

# 1 Representation of Distance and Direction of Nearby 2 Boundaries in Retrosplenial Cortex

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10

## 11 Abstract

12 **Borders and edges are salient and behaviourally relevant features for navigating the**  
13 **environment. The brain forms dedicated neural representations of environmental**  
14 **boundaries, which are assumed to serve as a reference for spatial coding. Here we**  
15 **expand this border coding network to include the retrosplenial cortex (RSC) in which**  
16 **we identified neurons that increase their firing near all boundaries of an arena. RSC**  
17 **border cells specifically encode walls, but not objects, and maintain their tuning in the**  
18 **absence of direct sensory detection. Unlike border cells in the medial entorhinal**  
19 **cortex (MEC), RSC border cells are sensitive to the animal's direction to nearby walls**  
20 **located contralateral to the recorded hemisphere. Pharmacogenetic inactivation of**  
21 **MEC led to a disruption of RSC border coding, but not vice versa, indicating network**  
22 **directionality. Together these data shed light on how information about distance and**  
23 **direction of boundaries is generated in the brain for guiding navigation behaviour.**

## 24 Introduction

25 Rodents travel great distances in their natural habitat, establishing foraging paths on which  
26 they hunt and search for food. These paths often follow (natural) edges along the  
27 environment, providing safety and cloaking from their predators as opposed to exposure in  
28 open fields. When first introduced into novel experimental environments, rats show high  
29 levels of anxiety and timidity, resulting in defecation (Hall, 1934) and thigmotaxis (or “wall  
30 hugging”; Valle, 1970; Walsh & Cummins, 1976). Rats display reduced locomotion and seek  
31 out the safety of walls and corners, spending up to 98% of their initial time away outside of  
32 the centre area (Valle, 1970). It is only after extensive habituation, coupled with scattering of  
33 food for motivation, that rats are nudged to explore.

34 Once they enter the open space however, rodents are able to discriminate positions within  
35 the arena, allowing them to navigate to a desired location. This ability is manifested in the  
36 activity of neurons that fire at particular locations in space, such as place cells or grid cells,  
37 and population activity of place cells can distinguish nearby positions at several centimeter  
38 resolution in an open field arena (Brown, Frank, Tang, Quirk, & Wilson, 1998). It has been  
39 suggested that this ability is based on the estimation of distance and direction relative to  
40 landmarks in the environment, and previous studies have pointed to the importance here of  
41 environmental boundaries, such as walls or edges (Barry et al., 2006; O’Keefe & Burgess,  
42 1996). For example, a subpopulation of neurons in the medial entorhinal cortex (MEC) or the  
43 subiculum increase firing rates near the environmental boundaries, called border cells or  
44 boundary-vector cells (Lever, Burton, Jeewajee, O’Keefe, & Burgess, 2009; Solstad,  
45 Boccara, Kropff, Moser, & Moser, 2008). The presence of dedicated representations of  
46 environmental borders in the hippocampus and parahippocampal regions implies a pivotal  
47 role of boundary information in generating accurate spatial representations in the brain. In  
48 accordance with this idea, border cells in MEC develop earlier than grid cells after birth,  
49 exhibiting adult-like firing fields at postnatal days 16-18, while grid cells still exhibit immature  
50 irregular firing fields (Bjerknes, Moser, & Moser, 2014). It has further been shown that  
51 position errors of firing fields of grid cells accumulate after the animal leaves a wall of an  
52 open-field arena, suggesting an error-correcting role of environmental boundaries for internal  
53 spatial representations.

54 While these previous studies have indicated a key role of environmental boundaries in the  
55 brain’s spatial representation, it remains largely unclear how the boundary representation is  
56 generated and used in other brain regions for navigation. Recent work furthermore reported  
57 that the dorsomedial striatum contains cells that are active near the boundaries of the arena  
58 (Hinman, Chapman, & Hasselmo, 2019), leading to a question of functional relationships  
59 between these cells for boundary representations. This urges for detailed characterization  
60 and comparison of boundary coding between regions.

61 Here we report that a subpopulation of neurons in the retrosplenial cortex (RSC), a key brain  
62 region for navigation with reciprocal anatomical connections with MEC, increase their firing  
63 rate near environmental borders independent of wall identity. We discovered that firing of  
64 these RSC border cells is strongly modulated by the animal’s head direction relative to the  
65 closest wall, providing local information about the animal’s distance and direction to nearby  
66 boundaries. We explored under which environmental circumstances this information is  
67 generated by manipulating sensory and spatial cues in the environment. Furthermore, using  
68 decoding and pharmacogenetic inactivation techniques, we show the difference of boundary

69 information as well as functional dependence between border cells in MEC and RSC,  
70 obtaining insights into the circuit organization of boundary representation in the brain.

71

## 72 **Results**

### 73 **RSC cells fire in close proximity to the maze perimeter at specific distances.**

74 We performed electrophysiological recordings of neuronal activity in the retrosplenial cortex  
75 (**Fig. 1a, Supplementary Fig. S1**) of rats as they explored a squared open field arena and  
76 foraged for scattered chocolate pellets (**Fig. 1b**). All animals were sufficiently habituated to  
77 the environment and procedures, and actively explored the entire arena (**Fig. 1c**). The  
78 experimental setup was placed in the room with fixed landmarks to allow the animals to  
79 orient themselves relative to external features.

80 We recorded the activity of 4754 RSC neurons across 8 animals ( $n = 75$  sessions) and  
81 observed a subpopulation of cells that fired consistently at the edge of the arena (**Fig. 1c**).  
82 Across this subgroup there was a variety of preferred firing distances from the wall, ranging  
83 from the very near proximity up to a body-length (15-18 cm) away. Unlike traditional border  
84 cells found in MEC and Subiculum (Solstad et al., 2008; Stewart, Jeewajee, Wills, Burgess,  
85 & Lever, 2014), these border responses occurred throughout the environment on each of the  
86 four available walls. RSC border cells furthermore form multiple firing fields that are not  
87 necessarily directly connected to the wall. Typical border cell classification using the original  
88 border score (Solstad et al., 2008) identified only a small fraction of border cells in RSC, as  
89 this score is based on the occupancy of a single firing field along a wall and is strongly  
90 biased to connected bins (**Supplementary Fig. S2**). We thus developed a new model-based  
91 approach using a template-matching procedure to classify these border cells in RSC (**Fig.**  
92 **1d-1f**), based on (Grossberger, Battaglia, & Vinck, 2018).

93 This method uses two-dimensional (2D) information of the firing rate maps and builds on the  
94 assumption that border cells have their spikes concentrated at the entire outer ring of the  
95 arena, incorporating geometric information into the classification procedure. The dissimilarity  
96 between a cell's spatial firing rate map and a "border" template (**Fig. 1d, 1e**) was assessed  
97 by the algorithm based on the Earth Mover's Distance (Hitchcock, 1941; Rubner, Tomasi, &  
98 Guibas, 1998) (EMD; see methods), a distance metric from the mathematical theory of  
99 optimal transport. While the metric is sensitive to a change in the geometric shape of rate  
100 maps, it is robust to small variations of preferred firing distances or pixel-by-pixel jittering,  
101 giving a single tuning metric that can assess changes in the cell's firing as a function of  
102 experimental manipulations.

