$, arena vs tilted: $p = .0067$, aligned vs tilted: $p = .56$, Supplementary Fig. 14c). These reduced firing rates did not negatively affect the accuracy of the speed-rate linear regressions as fit error was significantly smaller in the lattice maze environments (arena, aligned & tilted sum of squared errors: 1.22, 0.74 & 0.67; $\chi^2(2) = 207.7, p < .0001, \eta_p^2 = 0.18$, KW; arena vs aligned: $p < .0001$, arena vs tilted: $p < .0001$, aligned vs tilted: $p = .042$). Dwell time was significantly inversely related to running speed ($F(24,1225) = 126.0, p < .0001, \eta_p^2 = 0.71$) but this did not differ significantly between environments ($F(2,1225) = 0.2, p = .82, \eta_p^2 < 0.001$) nor was there a significant interaction between the two ($F(48,1225) = 1.2, p = .17, \eta_p^2 = 0.045$, Univariate ANOVA comparing effects of speed and environment on dwell time, Supplementary Fig. 14d).



Supplementary Fig. 13: The relationship between theta and running speed. **a** Markers represent sessions. The maximum power found in the theta band (6-12Hz) of the Welch power spectral density estimate (PSD) calculated using whole session LFPs. **b** Markers represent sessions. The frequencies associated with the maximum power in the theta band. **c** The mean PSD in each maze, averaged across sessions. Red lines denote the frequency band associated with theta rhythm. **d** For each maze, the mean and SEM theta power (amplitude of the Hilbert transform) observed at various running speeds, averaged across sessions. Below this are boxplots where markers represent sessions. These show the Pearson's correlation between power and speed (left) and the b parameter extracted from each session's speed-power curve (right). **e** Same as **d** but for frequency (derivative of the phase of the Hilbert transform). Source data are provided as a Source Data file.



488

489 **Supplementary Fig. 14:** The relationship between place cell activity and running speed. Similar
 490 relationships were also observed in non-spatially modulated pyramidal cells and interneurons (data
 491 not shown). **a** Firing rate by speed profiles for two place cells. Left two plots are for a cell recorded in
 492 the open field and aligned lattice respectively. Right two plots are for a cell recorded in the open field
 493 and tilted lattice respectively. Markers show the cell's average firing rate at that running speed,
 494 calculated across the whole session, red lines show the result of a linear least squares line of best fit.
 495 We extract two main features from these fits, the first is the y-axis intercept and the second is the
 496 slope of the line. Both cells show a reduced slope and thus speed modulation in the lattice mazes. **b**
 497 The mean and SEM firing rate of cells at various running speeds, averaged across place cells. **c**
 498 Markers represent place cells. Left) for each cell we extracted the slope of the line of best fit (red lines
 499 in **a**). These slopes are significantly lower in the lattice mazes when compared to the open field.
 500 Right) The overall mean firing rate of cells in the three mazes. **d** The mean and SEM proportion of
 501 time animals spent moving at various running speeds, averaged across sessions. Source data are
 502 provided as a Source Data file.
 503

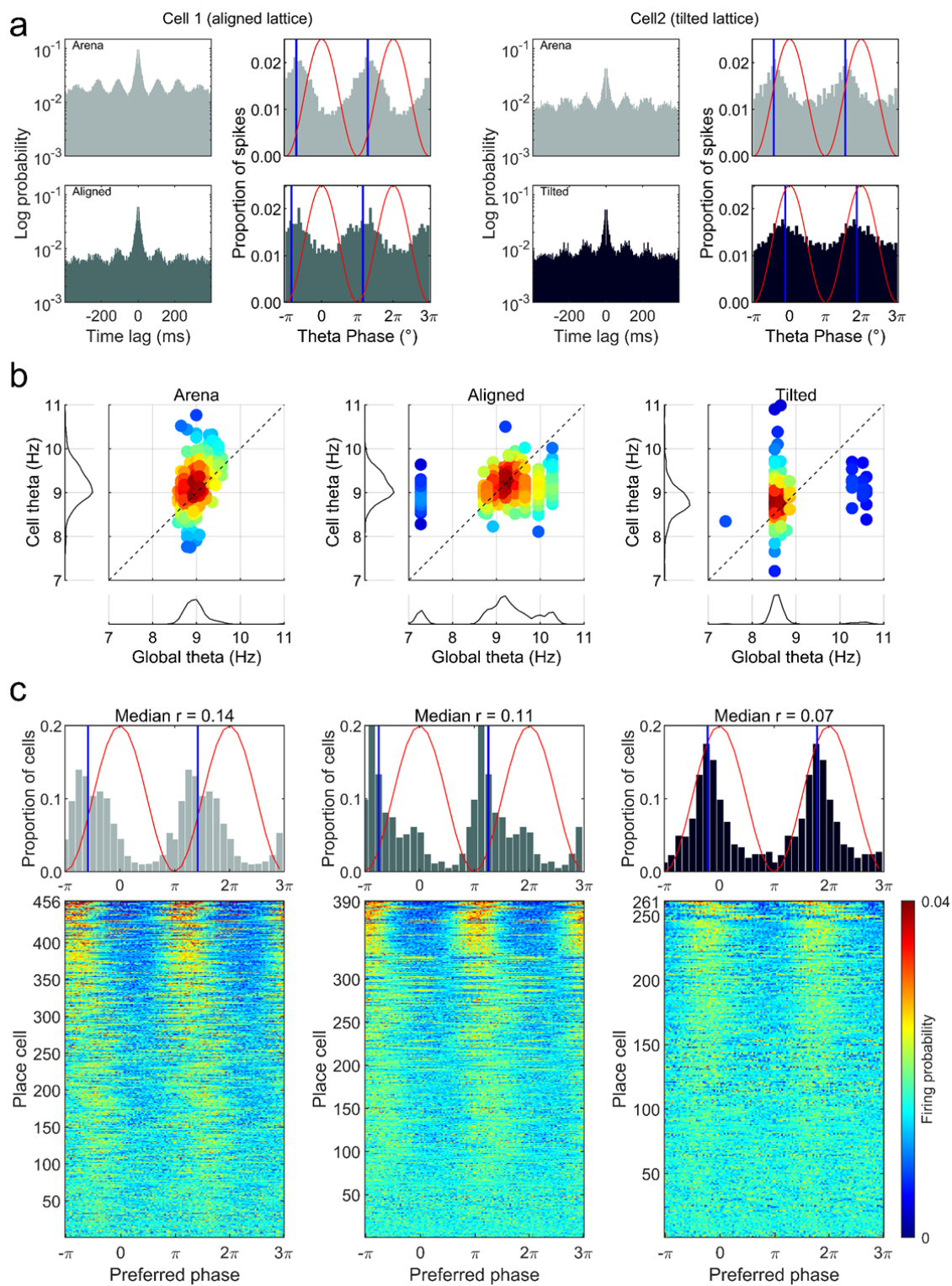
The relationship between theta and place cell spiking differed between the environments

Theta modulation of individual cells, assessed using spike autocorrelations, was significantly lower for place cells in the tilted lattice (arena, aligned & tilted median theta index: 0.26, 0.28 & 0.21 Hz; $\chi^2(2) = 38.4$, $p < .0001$, $\eta_p^2 = 0.033$, KW; arena vs aligned: $p > .99$, arena vs tilted: $p < .0001$, aligned vs tilted: $p < .0001$, Supplementary Fig. 15a). Place cells usually burst at a rate slightly faster than theta due to precession of their firing phase¹ which possibly reflects odometry². Overall intrinsic frequency of cells in the open field was faster than the global theta rhythm (median intrinsic vs. global frequency: 9.12 vs. 8.92 Hz, $z = -10.7$, $p < .001$, WSR; 275 cells or 79% faster than global, $\chi^2(1) = 115.8$, $p < .0001$, Chi-square test of expected proportions). The same was also true in the aligned lattice (intrinsic vs. global frequency: 9.21 & 9.07 Hz, $z = -2.4$, $p = .016$, WSR; 208 cells or 58% faster than global, $\chi^2(1) = 9.4$, $p = .002$, Chi-square test of expected proportions) but this proportion was significantly lower than in the open field ($\chi^2(1) = 36.0$, $p < .0001$, Chi-square test of expected proportions). Lastly, in the tilted lattice, overall burst frequency was faster than theta (median intrinsic vs. global frequency: 8.82 vs. 8.51 Hz, $z = -6.8$, $p < .001$, WSR; 170 cells or 78% faster than global, $\chi^2(1) = 66.9$, $p < .0001$, Chi-square test of expected proportions) and this proportion was more similar to the open field ($\chi^2(1) = 0.1$, $p = .78$, Chi-square test of expected proportions) but this was founded on a much smaller number of sessions (arena, aligned & tilted total sessions: 50, 34 & 16). These effects can be seen in Supplementary Fig. 15b.