103 Border cells were defined as stable cells with a low dissimilarity EMD score below the 1<sup>st</sup>  
104 percentile of a spike-shuffled null distribution of 0.191, and an average firing rate above 0.5  
105 Hz. In total 407 out of 4754 RSC cells (8.6%) passed this criterion (**Fig. 1e, 1f**). Selected  
106 border cells had a similar distribution of average firing rates compared to other recorded  
107 cells (border cells:  $FR = 1.70 \pm 0.20$  Hz, others:  $FR = 1.68 \pm 0.08$  Hz; Wilcoxon ranksum  
108 test,  $z = 0.024$ ,  $p = 0.981$ ; **Fig. 1g**), but had significantly higher spatial correlations between  
109 the first and last recording sessions (border cells:  $r = 0.52 \pm 0.01$ , others:  $r = 0.20 \pm 0.003$ ;  
110 Wilcoxon ranksum test:  $z = -23.46$ ,  $p = 1.15 \times 10^{-121}$ ; **Fig. 1h**).

111 **Border cells form new firing fields nearby added walls but not to objects.**

112 We next asked if the firing of these border cells is limited to walls, or whether these cells also  
113 encode information about other features of the environment (e.g. local cues or objects  
114 (Hoydal, Skytoen, Andersson, Moser, & Moser, 2019; Jacob et al., 2017)). Our first  
115 manipulation was to temporarily add an additional wall, protruding from one side into the  
116 centre of the maze (**Fig. 2a, 2b**). Border cells formed new firing fields around the added  
117 walls accordingly, as their firing rate inside a region-of-interest (ROI) around the wall  
118 increased significantly in the added wall sessions (Regular: FR =  $1.19 \pm 0.13$  Hz; Added  
119 wall: FR =  $1.58 \pm 0.21$  Hz; Wilcoxon signed rank test:  $z = -2.67$ ,  $p = 0.0076$ ;  $n = 42$  border  
120 cells; **Fig. 2c**). This was accompanied by a sharp drop in spatial correlations between  
121 ratemaps of regular versus added wall sessions (Reg-Reg:  $r = 0.51 \pm 0.004$ , Reg-Wall:  $r =$   
122  $0.25 \pm 0.006$ ; Wilcoxon signed rank test:  $z = 4.43$ ,  $p = 9.31 \times 10^{-6}$ ; Bonferroni-corrected  $\alpha =$   
123  $0.025$ ; **Fig. 2d**), while correlations remained high when comparing within session types  
124 (Wall-Wall:  $r = 0.47 \pm 0.005$ ; Wilcoxon signed rank test with Reg-Reg correlation:  $z = 0.63$ ,  $p =$   
125  $0.53$ ; Bonferroni-corrected  $\alpha = 0.025$ ; **Fig. 2d**). The EMD metric furthermore showed a  
126 significant increase in dissimilarity between ratemaps of these added wall sessions and the  
127 original border template (EMD score template 1: R1,  $0.176 \pm 0.002$ , W1,  $0.207 \pm 0.005$ , W2,  
128  $0.215 \pm 0.005$ , R2,  $0.179 \pm 0.002$ ; Friedman test:  $\chi^2(3) = 77.9$ ,  $p = 8.6 \times 10^{-17}$ ; Post-hoc  
129 Wilcoxon signed rank test: R1-W1,  $z = -5.35$ ,  $p = 9.0 \times 10^{-8}$ , R1-W2,  $z = -5.58$ ,  $p = 2.37 \times 10^{-8}$ ,  
130 R1-R2,  $z = -1.27$ ,  $p = 0.20$ ; Bonferroni-corrected  $\alpha = 0.017$ ; **Fig. 2e**). In contrast, the  
131 dissimilarity between the same ratemaps and an "added wall" template decreased  
132 significantly (EMD score template 2: R1,  $0.145 \pm 0.002$ , W1,  $0.132 \pm 0.003$ , W2,  $0.135 \pm$   
133  $0.004$ , R2,  $0.153 \pm 0.003$ ; Friedman test:  $\chi^2(3) = 33.7$ ,  $p = 2.3 \times 10^{-7}$ ; Post-hoc Wilcoxon  
134 signed rank test: R1-W1,  $z = -3.89$ ,  $p = 9.8 \times 10^{-5}$ , R1-W2,  $z = 2.59$ ,  $p = 0.0095$ , R1-R2,  $z = -$   
135  $2.22$ ,  $p = 0.027$ ; Bonferroni-corrected  $\alpha = 0.017$ ; **Fig. 2e**), confirming that border cells indeed  
136 encode wall information.

137 To investigate generalization to other environmental features we further added additional  
138 objects to the arena and tested the specificity of border responses to the spatial layout (**Fig.**  
139 **2f, 2g**). Contrary to an added wall, RSC border cells maintained tuning only to the outer  
140 walls and did not fire whenever objects were inside their receptive field (Regular: FR =  $1.39$   
141  $\pm 0.26$  Hz; Added object: FR =  $1.44 \pm 0.20$  Hz; Wilcoxon signed rank test:  $z = -0.57$ ,  $p =$   
142  $0.57$ ;  $n = 23$  border cells; **Fig. 2h**). There were no significant changes when comparing  
143 spatial correlations across session types (Reg-Reg:  $r = 0.54 \pm 0.007$ , Reg-Object:  $r = 0.63 \pm$   
144  $0.009$ ; Wilcoxon signed rank test:  $z = -1.41$ ,  $p = 0.16$ ; Object-Object:  $r = 0.55 \pm 0.008$ ;  
145 Wilcoxon signed rank test with Reg-Reg correlation:  $z = -0.51$ ,  $p = 0.61$ ; Bonferroni-corrected  
146  $\alpha = 0.025$ ; **Fig. 2i**). EMD analyses showed a minor but significant increase in dissimilarity to  
147 the border template in the object sessions (EMD score template 1: R1,  $0.169 \pm 0.005$ , O1,  
148  $0.182 \pm 0.004$ , O2,  $0.181 \pm 0.006$ , R2,  $0.171 \pm 0.004$ ; Friedman test:  $\chi^2(3) = 14.7$ ,  $p = 0.002$ ;  
149 Post-hoc Wilcoxon signed rank test: R1-O1,  $z = -2.71$ ,  $p = 0.007$ , R1-O2,  $z = -2.80$ ,  $p =$   
150  $0.005$ , R1-R2,  $z = -0.79$ ,  $p = 0.43$ ; Bonferroni-corrected  $\alpha = 0.017$ ; **Fig. 2j**), indicating small  
151 changes in the ratemaps of the object sessions. The cells did not form new firing fields  
152 around the object however, as fitting an "object" template led to a similar increase rather  
153 than decrease in dissimilarity (EMD score template 3: R1,  $0.149 \pm 0.003$ , O1,  $0.160 \pm 0.004$ ,  
154 O2,  $0.155 \pm 0.004$ , R2,  $0.150 \pm 0.003$ ; Friedman test:  $\chi^2(3) = 12.4$ ,  $p = 0.006$ ; Post-hoc  
155 Wilcoxon signed rank test: R1-O1,  $z = -2.65$ ,  $p = 0.008$ , R1-O2,  $z = -1.55$ ,  $p = 0.12$ , R1-R2,  $z =$   
156  $-0.30$ ,  $p = 0.76$ ; Bonferroni-corrected  $\alpha = 0.017$ ; **Fig. 2j**). Taken together these results

157 imply that RSC border cells encode information that is specific to boundaries of the spatial  
158 layout where cell responses differentiate between the types of added features.