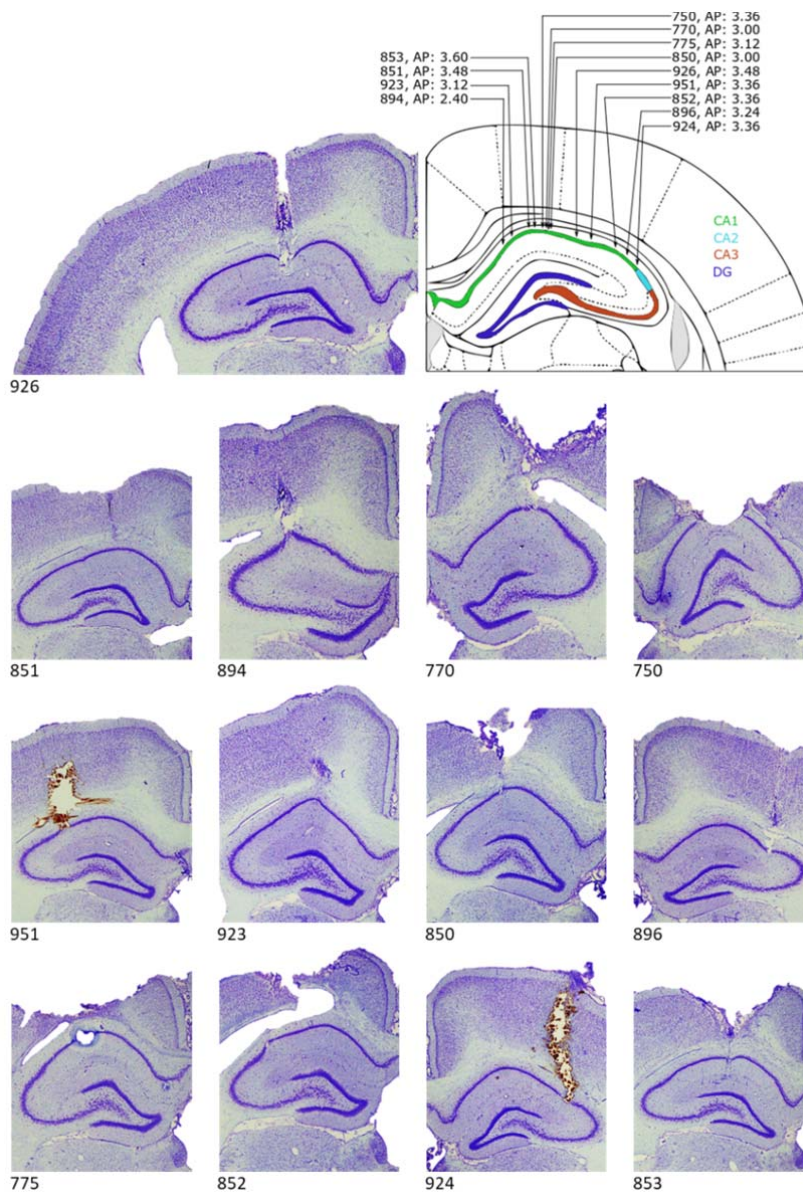
Place cells were similarly modulated by theta phase in the open field arena and aligned lattice but less strongly modulated in the tilted lattice (arena, aligned & tilted median Rayleigh vector lengths: 0.14, 0.11 & 0.07; $\chi^2(2) = 47.1$, $p < .0001$, $\eta_p^2 = 0.01$, KW; arena vs aligned: $p = .62$, arena vs tilted: $p < .0001$, aligned vs tilted: $p < .0001$). Nevertheless, in all three mazes place cells exhibited a preference for a specific phase of theta at the population

529 level (arena, aligned & tilted Rayleigh vector lengths: 0.53, 0.54 & 0.50, $p < .0001$ in all
530 cases, Supplementary Fig. 15c).

531 However, these distributions differed significantly (arena vs aligned: $k = 77692$, $p =$
532 $.003$; arena vs tilted: $k = 47963$, $p = .003$, aligned vs tilted: $k = 51130$, $p = .003$, two-sample
533 Kuiper tests with Bonferroni correction) and cross-correlation confirms that the aligned lattice
534 preferred phase was significantly earlier in phase than the arena (arena & aligned circular
535 mean: -1.84 & -2.29 rad, correlation lag = -0.4 rad) while the tilted lattice was significantly
536 later in phase than both of these (arena & tilted circular mean: -1.84 & -0.67 rad, correlation
537 lag = $+1.2$ rad; aligned vs tilted correlation lag = $+2.0$ rad). These effects can be seen in
538 Supplementary Fig. 15c.



Supplementary Fig. 15: Place cell activity and the theta oscillation. **a** Spike probability properties of two place cells. Left four plots are for a cell recorded in the open field (top row) and aligned lattice (bottom row). Right four plots are for a cell recorded in the open field (top row) and tilted lattice (bottom row). For each maze the left plot shows the 400ms spike autocorrelogram (1ms bins) and the right plot shows the spike-theta phase histogram. Red lines trace the amplitude of a scaled theta wave at every phase, blue lines show the circular mean of the phase angles. **b** Markers represent cells, color denotes marker density. Scatter plots showing the frequency of intrinsic theta modulation (extracted from the spike autocorrelograms) vs the overall frequency of global theta (extracted from the power spectral density estimate of the LFP). Markers above the dashed line represent cells firing at a rate faster than global theta, an indicator of phase precession. **c** Histograms showing the preferred theta phase (location of circular mean or blue lines in **a**) of all place cells. Red lines trace the amplitude of a scaled theta wave at every phase, blue lines show the circular mean of the preferred phases. Shown below each plot is the firing probability of all place cells relative to theta phase. Each row represents a cell and rows are sorted from top to bottom by the Rayleigh vector length of the cell's phase angles (i.e. the strength of phase-locking) from strong to weak. Source data are provided as a Source Data file.



558

559 **Supplementary Fig. 16:** Histological sections showing electrode tracks. Following fixation and
 560 sectioning, brains were imaged and recording electrode paths were reconstructed to determine the
 561 locations of recorded cells. In all animals the electrode tracks descended into dorsal CA1 and crossed
 562 the pyramidal cell layer without reaching dentate gyrus (DG) or CA3 below. Top: example histological
 563 slice (left) and the histological map corresponding to our intended coordinates (AP: -3.48, ML: ± 2.4 &
 564 DV: -1.5mm). Arrows show the estimated trajectory of each animals' electrode bundle, annotations
 565 give the animal number and the estimated AP of the electrode track. Bottom: smaller hippocampal
 566 sections for every animal which best show the electrode bundle track. The proportion of data obtained
 567 from each animal can be seen in Supplementary Table 2. Animal 775 received an electrolytic lesion
 568 under anesthesia prior to perfusion which can be seen as a small hole and surrounding tissue
 569 discoloration. Animals 951 and 924 lost their drive prior to perfusion resulting in staining of the
 570 electrode tracks. Source data are provided as a Source Data file.

571

572 **Supplementary methods**

573 *Apparatus*

574 All experiments were conducted in the same room (3.2×2.1×2.2m) under moderately
575 dimmed light conditions. Three of the room walls were covered with black material to aid
576 position tracking; on two of these walls were large high-contrast cues (1.5×1.2m cardboard
577 sheet and a 1×1.7m yellow plastic sheet). The last wall was covered with white material
578 (2.2×2.2m white cotton). The floor of the room was covered with black anti-static linoleum
579 flooring. We used three pieces of experimental apparatus; the first was a square open field
580 environment ('arena'), the second was a cubic lattice composed of horizontal and vertical
581 climbing bars ('aligned' lattice), the third was the same lattice rotated 45° around its Z-axis
582 and 54.74° around its X-axis so that two of its vertices were vertically aligned ('tilted' lattice).

583 The arena was a 1.8×1.8m square high-walled wooden enclosure, composed of four
584 1.8×0.65m matte black painted walls. This enclosure was placed directly on the black
585 linoleum flooring of the room. The top edges of these walls were covered with large
586 corrugated tubing to prevent the rats from exploring this area. The bottom edge of this
587 square was highlighted with a strip of 50% grey paint. One 0.45×0.65m matte white wooden
588 cue was affixed to one wall of this enclosure; the position of this cue remained the same
589 throughout the experiment. Rats were recorded freely foraging in the arena for randomly
590 dispersed flavored puffed rice (CocoPops, Kelloggs, Warrington, UK).

591 The cubic lattice maze (Fig. 1) was constructed from a children's toy-set (Quadro,
592 Hamburg, Germany). Hollow cubes were created by attaching red plastic tubes (length:
593 150mm, diameter: 10mm) using 6- or 4-way connectors (each 10mm wide). These cubes
594 were then assembled into a 6×6×6 cubic maze (0.97×0.97×0.97m). The maze was raised
595 0.45m above the ground, initially on black metal stools but later on a narrow wooden frame.
596 To encourage exploration, malt paste (GimCat Malt-Soft Paste, H. von Gimborn GmbH) was

affixed to bars of the lattice by the experimenter. This paste was spread evenly throughout the maze, midway along bars, equally between horizontal and vertical bars and reapplied every 15 minutes. This maze could be placed on one side, with the bars running vertically and horizontally; we refer to this as the ‘aligned’ configuration. Alternatively, the maze could be rotated (45° around its Z-axis and 54.74° around its X-axis) so that two vertices were vertically aligned – essentially standing the lattice vertically on one corner. We refer to this as the ‘tilted’ lattice configuration (Fig. 1).

Recording setup and procedure

Single unit activity was observed and recorded using a custom built 64-channel recording system (Axona, St. Albans, UK). Mill-Max connectors built into the rat’s microdrive were attached to a wireless headstage (custom 64-channel, W-series, Triangle Biosystems Int., Durham, NC). Analog signals were transmitted to a wireless base station via dual receiver antennae situated approximately 1m above the maze environments. Unfiltered signals were sampled at 50 kHz, amplified 100 times and transmitted at approximately 3.375 GHz (300 μ W at 3m). They were then passed to an Axona pre-amplifier where they were amplified a further 100 times. The signal was then passed to a system unit and for single unit recording the signal was band-pass (Butterworth) filtered between 300 and 7000 Hz. Signals were digitized at 48 kHz and could be further amplified 10–40 times at the experimenter’s discretion. For LFP recording a 4.8 kHz signal was saved as above which was then band-pass filtered between 6 and 12 Hz (4th order butterworth filter, Matlab *butter* and *filtfilt*) for theta analyses described below. The position of the animal was recorded using four wide-angle infrared LEDs (Osram Opto SFH 487P, 880nm) fixed to and powered by the wireless headstage. Five infrared sensitive CCTV cameras (Samsung SCB-5000P) tracked the animal’s position at all times (Supp. Methods: *Trajectory reconstruction*). Tracking artefacts and reflections were not observed during recording or in the trajectory data (Supplementary Fig. 18 and Supplementary Movie 4).

623 After recovery from surgery, rats were screened for single unit activity and for the
624 presence of theta oscillations once or twice a day, five days a week. Screening was
625 performed in the open field apparatus, after which rats were given approximately equivalent
626 experience freely foraging on the lattice maze. Once the presence of place cells was
627 confirmed rats were recorded in the experimental apparatus.

628 In these sessions, rats were recorded for a minimum of 18 minutes in the open field
629 environment and until they had sufficiently explored the environment (median, min & max
630 session time: 20.1, 18.0 & 30.7 minutes). Without unplugging the wireless headstage, they
631 were then removed and allowed to rest in an opaque, lidded box for approximately 10
632 minutes with access to drinking water. During this time, the open field environment was
633 dismantled and replaced with the lattice maze in one of the two configurations described
634 above. Rats were then placed on the bottom layer of the lattice maze (or the bottom front
635 face of the diagonal lattice) and explored this environment for a minimum of 45 minutes and
636 until they had sufficiently explored the environment (median, min & max session time: 60.2,
637 41.1 & 94.2 minutes). When this was complete, the rats were returned to the opaque box as
638 before and the open field was restored. For a subset of recordings (41 or 71.9% of sessions)
639 rats were then recorded for a further minimum of 16 minutes in the open field and until they
640 had sufficiently explored the environment (median, min & max session time: 20.1, 10.9 &
641 30.9 minutes). During recordings the experimenters monitored progress from a connected
642 room which housed the recording equipment and computers and was separated from the
643 experimental room by a black opaque curtain.

644 At the end of the recording session, the animals were removed from the apparatus
645 and the electrodes were lowered by at least 20 μm in order to maximize the chance of
646 recording from a different population of cells on the following day. No attempt was made to
647 track cells across days, and thus a subset of the cells may have been recorded on more
648 than one session, although inspection of the cluster space did not suggest that this was the

case and almost identical results to those reported in main text were also observed when only analyzing one session per animal (the session with the most place cells). These analyses can be replicated using the provided data set and code. Rats were tested until cells were no longer observed (median, min & max: 3, 1 & 14 sessions).

Trajectory reconstruction

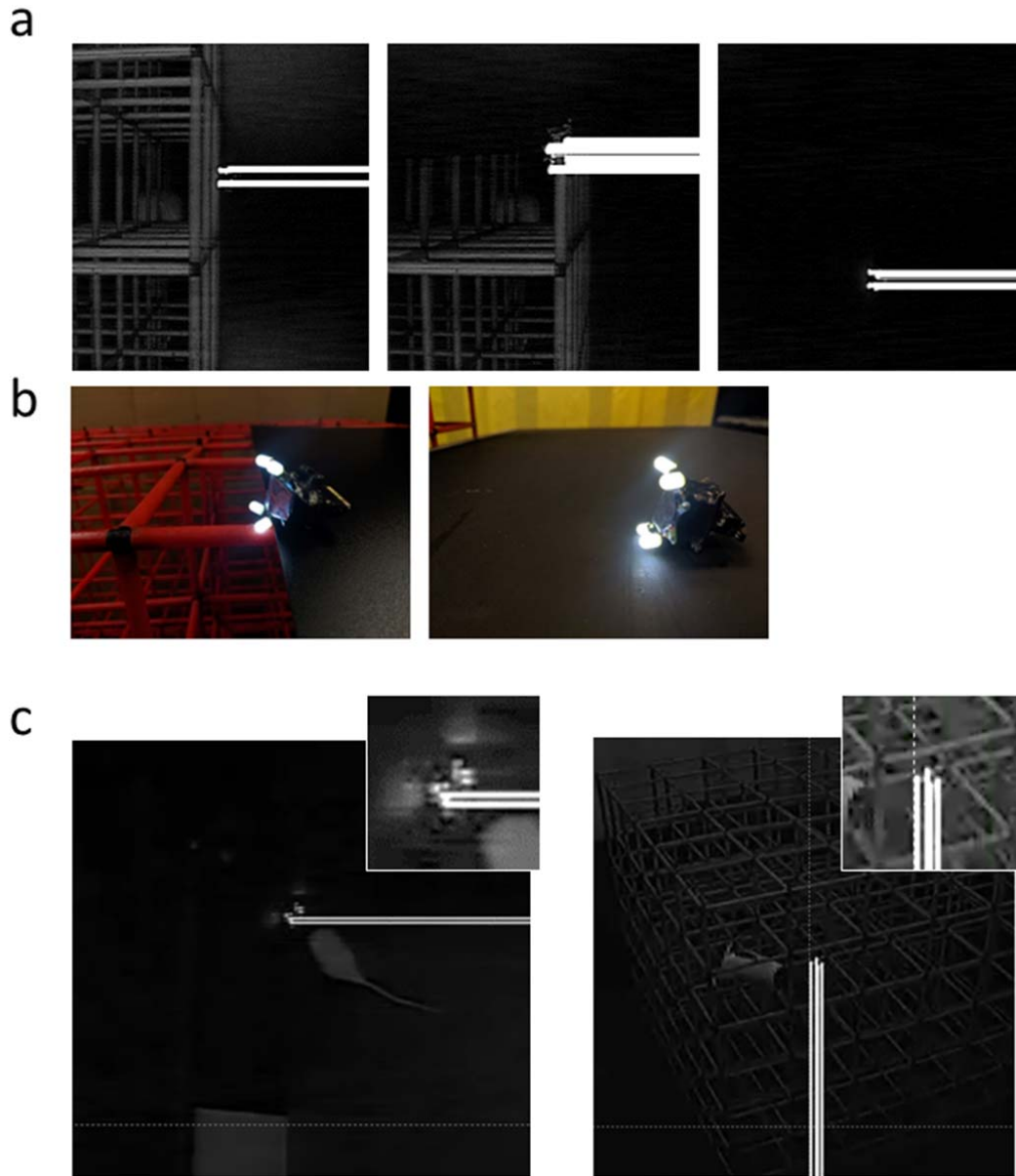
Each animal's movements were monitored using five infrared CCTV cameras (Samsung SCB-5000P) mounted at the four corners of the room, with one camera directly above the environment. These tracked the position of four light-emitting diodes connected to the head-stage on the rat's head. Its position was then tracked, in real time, using custom software (DacqTrack, Axona, St. Albans, UK) at a 25Hz sampling frequency. The data from these cameras was synchronized with neural data using a pulsed optic interface – each camera monitored a 1Hz TTL initiated light source, controlled by the recording system, which allowed accurate, offline synchronization. The onset of these light pulses was used to continually re-align the position data using nearest neighbor interpolation (Matlab function *interp1*).