### 159 **Border cells retain their tuning in darkness or to an edge without a wall.**

160 One way for border cells to compute information of boundaries is through direct sensory  
161 detection of the walls, for example by whisking or visual observation (Raudies & Hasselmo,  
162 2012). We next investigated the importance of direct sensory input on border tuning by  
163 removing either visual or somatosensory information of the boundary (**Fig. 3a, 3e**). We first  
164 recorded in complete darkness using an infrared position tracking system, but observed no  
165 significant changes in EMD dissimilarity scores across the sessions (EMD score template 1:  
166 R1,  $0.183 \pm 0.001$ , D1,  $0.185 \pm 0.003$ , D2,  $0.177 \pm 0.003$ , R2,  $0.182 \pm 0.002$ ; Friedman test,  
167  $\chi^2(3) = 1.23$ ,  $p = 0.75$ ;  $n = 21$  border cells; **Fig. 3b, 3d**). There were also no changes across  
168 spatial correlations between different session types (Reg-Reg:  $r = 0.42 \pm 0.007$ , Reg-Dark:  $r$   
169  $= 0.38 \pm 0.007$ ; Wilcoxon signed rank test  $z = 0.61$ ,  $p = 0.54$ ; Dark-Dark:  $r = 0.42 \pm 0.01$ ,  
170 Wilcoxon signed rank test with Reg-Reg correlation,  $z = 1.20$ ,  $p = 0.23$ ; Bonferroni-corrected  
171  $\alpha = 0.025$ ; **Fig. 3c**), indicating that activity is not generated solely through visual sensory  
172 input.

173 Similarly, we removed one of the outer walls that left a drop-edge above the floor, limiting  
174 movement of the animal in the absence of direct somatosensory information of a physical  
175 barrier (**Fig. 3e**). Again there were no major changes in EMD dissimilarity scores of the  
176 original border template for the regular versus drop-edge sessions (EMD score template 1:  
177 R1,  $0.171 \pm 0.001$ , Drop,  $0.174 \pm 0.002$ , R2,  $0.173 \pm 0.002$ ; Friedman test:  $\chi^2(2) = 7.0$ ,  $p =$   
178  $0.03$ ; Post-hoc Wilcoxon signed rank test: R1-Drop,  $z = -2.04$ ,  $p = 0.041$ , R1-R2,  $z = -2.03$ ,  $p$   
179  $= 0.041$ ; Bonferroni-corrected  $\alpha = 0.025$ ;  $n = 78$  border cells; **Fig. 3f, 3h**). We also observed  
180 no relevant changes in dissimilarity for a "drop-edge" template across all sessions, besides a  
181 small though significant drop in the final regular session (EMD score template 4: R1,  $0.274 \pm$   
182  $0.002$ , Drop,  $0.271 \pm 0.003$ , R2,  $0.263 \pm 0.003$ ; Friedman test:  $\chi^2(2) = 17.5$ ,  $p = 0.0002$ ;  
183 Post-hoc Wilcoxon signed rank test: R1-Drop,  $z = 0.76$ ,  $p = 0.44$ , R1-R2,  $z = 4.14$ ,  $p = 3.45 \times$   
184  $10^{-5}$ ; Bonferroni-corrected  $\alpha = 0.025$ ; **Fig. 3f, 3h**), indicating that RSC border cells do not  
185 change their firing properties alongside the drop-edge compared to a physical wall, in a  
186 similar manner as border cells in MEC (Solstad et al., 2008). This is supported by stable  
187 spatial correlations across session type comparisons (Reg-Reg:  $r = 0.57 \pm 0.002$ , Reg-Drop:  
188  $r = 0.55 \pm 0.002$ ; Wilcoxon signed rank test:  $z = 0.60$ ,  $p = 0.55$ ; **Fig. 3g**). These results  
189 suggest that neural activity of RSC border cells is not driven by pure sensory detection of  
190 boundaries, as cells are unaffected by the removal of unimodal sensory input.

### 191 **RSC cells have a biased directional tuning to boundaries in the contralateral side of** 192 **the recorded hemisphere.**

193 Recent reports pointed to egocentric anchoring of spatial representations to environmental  
194 features such as the maze centre or walls (Hinman et al., 2019; LaChance, Todd, & Taube,  
195 2019). RSC border cells described here have a similar direction tuning, where spikes that  
196 occur in close proximity to a wall are constraint by specific directions of the animal relative to  
197 the boundary (**Fig. 4a**). Projecting this trajectory data onto new body-centric axes, where  
198 coordinates indicate distance and direction of the nearest wall relative to the animal, indeed  
199 shows that cells fire predominantly whenever the wall occupies proximal space on the  
200 contra-lateral side of the recorded hemisphere (**Fig. 4b, 4c**).



201 We sought to establish whether this egocentric constraint was imposed by the head direction  
202 signal, as RSC receives inputs from the anterior limbic system that is a major source of head  
203 direction signals, and a subpopulation of RSC cells are tuned to allocentric head direction  
204 (Chen, Lin, Green, Barnes, & McNaughton, 1994; Mitchell, Czajkowski, Zhang, Jeffery, &  
205 Nelson, 2018). If the boundary representation of RSC border cells is driven by internally  
206 generated global direction signals, realignment of the head direction cells may affect the  
207 preferred tuning direction of RSC border cells. In order to manipulate the tuning of head  
208 direction cells, four blue landmark LEDs were placed on one side of the maze while all other  
209 sensory cues were kept invariant across the environment. The entire experimental setup  
210 was then rotated 90° clockwise in the middle sessions (**Fig. 4d**). As a result, all allocentric  
211 head direction (HD) cells rotated their tuning curves accordingly, although not a full 90° (A-  
212 A': median shift = 2.6°,  $z = 1.23$ ,  $p = 0.23$ ; B1-B2: median shift = 0.8°,  $z = 0.61$ ,  $p = 0.54$ ; A-  
213 B1: median shift = 62.9°,  $z = 4.62$ ,  $p = 3.8 \times 10^{-6}$ ; A-B1 rotated: median shift = -27.3°,  $z = -$   
214 3.07,  $p = 0.002$ ; Wilcoxon signed rank test; Bonferroni-corrected  $\alpha = 0.013$ ;  $n = 28$  HD cells;  
215 **Fig. 4g**). The direction tuning of border cells in contrast remained unchanged (examples in  
216 **Fig. 4e, 4f**; A-A': median shift = 0°,  $z = 0.085$ ,  $p = 0.93$ ; B1-B2: median shift = 0°,  $z = -0.85$ ,  $p$   
217 = 0.40; A-B1: median shift = 0°,  $z = 1.61$ ,  $p = 0.11$ ; A-B1 rotated: median shift = -79°,  $z = -$   
218 3.95,  $p = 7.7 \times 10^{-5}$ ; Wilcoxon signed rank test; Bonferroni-corrected  $\alpha = 0.013$ ;  $n = 46$  border  
219 cells; **Fig. 4e-4g**). This result indicates that the direction tuning of RSC border cells is either  
220 generated by local place and direction information independent of allocentric head direction  
221 cells, or is dependent on the integration of tightly-bound allocentric position and head-  
222 direction coding that rotated together.