The rat's 3D position was then reconstructed using the direct linear transform algorithm³, applied to the data from all five cameras, in pairs. Briefly, these cameras were first calibrated in order to reverse any distortion introduced by their optical elements (Matlab functions *estimateCameraParameters*, *undistortImage* and *undistortPoints*). We then imaged the same checkerboard pattern with each camera and used its 3D pose to calculate the distance and orientation of each camera relative to it and thus to each other (Matlab functions *extrinsics* and *cameraMatrix*). Using this information, we constructed a fundamental matrix. If x are some points viewed by camera 1 and x' are the same points viewed by camera 2, the fundamental matrix, F , represents the relationship between points x and x' :

$$x_i' F x_i = 0$$

This relationship can be used to triangulate any given pair of points imaged by two cameras into three-dimensional space³. For each recording session we reconstructed the animal's path using every possible pair of cameras (Matlab function *triangulate*) and we then combined these reconstructions into one single trajectory. This was achieved by taking the weighted mean of each point, where the weighting was the reliability of the point's estimated location. Reliability was assessed using each point's reprojection error; after triangulation each point was projected back into both camera images; the reprojection error was then calculated as the distance between the original and reprojected position of the point.

In this setup, the rat need only be viewed by two cameras at any one time for a successful reconstruction, allowing for near continuous tracking even in cluttered, complex environments such as the lattice maze. For the lattice sessions reported here, rats were in view of at least two cameras for an average of 98.0% (geometric mean, geometric SD = 3.3%) of the session, this value was similar for the open field sessions (geometric mean = 97.6%, geometric SD = 2.5%). Based on reprojection errors, we estimate a median error in reconstruction of just 1.8 mm (median absolute deviation = 1.1mm). Camera trajectory reconstructions from different pairs of cameras were within a median of 13.8mm agreement (median absolute deviation = 7.4mm) of the weighted mean trajectory. Our cameras were extremely stable; however, re-calibrations were conducted once every two to four weeks to ensure continued reconstruction accuracy. For segments of missing tracking data, we simultaneously interpolated and smoothed the existing data using an unsupervised, robust, discretized, n-dimensional spline smoothing algorithm (Matlab function *smoothn*^{4,5}). We did not observe reflections from the maze surfaces being tracked during recording, indeed the recording backdrop and lighting were specifically designed to eliminate tracking artefacts such as this. Examples of reflection-less tracking can be seen in Supplementary Fig. 17 and Supplementary Movie 4.



699

700 **Supplementary Fig. 17:** Apparatus reflectively and tracking. The arena and lattice maze were both
 701 designed and colored with optimal tracking as a priority. We did not observe the mazes being tracked
 702 or reflections of the headstage LED in the mazes being tracked at any point. **a** Frames from footage
 703 recorded using our overhead camera (Fig. 1) showing the headstage LEDs pointing at the side of the
 704 lattice (left) pointing along the top bars of the lattice close to the camera (middle) or laying on a
 705 section of the arena walls (right). White lines end at tracked pixels which in all examples correspond
 706 to the 4 tracking LEDs on the headstage. **b** Color photographs showing how the tracking LEDs
 707 appear to the naked eye. **c** Frames from footage taken during recording sessions. Left) an animal
 708 faces into one corner of the arena very close to the walls, reflections can be seen but these are not
 709 tracked. Inset shows a close-up of the animal's head position with the brightness increased to
 710 highlight the reflections. Right) An animal explores the lattice maze and has his head close to the bars
 711 near one side of the maze. Again, in this case no reflections are observed or tracked.
 712

713 *Tilted data rotation*

714 For some firing rate map analyses we computed values for the A, B and C axes of
715 the tilted lattice. In these cases we un-tilted the tilted lattice firing rate map to align it with
716 horizontal/vertical axes (54.735° clockwise around the X-axis and 45° clockwise around the
717 Z-axis) using nearest neighbor interpolation (Matlab functions *affine3d* and *imwarp*), and
718 then used the same analyses as for the aligned lattice. A similar approach was also applied
719 to position data where necessary (Matlab functions *transformPointsForward* and
720 *worldToIntrinsic*) using the transformation defined above or using appropriate rotation
721 matrices (Matlab function *Axe/Rot*, Jacobson, M.).

722 *Behavior and spherical heat maps*

723 Using smoothed and interpolated 3D reconstructed position data we calculated the
724 instantaneous three-dimensional heading of the animal as the normalized change in
725 position:

$$\hat{u} = \frac{\vec{u}}{||\vec{u}||}$$

726 where;

$$\vec{u} = (\Delta_X(t), \Delta_Y(t), \Delta_Z(t))$$

727 and;

$$||\vec{u}|| = \sqrt{\Delta_X(t)^2 + \Delta_Y(t)^2 + \Delta_Z(t)^2}$$

728 this gives a unit vector representing the animal's heading at time t . We then projected these
729 vectors on to a unit sphere and extracted position data falling within regions on the surface
730 of the sphere corresponding to the intersection of the sphere and the 6 axes of interest: the
731 Cartesian X (Pitch = 0°, Azimuth = 0° & 180°), Y (Pitch= 0°, Azimuth = 90° & 270°), Z
732 (Pitch,= ±180°, Azimuth = 0°) axes and the diagonal lattice maze relative A (Pitch = ±35.26°,

Azimuth = -60° & 120°), B (Pitch = ±35.26°, Azimuth = 60° & -120°) and C (Pitch = ±35.26°, Azimuth = -180° & 0°) axes. The regions were equivalent to 60° conic sections centered on each respective axis in one direction from the origin. For each axis we combined the two corresponding directional regions. The length of time spent moving along each of these axes was calculated as the number of position samples falling along each of these axes multiplied by the sampling rate of the system. For this analysis we used only data when the animal was travelling at a speed >20cm/s. The average speed of movement along each axis was calculated separately as the total distance travelled in each axis divided by the total session time.

We also calculated the kernel smoothed density estimate of these spherical points using a Von Mises–Fisher distribution. Briefly, the Gaussian used was defined as:

$$g(x) = e^{(-0.5(\frac{x}{\sigma})^2)}$$

where x was defined as the inverse cosine of the inner dot product between each vector point and points across a sphere's surface (Matlab function *sphere*) and σ was the standard deviation of the Gaussian, which was set to 10. In this way, the resulting three-dimensional heat plots give a density estimate of points on the sphere, where density is estimated as the sum of the Gaussian weighted distances (along the surface of the sphere) to every data point. These processes can be seen in Supplementary Movie 3.

For a measure of three-dimensional thigmotaxis we calculated the total length of time spent in the inner and outer half volumes of the lattice – the inner half being an approximately 77×77×77cm cube centered on the center of the lattice. For the diagonal lattice, this central cube was rotated to match the geometry of the maze. Dwell time ratio was calculated as the ratio of these two values for each session. For the arena environment, we omitted the Z-dimension, instead the inner half was defined as the square region with half the surface area of the arena centered on the middle of the maze.

757 To determine if the rats displayed a bias in their occupancy of 3D space, we also
 758 calculated the length of time the rats spent in the top and bottom of the two lattice mazes.
 759 For this we categorized data as either above the center node of the lattice (top) or below it
 760 (bottom). Dwell time ratio was calculated as the ratio of these two values for each session.
 761 As a measure of exploration coverage, for each session we calculated the proportion of
 762 lattice maze nodes (climbing bar intersections) enclosed by the convex envelope of that
 763 session's position data.

764 *Cluster cutting*

765 Single unit activity was analyzed offline using a combination of Matlab functions and
 766 custom spike sorting software. First, the dimensionality of the waveform information was
 767 reduced to the first three principal components and amplitude. Based on these parameters,
 768 an automated spike sorting algorithm (Klustakwik v3.0, ⁶) was used to distinguish and isolate
 769 separate clusters. The clusters were then further checked and refined manually using a
 770 manual cluster cutting GUI (TINT v4.4.12, Axona, UK). As well as the previously mentioned
 771 features, manual cluster cutting also made use of spike auto- and cross-correlograms.

772 Cluster quality was operationalized by calculating isolation distance (Iso-D), L_{ratio} ,
 773 signal to noise ratio (S/N), refractory period contamination and peak waveform amplitude,
 774 taken as the highest amplitude reached by the four mean cluster waveforms. For cluster C ,
 775 containing n_c spikes, Iso-D is defined as the squared Mahalanobis distance of the n_c -th
 776 closest non- c spike to the center of C . The squared Mahalanobis distance was calculated as:

$$D_{i,C}^2 = (x_i - \mu_C)^T - \sum_c^1 (x_i - \mu_C)$$

777 where x_i is the vector containing features for spike i , and μ_c is the mean feature vector for
 778 cluster C . A higher value indicates better isolation from non-cluster spikes ⁷. The L quantity
 779 was defined as:

$$L(c) = \sum_{i \notin C} 1 - CDF_{x_{df}^2}(D_{i,C}^2)$$

780 where $i \notin C$ is the set of spikes which are not members of the cluster and $CDF_{x_{df}^2}$ is the
 781 cumulative distribution function of the distribution with 8 degrees of freedom. The cluster
 782 quality measure, L_{ratio} was thus defined as L divided by the total number of spikes in the
 783 cluster⁸. As the signal and noise are both measured across the same impedance, signal to
 784 noise ratio (S/N) was defined as:

$$Signal\ to\ Noise\ Ratio = \left(\frac{RMS_{signal}}{RMS_{noise}} \right)^2$$

785 where RMS is the root mean squared amplitude (maximum of the mean waveform). For
 786 RMS_{noise} we used the noise cluster which accompanied unit spikes on that tetrode. This is a
 787 conservative noise measure as the noise cluster contains only noise with a high enough
 788 amplitude to breach the threshold used during recording rather than the true background
 789 noise amplitude. All four quality measures were assessed for their potential impact on our
 790 analyses by assessing the relationship between these measures and our main experimental
 791 statistics (as suggested by⁷).

792 *Field detection*

793 Unless otherwise stated, all analyses were performed on the unsmoothed firing rate
 794 maps. When detecting place fields, we looked for areas of more than 64 contiguous voxels
 795 (voxels were 50×50×50mm) with a firing rate > 20% of the maximum value. Contiguity was
 796 defined as a three-dimensional 18-connected neighborhood, which includes all voxels
 797 sharing an edge or face but not only a vertex. This was calculated using the Matlab function
 798 *bwlabeln*. Once connected regions were identified we applied two analyses: we first found
 799 their convex hull (Matlab function *convhulln*), and second, we extracted the main features of
 800 each field using the Matlab function *regionprops3*. These main features are discussed in

more detail below. To be analyzed further a place field had to be visited more than 5 times, where a visit was defined as 1 second of contiguous time spent within the place field convex hull. The position of each place field was defined as its centroid: the average position of all in-field voxels. Although the arena was flat, animals were free to move vertically in the area (i.e. crouching, standing and rearing) and so we treated the arena data as a thin volume; nevertheless, the analyses for the z dimension (height) in the arena must be treated with caution.

Field volume and density

We calculated the volume of place fields as being the sum of all its voxels. We also extracted the enclosing diameter of each place field, which was defined as the diameter of the smallest sphere capable of enclosing this convex polygon.

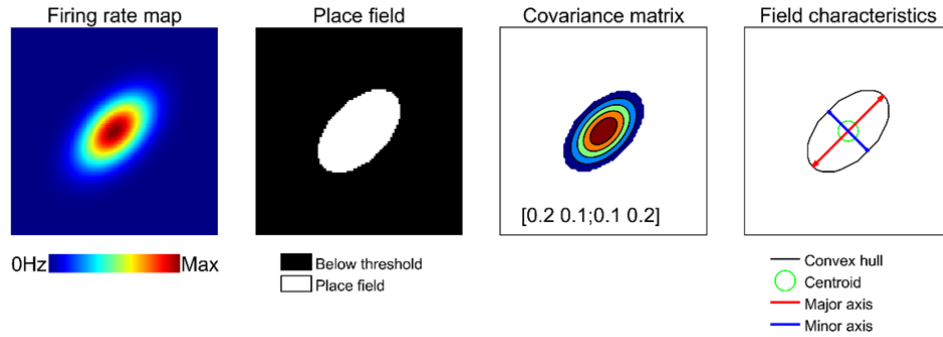
To quantify the relative volume of place fields and the density of fields per m^3 in each environment we estimated the practical volume of the lattice mazes and arena. For each recording session we calculated the volume of the convex hull (Matlab *convhulln*) of that session's position data. The practical volume of each maze was the average of these values (arena, aligned and tilted mean & SD = $0.45 \pm 0.12\text{m}^3$, $1.45 \pm 0.16\text{m}^3$ and $1.63 \pm 0.26\text{m}^3$ respectively). Field density (fields per m^3) was then calculated as the number of fields expressed by each cell divided by the volume of the maze.

Field orientation and size

We extracted the Cartesian height and width of the fields, defined as the side length of the field region when projected on to the X, Y and Z-axes respectively (i.e. the side lengths of the minimum cuboid that could enclose the field). To examine these lengths in more detail we compared the shape of their distributions (Fig. 6d and Supplementary Fig. 7). The distribution of field heights (side length along Z) was weakly bimodal in the aligned lattice, to test this we used the bootstrapped modality test described by ⁹ (Matlab function

826 *bootmode*; A. C. Penn), similar results were also found using Akaike's Information Criterion
 827 (AIC, Matlab function *fitgmdist*).

828 We extracted the field's orientation and principal axes, which were defined as the
 829 orientation and major axes of an ellipsoid with the same normalized second central moments
 830 as the field region. In more detail, we calculated the second central moments or covariance
 831 matrix which best described a thresholded place field, in effect, fitting a multivariate normal
 832 distribution to the field. The direction and magnitude of the best fit ellipse which describes
 833 the place field are then given by the eigenvectors and eigenvalues of this covariance
 834 matrix respectively (Supplementary Fig. 18; Matlab function *regionprops3*).



835

836 **Supplementary Fig. 18:** Schematic demonstrating place field feature extraction. These examples are
 837 two-dimensional but the same principles were applied to three dimensional firing rate maps in our
 838 data. A firing rate map (left; in this case a simulated 2D Gaussian distribution) is thresholded at 20%
 839 of the peak firing rate to generate a binary field map (middle left). The second central moments of the
 840 field pixel locations give the covariance matrix which describes the best fit multivariate normal
 841 distribution of the field (middle right, visualized as a contour plot). The eigenvectors and eigenvalues
 842 of this covariance matrix give the direction and length of the place field axes. The average pixel
 843 location gives the field centroid and the convex hull of the thresholded ratemap gives the field
 844 perimeter.

845

846 We calculated the elongation index of the principal axes as:

$$Elongation = \frac{P1}{0.5(P2 + P3)}$$

where $P1$, $P2$ and $P3$ are the principal axes from largest to smallest respectively. This gives a measure of the curvature of the place field; large elongation values represent elongated fields while a value of 1 would represent a sphere. In the case of the arena, elongation was calculated using the first two largest principal axes ($P1/P2$). As an additional geometric measure, independent of the axis lengths, we also calculated the sphericity of each place field's convex hull, defined as:

$$sphericity = \frac{\pi^{\frac{1}{3}}(6V)^{\frac{2}{3}}}{A}$$

where V is the volume of the place field and A is its surface area. A sphericity of 1 would represent a sphere and any deviation from a sphere would result in a value lower than this.

To determine if fields were oriented in three-dimensions along one or more arbitrary axes we projected the place field eigenvectors (and their antipodal equivalents) onto a unit sphere. Using a similar analysis as the one described for position data, we then extracted the number of fields falling within regions on the surface of the sphere corresponding to the intersection of the sphere and the axes of interest; the Cartesian XYZ axes and the lattice maze relative ABC axes. These regions are equivalent to $\sim 60^\circ$ conic sections centered on each respective axis in one direction from the origin, thus for each axis we combined the two corresponding directional regions.

To determine whether the number of fields oriented parallel to one axis was greater or less than another axis we calculated 95% confidence intervals for each point. To do this we extracted the orientations of random place fields with replacement so that the number of random field orientations was equal to the number of observed fields. We then recalculated the proportion of fields in this shuffle that were oriented parallel to each axis as above. We did this 1000 times for each maze. The error bars shown in Fig. 7c represent the 2.5th and

97.5th percentile ranks of these shuffled counts. If the observed value for one axis fell within the error bars of another axis the two axes were not considered to differ significantly.

To determine whether more fields were parallel to an axis than would be expected by chance, we generated 1000 random points on the face of a sphere and counted the proportion of points falling within the area around each axis. We did this 1000 times. Chance was calculated as the interval between the 2.5th and 97.5th percentile ranks of this distribution. If the observed field count for an axis exceeded the upper threshold it was considered to be overrepresented with respect to chance.