223 Across the population, border cells were tuned predominantly to the very near proximity  
224 (main peak at 5.5 cm; **Fig. 4j**), although some cells had fields at extended distances up to 20  
225 cm away from the wall. Border cells showed a similar disproportionately biased distribution  
226 of preferred directions, dependent on the hemisphere where cells were recorded (Left  
227 hemisphere: mean direction = -102.9°,  $z = 10.11$ ,  $p = 3.0 \times 10^{-5}$ ; Right hemisphere: mean  
228 direction = 32.0°,  $z = 32.54$ ,  $p = 3.4 \times 10^{-15}$ ; Rayleigh test; comparing both probability  
229 distributions: two-sample Kolmogorov-Smirnov test,  $p = 0.021$ ;  $n = 333$  border cells; **Fig. 4h,**  
230 **4i**). The majority of border cells were tuned to the contra-lateral side of the implanted  
231 electrode (e.g. whenever the wall is on the right side while the cell is recorded in the left  
232 hemisphere), although not exclusively (**Fig. 4h, 4i**). This hemisphere-specific tuning bias  
233 implies that boundary representations in RSC may either be generated by direct sensory  
234 signals, or reflect the command of motor actions, in both of which this bias arises along the  
235 right-left body axis.

### 236 **Inhibition of MEC disrupts border cell activity in RSC but not vice versa.**

237 The retrosplenial cortex is known to have direct, bi-directional connections with the medial  
238 entorhinal cortex (Bethany F Jones & Witter, 2007; Ohara et al., 2018), in particular with  
239 MEC layer 5 where the majority of border cells are located (Boccarda et al., 2010), although  
240 their function remains unknown. Given the presence of border cells in both RSC and MEC,  
241 albeit with different properties, it is crucial to establish the direction and extent of functional  
242 interactions between these brain regions. We thus performed electrophysiological recordings  
243 of border cells in RSC and MEC and quantified their boundary information.

244 Border cells in MEC are different from those in RSC by having fields attached to only one or  
245 two walls rather than all (**Fig. 5a**) (Solstad et al., 2008), but both populations have similar

246 peak firing rates when the animal's distance and direction to the wall were in the optimal  
247 range (RSC: FR =  $4.08 \pm 0.50$  Hz, MEC: FR =  $5.39 \pm 1.00$  Hz, Wilcoxon ranksum test:  $z =$   
248  $0.72$ ,  $p = 0.47$ ; **Fig. 5b**). We first examined whether border cells in the two regions carry  
249 similar distance information on a population level. A decoder based on support vector  
250 machines estimated the animal's distance away from the wall using population spiking  
251 activity, and performed with high accuracy for both MEC and RSC in the lower distance  
252 range ( $p < 0.05$  for 0-20 cm, compared with a chance level of 20%; **Fig. 5c**). However,  
253 decoding performance from RSC activity dropped to chance level in the higher distance  
254 range ( $p > 0.05$  for 30-50 cm; **Fig. 5c**), suggesting RSC border cells mainly encode local  
255 information. This matches the firing properties of RSC border cells which have preferred  
256 distance tuning up to 20cm away from the wall (**Fig. 4j**). Conversely, MEC computes  
257 distance information that extends well into the arena, with decoding performance above  
258 chance-level until the maximum range of 50 cm (e.g. in the centre of the maze;  $p < 0.05$ ;  
259 **Fig. 5c, 5d**).

260 Finally, we addressed the question of whether there is any communication between MEC  
261 and RSC in terms of encoding border information using a pharmacogenetic inactivation  
262 technique (Armbruster, Li, Pausch, Herlitze, & Roth, 2007). We first injected an AAV  
263 encoding the inhibitory DREADDs hM4Di into MEC, while simultaneously implanting a 28-  
264 tetrode hyperdrive into RSC (**Fig. 5e, Supplementary Fig. 4**). Subcutaneous administration  
265 of agonist-21 (DREADDs agonist) resulted in a drastic reduction of firing after 20 min for  
266 MEC cells infected with the virus (**Fig. 5f**). Inactivation of MEC led to a subsequent  
267 disruption of firing in a subset of RSC border cells (**Fig. 5g**), worsening border tuning that  
268 resulted in higher EMD scores (before: EMD score =  $0.181 \pm 0.002$ , after: EMD score =  
269  $0.186 \pm 0.003$ ; Wilcoxon signed rank test:  $z = -2.40$ ,  $p = 0.016$ ;  $n = 102$  border cells; **Fig. 5h**)  
270 and lower overall firing rates after the manipulation (before: FR =  $1.52 \pm 0.20$  Hz, after: FR =  
271  $1.12 \pm 0.24$  Hz, Wilcoxon signed rank test:  $z = 3.15$ ,  $p = 0.0016$ ; **Fig. 5i**). We next performed  
272 a reversed manipulation, injecting the virus encoding DREADDs hM4Di into RSC while  
273 recording neural activity in MEC (**Fig. 5j, Supplementary Fig. S4**). Administration of  
274 agonist-21 led to similar decreased activity in RSC for the infected cells (**Fig. 5k**), but RSC  
275 inhibition had no significant effect on MEC border cell tuning (before: border score =  $0.55 \pm$   
276  $0.015$ , after: border score =  $0.54 \pm 0.014$ ; Wilcoxon signed rank test:  $z = -0.014$ ,  $p = 0.989$ ;  $n$   
277 = 96 border cells; **Fig. 5m**) or average firing rates (before: FR =  $1.17 \pm 0.11$  Hz, after: FR =  
278  $1.19 \pm 0.13$  Hz; Wilcoxon signed rank test:  $z = 1.153$ ,  $p = 0.249$ ; **Fig. 5l, 5n**). Given the  
279 presence of border cells in both RSC and MEC and their bidirectional connectivity, it seems  
280 plausible that both regions are part of a broader border coding network. Our results here  
281 indeed show this to be the case, although only in one direction, suggesting that RSC border  
282 coding is partly dependent on MEC but not vice versa.

283

## 284 Discussion

285 We have shown that a subpopulation of neurons in the RSC increase their firing rates when  
286 the animal approached the proximity of walls. We used a metric of the earth mover's  
287 distance to quantify the boundary coding of cells, and found that border responses are  
288 specific to boundaries that impede the movement of animals, while they are invariant to an  
289 object introduced into the maze. Border responses were maintained in complete darkness  
290 and to an environmental edge without a physical wall. These results together suggest that

291 RSC border cells are not simply driven by local sensory cues, but likely discriminate  
292 boundaries from a global perspective of the environment.

293 Notably, we found that firing of RSC border cells is strongly constrained by the animal's head  
294 direction toward nearby boundaries, rather than to the environment, indicating body-centred  
295 or egocentric border representation. Furthermore, we assessed the spatial information  
296 provided by a population of border cells in RSC and MEC by implementing a decoding  
297 analysis and found that RSC border cells provide only local information at the wall proximity,  
298 whereas MEC border cells provide long-range distance information of a boundary. Finally, by  
299 inactivating neurons in either MEC or RSC, we found that the activity of RSC border cells is  
300 partly driven by MEC, but not vice versa. Altogether our results clarify the features of  
301 boundary representations in RSC, as well as key differences of their codes from border cells  
302 in MEC.

303 Anatomically, RSC locates at an interface region of the hippocampus and MEC with sensory  
304 and motor cortices (Bethany F Jones & Witter, 2007; Sugar, Witter, van Strien, & Cappaert,  
305 2011; T van Groen & Wyss, 1990, 1992; Thomas Van Groen & Wyss, 2003). While both  
306 human patients and rodents with lesions in RSC exhibited severe impairment in navigation  
307 ability (Takahashi, Kawamura, Shiota, Kasahata, & Hirayama, 1997; Vann, Aggleton, &  
308 Maguire, 2009), the exact role of RSC has been largely unclear until recently. Several recent  
309 studies have provided clues for understanding RSC function. An fMRI study in humans  
310 demonstrated that RSC is particularly engaged in representing permanent landmarks in the  
311 environment (Auger, Mullally, & Maguire, 2012), which is consistent with the present finding  
312 of border cells as walls can serve as permanent landmarks in an open field arena, especially  
313 in the absence of local cues. On the other hand, recording studies in rats have identified  
314 several types of spatially-tuned cells in RSC, such as head-direction cells, place cells, and  
315 the cells that represent geometric features of the environment (Alexander & Nitz, 2015; Cho  
316 & Sharp, 2001; Mao, Kandler, McNaughton, & Bonin, 2017). Because of the existence of  
317 these spatially-tuned cells as well as anatomical connections, RSC has been considered an  
318 ideal brain region to implement a transformation of spatial representations between  
319 egocentric and allocentric coordinate systems (Byrne, Becker, & Burgess, 2007; Mitchell et  
320 al., 2018). The allocentric-egocentric transformation is an essential computational step for  
321 navigation because, while spatial representations in the parahippocampal regions about  
322 head direction, places, or borders, are anchored to external features of the environment (i.e.  
323 in allocentric coordinates), experiencing the world through sensory organs and executing  
324 motor plans to move through space is referenced to the actor's body and viewpoint (i.e. in  
325 egocentric coordinates). Recent studies have reported neurons with egocentric tuning to  
326 navigational landmarks, such as the maze centre, objects, or boundaries, in brain regions  
327 including the lateral entorhinal cortex, the postrhinal cortex, and the dorsomedial striatum  
328 (Hinman et al., 2019; LaChance et al., 2019; Wang et al., 2018), and a picture is emerging of  
329 a functional network across brain regions that encode a wide-range of environmental  
330 features from a self-centred perspective.

331 Our findings are consistent with the RSC's role in coordinate transformation because both  
332 allocentric head-direction cells and egocentric border cells co-exist in RSC. The question is  
333 how such egocentric representation is generated. One possibility is that egocentric border  
334 firing is directly driven by sensory perception, such as optic flow or whisker sensation, which  
335 is egocentric in nature. However, our present results argue against this possibility as firing of  
336 RSC border cells was not affected by the absence of direct visual or somatosensory

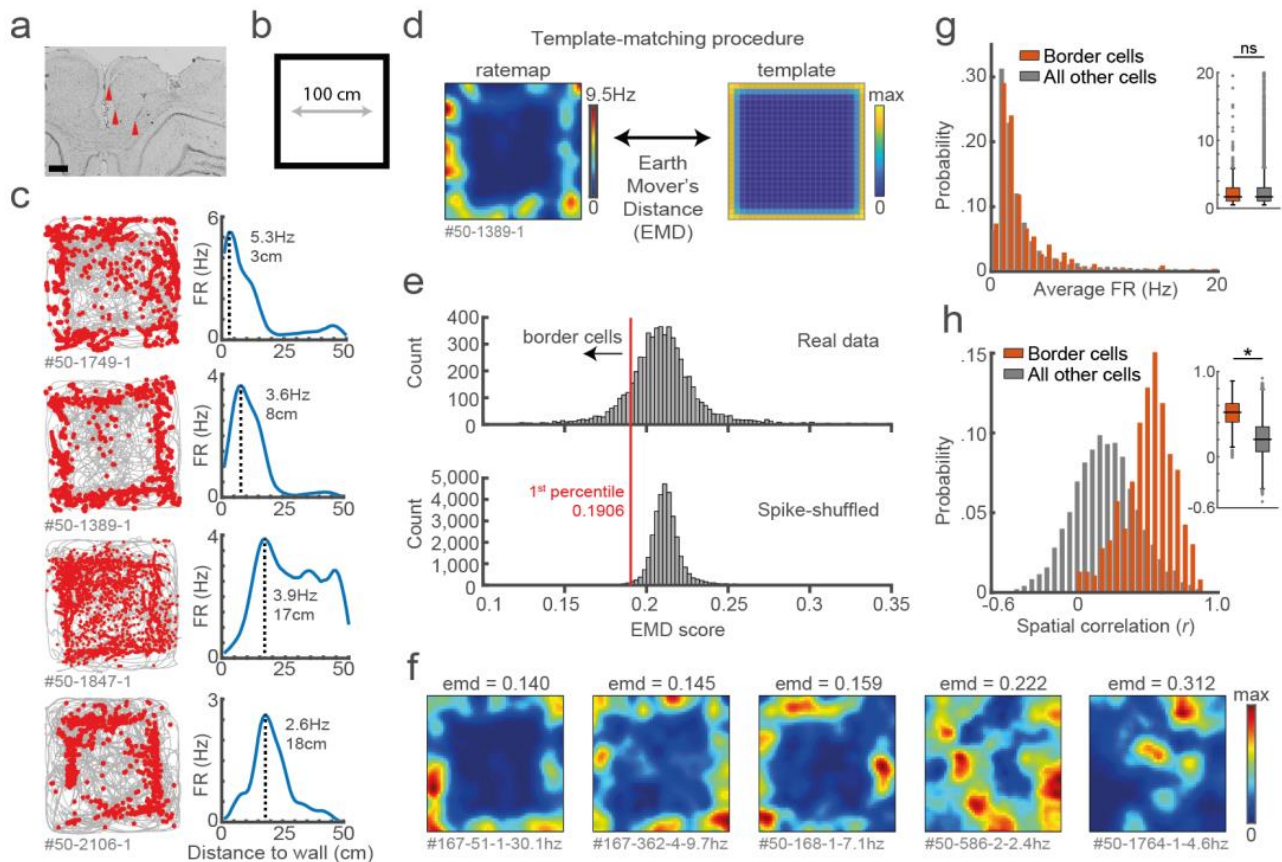


337 detection. Instead, our results favour the idea that RSC border cells are driven, at least in  
338 part, by MEC cells. This idea was proposed as a theoretical model (Byrne et al., 2007), in  
339 which the information about allocentric boundary locations is integrated with head-direction  
340 signals to form egocentric border representations. We found that the rotation of head-  
341 direction cells in RSC, elicited by a cue rotation of the environment, did not affect the  
342 egocentric tuning of RSC border cells, indicating that head-direction and position coding in  
343 RSC border cells must be bound and rotated together during environmental manipulations,  
344 consistent with the proposed circuit model (Byrne et al., 2007). This idea is further supported  
345 by our experiments with DREADDs-mediated activity manipulations, in which RSC border  
346 cells were significantly impaired by the inactivation of MEC, whereas RSC inactivation did  
347 not change the quality of border coding in MEC, suggesting that RSC border cells are partly  
348 dependent on MEC activity, but not likely the source of boundary information in MEC.