As fields were observed to fall in alignment with the XYZ axes in the aligned lattice maze and the ABC axes in the diagonal lattice we also computed an 'axis ratio' for comparison. This was defined as:

$$axis\ ratio = \sum XYZ / \sum ABC$$

To test the likelihood of observing these axis ratios by chance we compared them to a shuffled distribution. For this, we randomly distributed 1000 points across the surface of a sphere 1000 times and recomputed the above values. If the axis ratio of a maze exceeded the 1st or 99th percentile of the ratios obtained in the shuffle it was defined as significantly deviating from 1 (no axis bias of any kind). In this case an observed value less than the 1st percentile would represent a significant overrepresentation of fields aligned to the ABC axes, while a value exceeding the 99th percentile would represent a significant overrepresentation of fields aligned to the XYZ axes.

For visualization, we calculated the Von Mises–Fisher kernel smoothed density estimate of these place field vectors across the sphere's surface (as described in Behavioral analyses). These 3D spherical maps are presented in the main text for visualization only (Fig. 2&7).

In addition to the place field orientation analyses presented in the main text, we also generated spherical field maps predicting the pattern of results in each maze if all fields were parallel to the maze axes. These maps were produced by normalizing the output of our Von Mises–Fisher kernel smoothed density estimate computed on the directional angles associated with each maze axis (i.e. the intersection points of each maze axis with a unit sphere). In the case of the arena we included only the X and Y axes, for the aligned lattice we included the X, Y and Z axes and for the tilted lattice we only included the A, B and C axes. This process can be seen in Supplementary Fig. 8a. These maps essentially highlight the pitch $\times$ azimuth regions associated with the maze axes.

Next, we correlated the 2D cylindrically projected maps observed in each maze (i.e. the actual, collected data) with each of these possible predictive maps (Pearson, pairwise correlation, Matlab function *corr*) also projected cylindrically. In this way, if the fields in a maze are parallel to the maze axes we would expect a high correlation between the observed and predicted maps. To test if these correlations were significantly higher than would be expected by chance (and to account for the loss of sphericity in these cylindrical projections) we generated distributions of shuffled maps for each maze. For these shuffles we generated 1000 maps as above using 10000 random spherical points (an example can be seen in Supplementary Fig. 8a). We correlated the shuffle maps with the observed data and extracted the 99th percentile of this distribution. The original correlations were deemed statistically significant if they exceeded this value.

Field elongation and sphericity

For each place field we tested whether its elongation index and sphericity deviated significantly from a distribution that would be expected by chance using an analysis inspired by one reported previously¹⁰. For each place field we defined a perfect sphere, centered on the field's centroid. The diameter of this sphere was calculated such that it would share the

917 same convex volume (the volume of the convex hull enclosing the field voxels) as the place
918 field. This was calculated as:

$$equivalent\ diameter = 6\left(\frac{vf}{\pi}\right)^{\frac{1}{3}}$$

919 where vf is the convex volume of the place field. We found this to be more accurate than the
920 geometric mean approach reported previously¹⁰ which assumes all place fields are perfectly
921 elliptical and thus tends to underestimate equivalent diameter. Next, the spikes emitted
922 within the place field were randomly shuffled among the trajectories through this sphere
923 using a multivariate Gaussian process (Matlab *normrnd*). The mean of the Gaussian was the
924 sphere center and the standard deviation of the Gaussian was set to $1.8 \times$ the radius of the
925 sphere (to approximately match the 20% thresholding used during field detection). Each
926 spike was then assigned to the position of the nearest trajectory data point (Matlab
927 *knnsearch*). The result of this procedure was a normally distributed point cloud of spikes
928 centered on the centroid of the original field with the same equivalent diameter and firing
929 rate.

930 We then recomputed the firing rate map for these shuffled spikes and extracted its
931 elongation index as described above. This procedure was repeated 100 times for each
932 place-field. Place fields with an elongation index or sphericity that could be expected, by
933 chance, from an underlying spherical field (i.e. with an elongation index lower than the 95th
934 percentile rank of the shuffled distribution) were defined as spherical or isotropic: otherwise,
935 place fields were defined as non-spherical or anisotropic.

936 *Field distribution*

937 To test whether place fields in the mazes were distributed homogenously we
938 compared the distribution of place field centroids in X, Y and Z to a shuffled set of centroids.
939 We excluded place fields falling outside the lattice frame to limit the test to a consistent and

940 repeatable volume. We then calculated the median position of fields in the X, Y and Z axes.
 941 Next we generated N uniformly random points (Matlab function *rand*) within the lattice frame,
 942 where N was the number of real place fields falling within this volume. We repeated this
 943 1000 times and at each step calculated the median position of the centroids in X, Y and Z.
 944 Next we calculated the 2.5th and 97.5th percentile ranks of these distributions; if the observed
 945 median position in X, Y or Z was found to exceed these bounds we considered that place
 946 fields were not distributed uniformly around the center of the maze in that axis.

947 *Binary morphology*

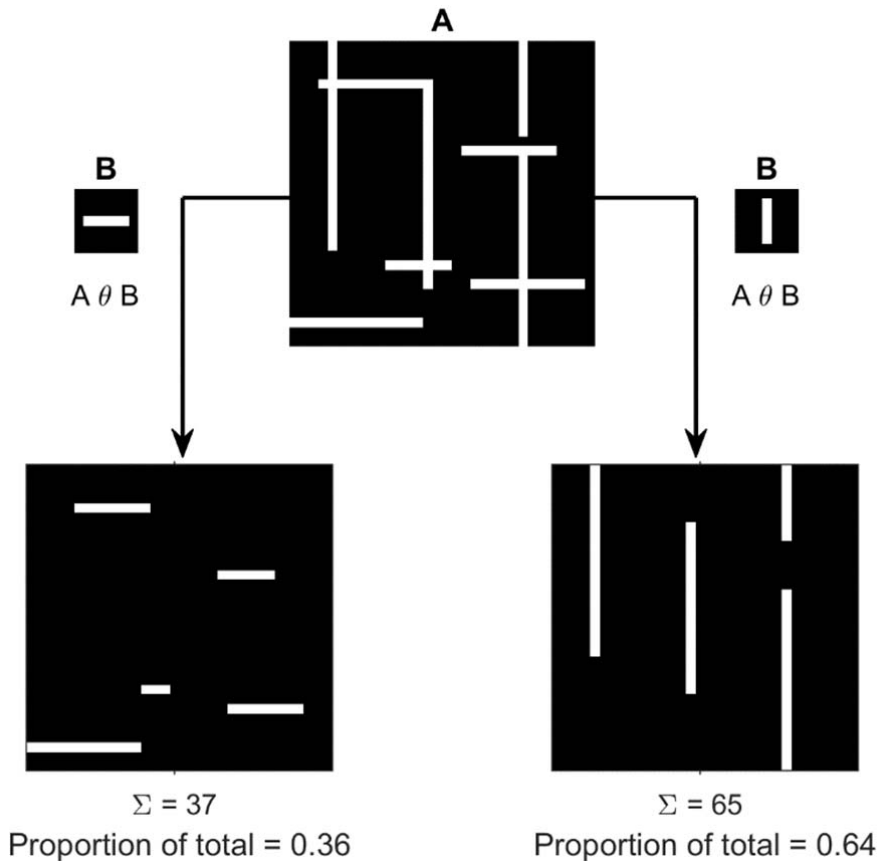
948 We also conducted a binary morphological analysis on thresholded firing rate maps
 949 to detect their maximal connectivity along each dimension (Supplementary Fig. 19). First,
 950 binary firing rate maps (A) were generated by thresholding firing rate maps (F) at 10% of
 951 their maximum value:

$$F(x) = \begin{cases} 1 & \text{if } x \geq 0.1(\max(F)) \\ 0 & \text{if } x < 0.1(\max(F)) \end{cases}$$

952 We then performed morphological erosion ($\ominus$) using structuring element vectors (B) with
 953 lengths ranging from 3-19 voxels along each cardinal axis (Matlab functions *imbinarize* and
 954 *bwhitmiss*):

$$A \ominus B = \{z \in E \mid B_z \subseteq A\}$$

955 For each erosion we took the linear sum of the remaining voxels as a measure of the map's
 956 connectivity along that dimension and then expressed this as the proportion of all remaining
 957 voxels for that element length. This last step was necessary to account for the fact that as
 958 the structuring element increases in size the likelihood of voxel connectivity decreases
 959 substantially, although we achieved similar results without it.



Supplementary Fig. 19: Two dimensional representation of the binary morphological process. A firing rate map is thresholded to form a binary image (A), where 1's represent voxels with a value above 10% of the map's maximum and 0's represent all other voxels. This binary map is then eroded using structuring elements (B) which are vectors of varying lengths that are parallel to one of the primary cardinal axes. The result is a map where 1's represent voxels that can accommodate the structuring element and 0's are voxels that cannot. As a measure of relative connectivity along each dimension, we took the sum of each eroded map and expressed this as a proportion of the sum of all maps. In this example, A has longer vertical periods of connectivity than horizontal ones, this is confirmed by the fact that almost two thirds of the eroded voxels can be found after eroding with a structuring element parallel to the Y-axis.