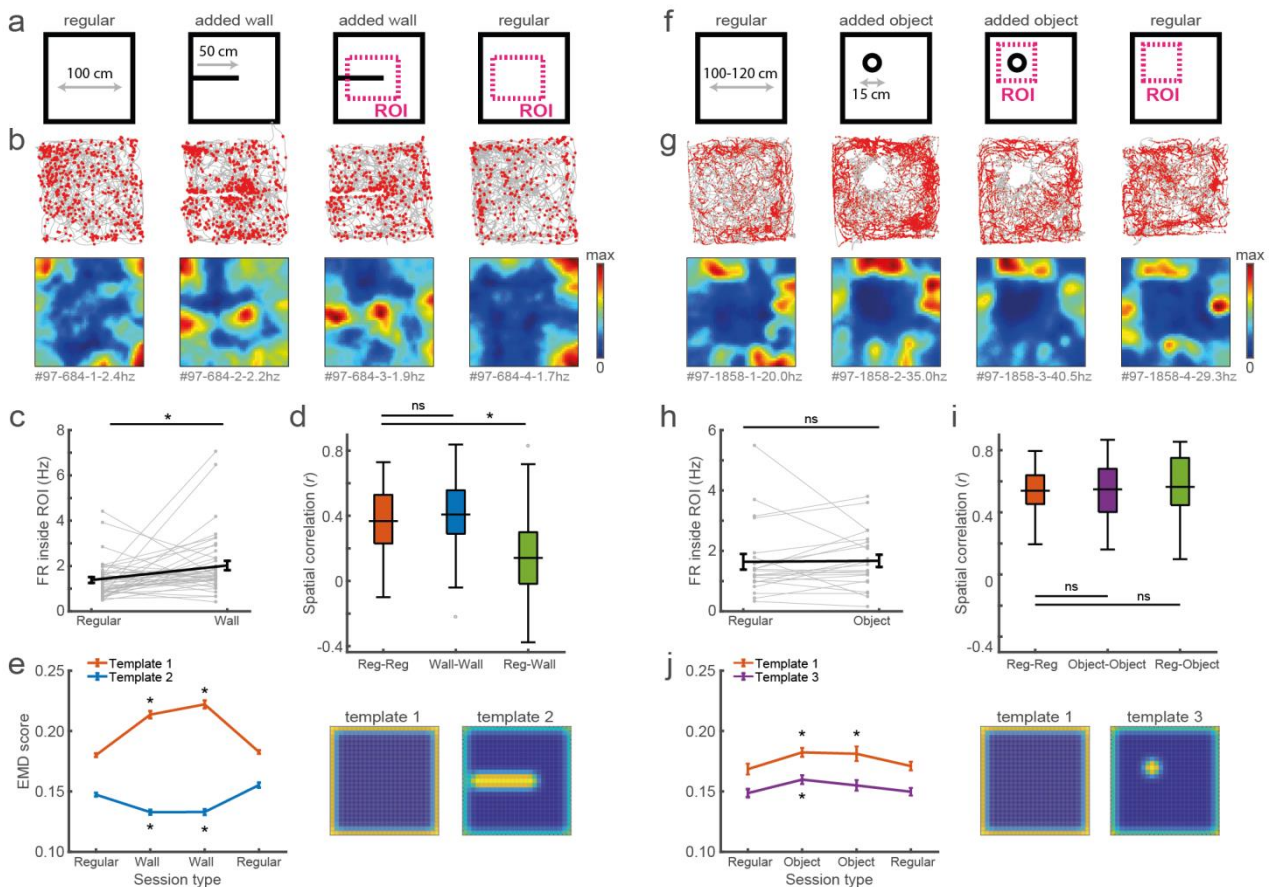
349 However, our results also clarify that RSC border cells are not necessarily a simple product  
350 of coordinate transformations from MEC cells. Our data clearly show a strong bias of tuning  
351 direction contra-lateral to the recorded hemisphere, an effect not observed in  
352 parahippocampal regions, which would indicate that a single hemisphere could transform  
353 only half of the potential behavioural space. Second, the range at which information about  
354 wall distance is present is different between MEC and RSC border cells. While RSC border  
355 cells provide local information about a nearby wall that is located less than 20 cm from the  
356 animal's position, border cells in MEC have extended distance information up to 50 cm (from  
357 a wall to the centre of the maze). These findings indicate that RSC border cells do not  
358 necessarily constitute an egocentric border map as a counterpart of an allocentric map in  
359 MEC.

360 What can be the cause of hemisphere-specific bias to boundaries in the animal's  
361 contralateral side, if RSC border cells are not directly driven by sensory perception? This  
362 bias may be a manifestation of the animal's immediate action control to the direction of an  
363 approaching wall. Collision detection and avoidance are fundamental roles of sensory-motor  
364 systems for many species of animals (Fotowat & Gabbiani, 2011), and rodents are also  
365 required to detect boundaries to avoid hitting walls or falling off edges. The boundary  
366 information in MEC and RSC may therefore be used in other brain regions to control the  
367 animal's next movements against walls or edges. RSC gives rise to inputs in brain regions  
368 necessary for motor control and initiation, such as premotor and motor cortices, cingulate  
369 cortex, as well as the dorsal striatum (Guo et al., 2015; B. F. Jones, Groenewegen, & Witter,  
370 2005; Yamawaki, Radulovic, & Shepherd, 2016). A recent recording study on the  
371 dorsomedial striatum has identified a type of neurons that fire near environmental borders in  
372 a similar manner as RSC border cells do. However, their egocentric tuning is largely  
373 dependent on the animal's movement direction (Hinman et al., 2019), rather than head  
374 direction as in RSC border cells. RSC border cells may thus provide the downstream  
375 striatum circuits with information about the direction of approaching wall in an egocentric  
376 perspective so that animals can initiate next appropriate actions against the wall direction.  
377 Our results thus support the idea that RSC implements a coordinate transformation of  
378 behaviourally relevant information, pointing to RSC as a key brain region linking between the  
379 brain's allocentric spatial representation and behaviours.