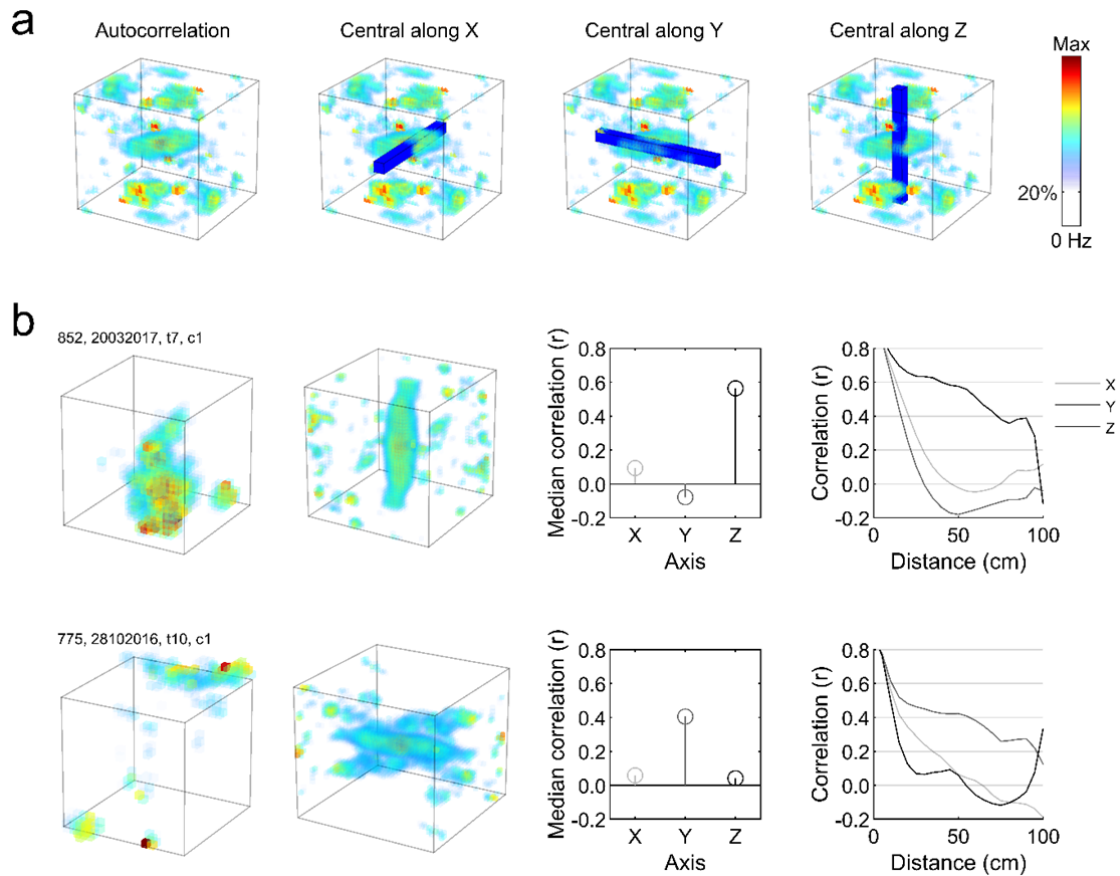
Autocorrelation and spatial information

Anisotropic place fields that are oriented parallel to a Cartesian axis (i.e. forming vertical columns or horizontal bands) will be visible on multiple two-dimensional slices through a three-dimensional rate map (Supplementary Fig. 9). To investigate this possibility we computed the three dimensional autocorrelation, r , of each place cell's firing rate map, defined as:

$$r(\tau_x, \tau_y, \tau_z) = \frac{M \sum_{x,y,z} \lambda(x, y, z) \lambda(x - \tau_x, y - \tau_y, z - \tau_z) - \sum_{x,y,z} \lambda(x, y, z) \sum_{x,y,z} \lambda(x - \tau_x, y - \tau_y, z - \tau_z)}{\sqrt{[M \sum_{x,y,z} \lambda(x, y, z)^2 - [\sum_{x,y,z} \lambda(x, y, z)]^2] [M \sum_{x,y,z} \lambda(x - \tau_x, y - \tau_y, z - \tau_z)^2 - [\sum_{x,y,z} \lambda(x - \tau_x, y - \tau_y, z - \tau_z)]^2]}}$$

978 where $\lambda(x, y, z)$ is the firing rate at the location (x, y, z) in the firing rate map, M is the total
 979 number of voxels in the rate map, and τ_x , τ_y and τ_z correspond to x , y , and z coordinate
 980 spatial lags¹¹. From this, we extracted the three voxel wide midline portion along the X, Y
 981 and Z axes and took the median value of the all the contained correlation scores. We also
 982 extracted the values falling exactly on these midlines (Matlab function *interp3*) for a measure
 983 of similarity over increasing distances or autocorrelation voxel lag (see Supplementary Fig.
 984 20 for a schematic and examples).

985 We also projected firing rate maps onto the three possible Cartesian planes by taking
 986 the average along each axis (ignoring empty voxels). We then calculated the spatial
 987 information content found in each projection and expressed these as the proportion of total
 988 spatial information. This last step was to account for cells with different overall spatial
 989 information content.



Supplementary Fig. 20: Example autocorrelations and schematic of the autocorrelation analysis procedure. To quantify the self-similarity of firing rate maps along each maze axis we generated firing rate map autocorrelations using the method described above. An example of one of these can be seen in **a**. Next we extracted the values falling along the midline of each axis (blue regions in **a**). For each of these we calculated the overall median correlation value. We also extracted values falling on the midline of each axis (i.e. a line running through the middle of each blue region). **b** The left column shows two example firing rate maps (one per row), the second column shows the result of an autocorrelation performed on each rate map. The third column shows the median autocorrelation value calculated for each axis (blue regions in **a**). The right column shows the values found along the midline of each axis of the autocorrelation; as the autocorrelations are symmetrical we have only shown the positive half of each axis. Note that for the top cell with vertically aligned firing the Z-axis median value is highest and this axis exhibits generally higher correlation values along this axis. The bottom cell has horizontally aligned firing and as a result the same effect is instead exhibited by the Y-axis.

Place field standard deviation analysis

To quantify the standard deviation of firing in the X, Y and Z dimensions for each place field, we summed the field along each of these dimensions and fitted a Gaussian (Matlab function *fit*, with curve *gauss1*) to the result. We then extracted the standard

1010 deviation of this Gaussian as a measure of place field spatial variance. Values resulting from
1011 poor Gaussian fits ($r^2 < 0.2$) were excluded.

1012 *Trajectory downsampling*

1013 To test if spatial information and autocorrelation were affected by the inhomogeneous
1014 sampling of space in the aligned lattice (i.e. animals move vertically much less than
1015 horizontally) we used a downsampling procedure. This consisted of filtering trajectory and
1016 spike data to include 50% vertical movements (defined as movements at a pitch $>30^\circ$ or $<-$
1017 30°) and 50% horizontal movements (defined as movements at a pitch $<30^\circ$ and $>-30^\circ$) for a
1018 total trajectory made up of sub-sampled but homogeneous data. We used a pitch angle of
1019 30° to delineate movements as this divides the face of a sphere into two halves with equal
1020 surface area (a 'belt' around the equator for one half and two spherical caps for the other).
1021 We generated firing rate maps using this sub-sampled trajectory and spike data and then
1022 repeated the analyses described in Supp. Methods: *Autocorrelation and spatial information*.
1023 We repeated the same test using data subsampled to include 50% inner (inner 50% volume
1024 of the lattice) and outer (outermost 50% volume) movements or 50% top (top 50% volume of
1025 the lattice) and bottom (bottom 50% volume) movements.

1026 *Zingg shape categorisation*

1027 To quantify the shape of place fields we used the method described by Zingg¹². If
1028 P1, P2 and P3 are the long, intermediate, and short axes of a place field and R is a number
1029 greater than one then four mutually exclusive shape classes can be defined (see
1030 Supplementary Table 4). In our approach we used $R = 3/2$ as suggested by Zingg¹². This
1031 approach classifies shapes into four mutually exclusive groups that can be used to evaluate
1032 the shape of individual fields; 'equant', 'prolate', 'oblate' and 'bladed'. Of greatest interest to
1033 us are the equant (spherical) and prolate (significantly elongated) classes. For each maze
1034 we calculated a shuffled distribution for comparison. To maintain a suitable underlying
1035 distribution we randomly shuffled the principal axis lengths of our place fields, grouped them

into triplets and sorted them from highest to lowest. We then recalculated the number of triplets falling into each shape class. We repeated this process 1000 times. If the number of fields fulfilling a shape class in a given environment exceeded the 99th percentile of the number of fields fulfilling the same class in the shuffles this was considered a significant deviation from chance.