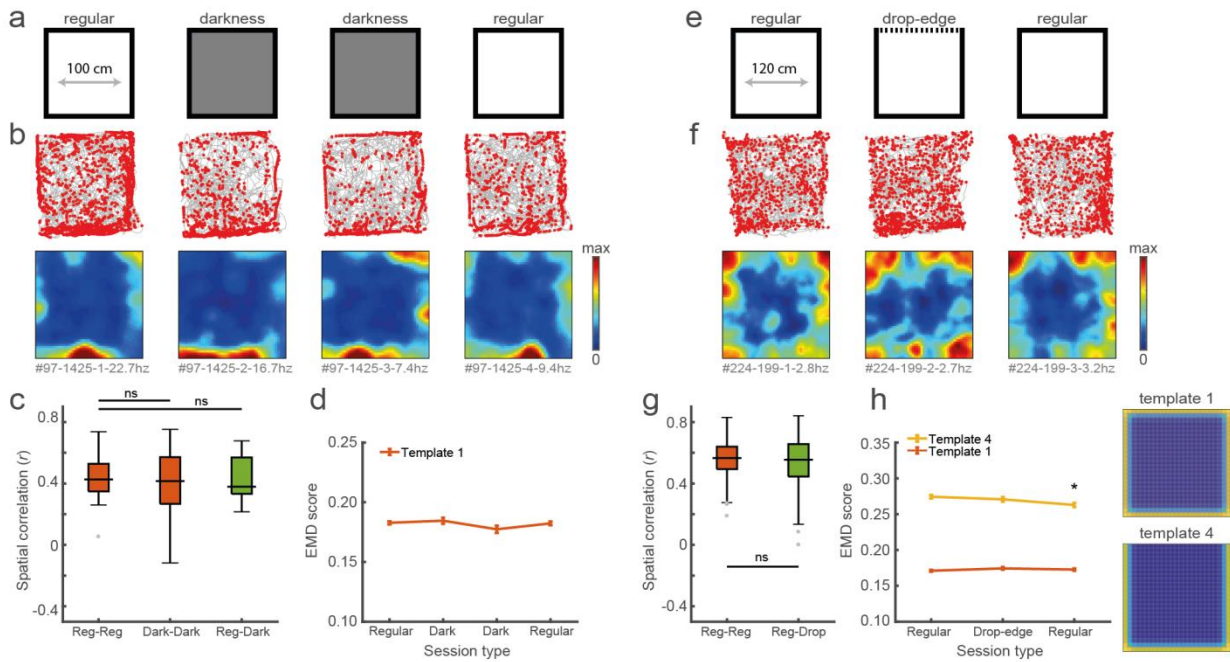
## 380 Figures



381 **Figure 1: Response profiles of border cells in RSC.** (a) Location of tetrode tracts marked  
 382 with red in an example Nissl-stained coronal section. Scale bar, 500 $\mu$ m. (b) Task behaviour  
 383 consisted of free exploration in a squared 1m<sup>2</sup> arena. (c) Trajectory spike plots (left column)  
 384 and distance FR plots (right column) of four example cells that fire at different distances  
 385 away from the wall, relative to the closest wall at any time. Grey lines indicate the animal's  
 386 trajectory and red dots the rat's position when a spike occurred. (d) A template-matching  
 387 procedure was applied to classify border cells by calculating the Earth Mover's Distance  
 388 (EMD) between each cell's spatial ratemap and an ideal template (see methods). (e) A cell  
 389 was classified as a border cell when its EMD score was below the 1<sup>st</sup> percentile of a shuffled  
 390 null distribution, together with an average FR above 0.5 Hz. (f) Colour-coded spatial  
 391 ratemaps of five example cells with different EMD scores, where warm colours indicate high  
 392 firing. From left to right: three typical border cells, a non-uniform firing cell and a cell with  
 393 focused firing fields. (g) Distribution of average FR over the entire recording day for border  
 394 cells and other recorded cells. (h) Distribution of spatial correlations between recorded  
 395 sessions for all neurons. \*p < 0.05, Wilcoxon ranksum test.

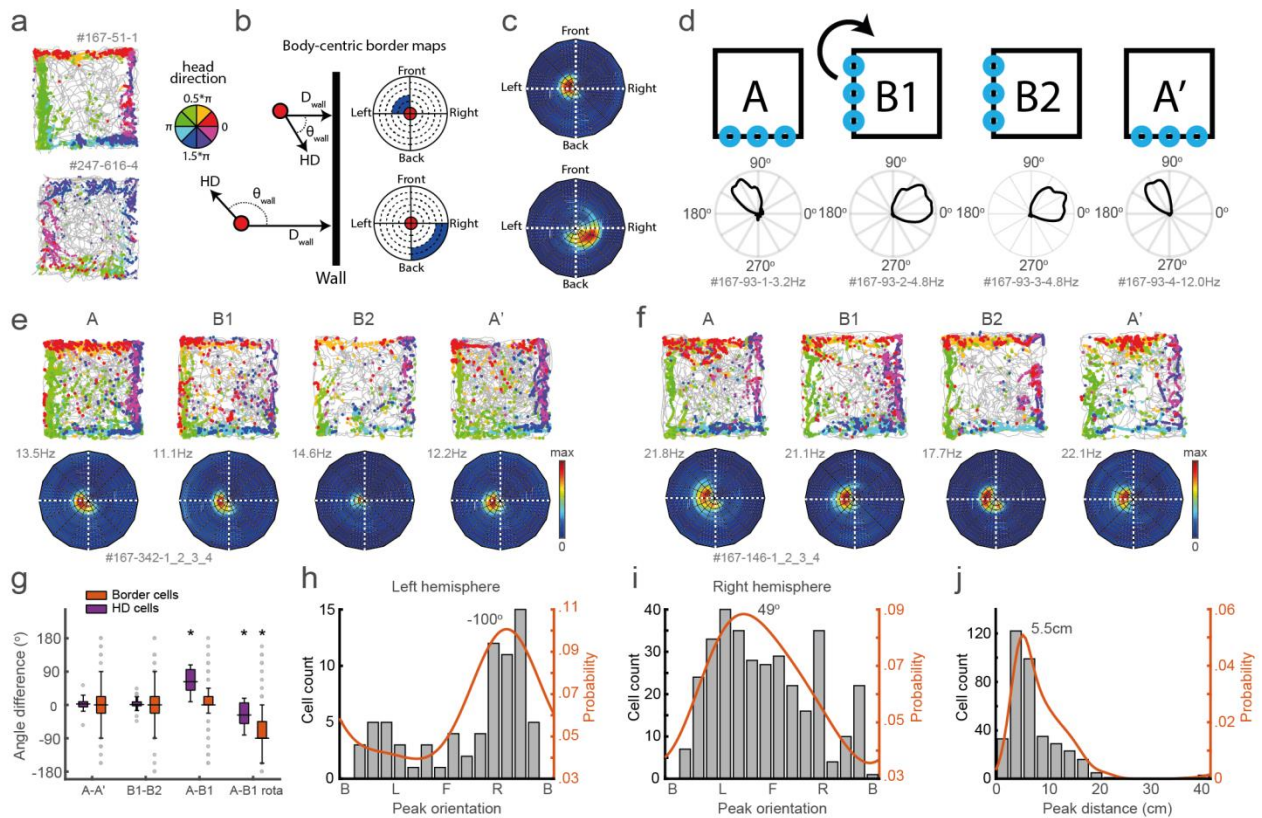


396 **Figure 2: Border cells respond to new walls but not to the addition of new objects. (a)**  
 397 **An additional temporary wall was added to the centre of the maze in the middle sessions. (b)**  
 398 **Trajectory spike plots and spatial ratemaps of an example border cell across regular and**  
 399 **added wall sessions during one recording day. (c) Border cells form new firing fields nearby**  
 400 **the added wall, as cells significantly increase their firing rate in the region-of-interest (ROI)**  
 401 **area around the central wall. (d) Spatial correlations between ratemaps of regular and wall**  
 402 **sessions are decreased, but remain high within session type. (e) The original border**  
 403 **template (#1) significantly increases in dissimilarity as cells form fields around the added**  
 404 **wall, opposite to an added wall template (#2) which decreases in dissimilarity. (f) A high,**  
 405 **non-climbable object was introduced in the north-west corner of the maze. (g) Trajectory**  
 406 **spike plots and spatial ratemaps of an example border cell across regular and object**  
 407 **sessions. (h) Border cells ignore the addition of objects as their FR in a ROI around the**  
 408 **object remains unchanged between session types. (i) There are no significant changes in**  
 409 **spatial correlations between the different session types. (j) There is a small increase in EMD**  
 410 **scores for template 1, but objects do not elicit a response from border cells as the object**  
 411 **template (#3) shows a similar increase. \* $p < 0.05$  (Bonferroni correction for multiple**  
 412 **comparisons), Wilcoxon signed rank test.**