Supplementary Table 4

Table showing Zingg (1935) shape classification criteria

Shape class	P1 and P2	P2 and P3	Description	Example
Equant	$P2 < P1 < R \cdot P1$	$P3 < P2 < R \cdot P3$	all dimensions are comparable	Sphere
Prolate	$P1 > R \cdot P2$	$P3 < P2 < R \cdot P3$	one dimension is much longer	Cigar
Oblate	$P2 < P1 < R \cdot P2$	$P2 > R \cdot P3$	one dimension is much shorter	Pancake
Bladed	$P1 > R \cdot P2$	$P2 > R \cdot P3$	all dimensions are very different	Sheet of paper

Comparing activity between mazes

We sought to compare some basic firing properties between mazes and determine if there was a link between the characteristics of fields in the arena and lattice mazes. Overall maze firing rates were calculated as the total spikes emitted in a maze divided by the total time spent there. Spatial information content was calculated as in Methods: *Place cell criteria*. Sparsity was defined as:

$$sparsity = \sum (P_i R_i^2) / R^2$$

where P_i is the probability of occupancy of bin i , R_i is the mean firing rate in bin i , and R is the overall mean firing rate¹³. To compare field elongation between mazes we correlated the elongation of fields in the arena (or average elongation if a cell expressed multiple fields) and the elongation of fields (or average) in the following lattice maze session.

1056 To investigate if multiple fields of the same cell shared similar characteristics we
1057 compared their length and orientation, these analyses were conducted only on those cells
1058 with more than 1 place field. For length we calculated the average pairwise difference in
1059 length between the place fields of a cell and compared this to a shuffled distribution
1060 containing the average pairwise differences between randomly paired fields selected from
1061 the overall dataset (for that maze) without replacement. For orientation we calculated the
1062 average pairwise inner angle between the major axes of place fields and compared this to a
1063 shuffled distribution containing the average pairwise angles between randomly paired fields
1064 selected from the overall dataset (for that maze) without replacement. Lastly, to determine if
1065 cells were likely to exhibit the same number of fields in each maze, we calculated the
1066 difference between total fields in the lattice mazes and arena. We compared these values to
1067 shuffles where we calculated the same difference between randomly paired cells selected
1068 from the overall dataset (for that maze) without replacement.

1069 *Local field potential (LFP) analyses*

1070 Before analysis, all LFP data were removed of their direct current offsets, slowly
1071 changing components, and running line noise using the Chronux toolbox ¹⁴ *locdetrend*
1072 function which subtracts the linear regression line fit within a 1s moving window. They were
1073 then resampled at 250 Hz using a polyphase anti-aliasing filter (MATLAB function *resample*,
1074 *pchip* interpolation).

1075 To obtain a theta phase angle for each spike, LFPs were first bandpass filtered in the
1076 6-12 Hz range (fourth-order Butterworth, Matlab functions *butter* and *filtfilt*) before a Hilbert
1077 transform was applied to obtain the instantaneous phase angle (Matlab function *hilbert*).
1078 Instantaneous frequency was calculated as the derivative of this analytic signal (Matlab
1079 function *instfreq*) and instantaneous amplitude was calculated as its magnitude.

1080 To assess the relationship between running speed and the theta oscillation we
1081 compared the instantaneous theta power/amplitude at every position data point (every 20
1082 ms) to the animals' instantaneous running speed. Instantaneous speed was estimated as the
1083 total distance travelled in every 40 ms window (i.e. the distance between every position data
1084 sample, the previous one and the next one divided by the time between them). To quantify
1085 the relationship between speed and power we fitted a linear regression model using a least-
1086 squares approach (Matlab function *polyfit*, 1 degree) and extracted the slope, y-intercept and
1087 sum of squared error. To assess the curvature of these relationships we also fitted a power
1088 function ($y(x) = ax^b + c$, where $a \geq 0$) to each session's speed-power curve and extracted
1089 the power parameter b (Matlab function *fit*). In this model $b=1$ denotes the linear function
1090 $f(x) = x$, $b<1$ denotes a downward curve and $b>1$ denotes an upward curve. We also
1091 performed the same procedures to test the relationship between running speed and
1092 instantaneous frequency.

1093 To calculate global/overall theta characteristics we computed average power spectral
 1094 densities (PSDs) for each recording session by first zero-padding data to the next highest
 1095 power of 2. A Welch spectral estimator was then applied to obtain the PSD (Matlab function
 1096 *pwelch*, Hamming window, 8 segments, 50% overlap). This was computed for 500
 1097 logarithmically spaced points between 0-250 Hz. Theta power was estimated as the
 1098 maximum power found in the theta band (6-12Hz), theta frequency was defined as the
 1099 frequency associated with this maximum power.

1100 *Running speed analyses*

1101 Instantaneous running speed was estimated as the total distance travelled in every
 1102 40ms window. For each cell, instantaneous firing rate was estimated as the smoothed spike
 1103 histogram (20 ms bins, 13 bin or 260 ms Gaussian smoothing window using Matlab function
 1104 *fspecial*). To quantify the relationship between speed and firing rate we used an analysis
 1105 similar to that described previously¹⁵. We binned the animals' running speeds in 2cm/s
 1106 increments and calculated the mean firing rate for each running speed bin and the total time
 1107 spent moving at that speed. We then fitted a linear regression model to the average firing
 1108 rate/speed data using a least-squares approach (Matlab function *polyfit*, 1 degree) and
 1109 extracted the slope, y-intercept and sum of squared error.

1110 *Spike phase and autocorrelation analyses*

1111 To quantify the intrinsic theta modulation of every place cell we used an analysis
 1112 described previously^{16,17}. For each cell we calculated the ± 500 ms spike autocorrelation in
 1113 10ms bins, normalized this to the maximum value found between 100 and 150 ms and
 1114 removed values >1 . Then we fit the following function to the remaining data:

$$y(t) = \left(a * \left(\sin \left(2\pi\omega t + \frac{\pi}{2} \right) + 1 \right) + b \right) * \exp \left(-\frac{|t|}{\tau_1} \right) + c * \exp \left(-\frac{r^2}{\tau_2^2} \right)$$

1115 where a, b, c, ω, τ_1 and τ_2 were fit to the data using a non-linear least squares method
 1116 (Matlab function *fit*) and t is the autocorrelogram time lag. In simple terms this function fits a
 1117 sine wave of frequency ω to the data and the exponential term allows for this to decrease
 1118 exponentially as the time lag increases (reflecting the exponential decay inherent to all spike
 1119 autocorrelations). The last Gaussian term helps to center the fit on the autocorrelogram
 1120 peak, which we found to be unnecessary in most cases. A measure of theta modulation
 1121 strength was defined as a/b , which intuitively corresponds to the ratio of the sine fit relative
 1122 to the baseline in the autocorrelogram. The parameter ω was extracted as the intrinsic theta
 1123 modulation of the cell. We restricted possible values for ω to $[6, 12]$, a and b were restricted
 1124 to non-negative values $[0, \infty]$, c was restricted to $[0, 0.8]$, τ_1 was unrestricted and τ_2 was
 1125 restricted to $[0, 0.05]$. This fitting procedure was only carried out on cells that fired at least
 1126 500 spikes.

1127 For each cell the instantaneous theta phase of every spike was calculated by linear
 1128 interpolation of the instantaneous theta phase signal described previously. These phase
 1129 angles were binned between $-\pi$ and π in 0.1 rad bins. The cell's preferred theta phase was
 1130 defined as the circular mean of these angles and the strength of this modulation was defined
 1131 as the mean resultant vector length of these angles (Matlab functions *circ_mean* and *circ_r*
 1132 respectively, circular statistics toolbox, ¹⁸). At the population level, all cell preferred phases
 1133 were collated, binned between $-\pi$ and π in 0.1 rad bins and again we calculated the
 1134 strength of this modulation using the mean resultant vector length as above. Distributions
 1135 were compared between mazes using a two-sample Kuiper test (Matlab function
 1136 *circ_kuipertest*, circular statistics toolbox, ¹⁸) and the phase lag between them was estimated
 1137 by cross-correlation (Matlab function *finddelay*).

1138 *Histology*

1139 At the end of the experiment animals were given an overdose of pentobarbital
 1140 intraperitoneally (Euthatal, Merial Animal Health Ltd., Essex, UK), and perfused with 0.9%

1141 saline solution followed by a 4% formalin solution. The brain was extracted and stored in 4%
1142 formalin for at least seven days prior to any histological analyses. The brains were sliced in
1143 30 μm sections on a freezing microtome at -20° . These sections were stained with a 0.1%
1144 cresyl violet solution and the slice best representing the electrode track was then imaged
1145 and color corrected.

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