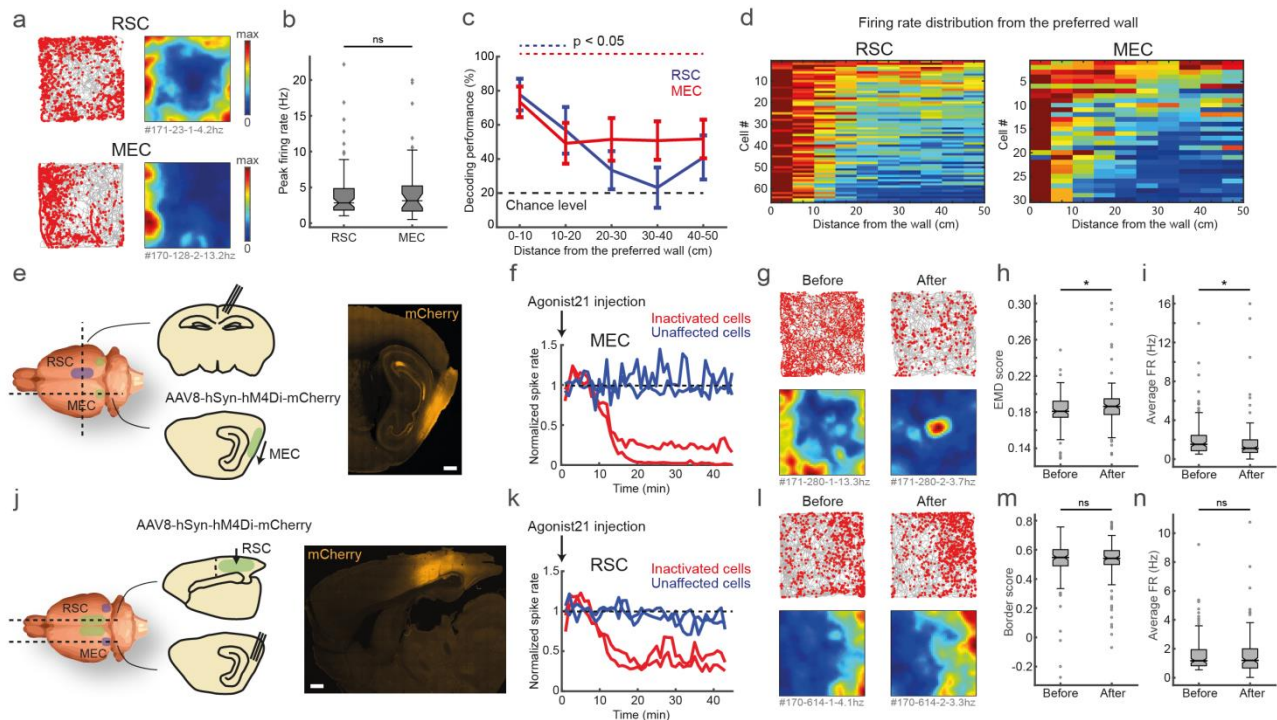
413 **Figure 3: Removing direct sensory detection of walls does not alter the border cell's**  
 414 **activity near boundaries. (a)** Recordings were performed in complete darkness for the  
 415 middle sessions, and animals were tracked in the non-visible infrared spectrum. **(b)**  
 416 Trajectory spike plots and spatial ratemaps of an example border cell recorded in light and  
 417 dark conditions. **(c)** Spatial correlations between ratemaps of regular and dark sessions  
 418 remain high, indicating border cells still fire nearby boundaries in darkness. **(d)** There were  
 419 no changes in EMD scores with template 1, confirming that cells maintain their tuning to the  
 420 outer walls without direct visual detection. **(e)** One of the outer walls was removed, leaving  
 421 only a drop-edge on one side to confine the arena. **(f)** Trajectory spike plots and spatial  
 422 ratemaps of an example border cell across recording sessions. **(g)** There are no significant  
 423 changes in spatial correlations across session types. **(h)** Spatial ratemaps remain  
 424 unchanged across session type, with no relevant changes in EMD scores for either template.  
 425 \*  $p < 0.05$  (Bonferroni correction for multiple comparisons), Wilcoxon signed rank test.



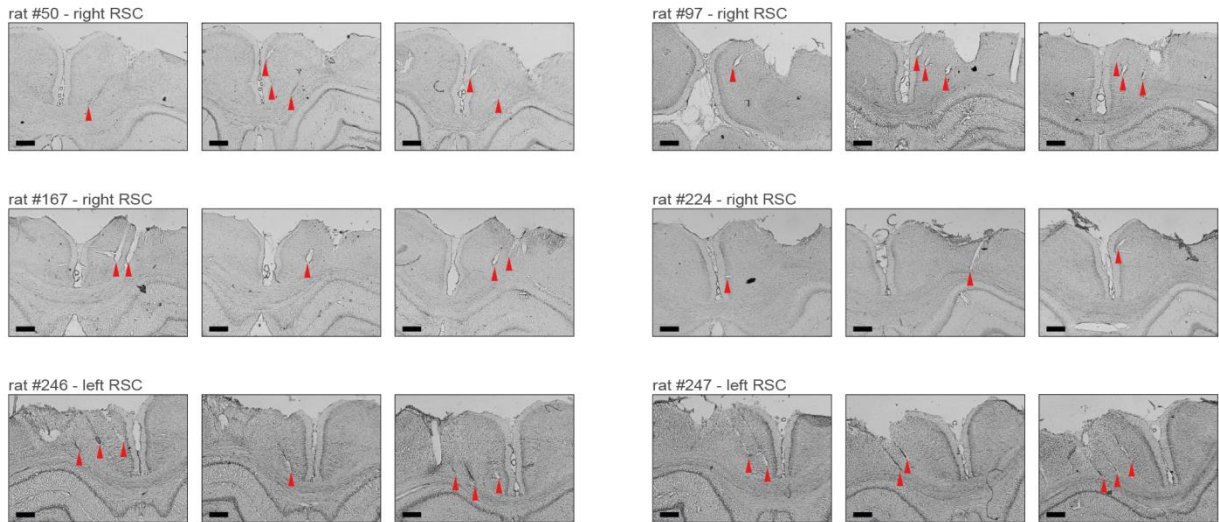


426 **Figure 4: Border responses have narrow directional constraints and are biased to the**  
 427 **contra-lateral hemisphere. (a)** Example trajectory spike plots with spike locations colour-  
 428 coded according to the direction of the animal. Most spikes alongside a wall occur only when  
 429 the animal is in a narrow range of directions. Top: recorded in right hemisphere. Bottom:  
 430 recorded in left hemisphere. **(b)** Trajectory data is projected onto new body-centric border  
 431 maps, where coordinates indicate the distance ( $D_{wall}$ ) and direction ( $\theta_{wall}$ ) of the closest wall  
 432 relative to the animal's position and head direction (HD) respectively. **(c)** Ratemaps in this  
 433 border space for the same example cells shown in (a). **(d)** Top: prominent blue landmark  
 434 LEDs were placed on one wall, and the entire experimental set-up was rotated 90° clockwise  
 435 in the middle sessions. Bottom: example HD cell showing its tuning shifted accordingly. **(e-f)**  
 436 Two example cells with trajectory spike plots and border ratemaps showing egocentric  
 437 border tuning is stable across rotation sessions. **(g)** Comparison of shifts in direction tuning  
 438 for head direction and border cells across the different sessions. **(h)** Preferred directional  
 439 tuning of all border cells recorded in the left hemisphere, with 0° being in front of the animal.  
 440 **(i)** Same as (h), but now for all border cells recorded in the right hemisphere. **(j)** Preferred  
 441 distance tuning of all border cells. \*  $p < 0.05$  (Bonferroni correction for multiple comparisons),  
 442 Wilcoxon signed rank test.

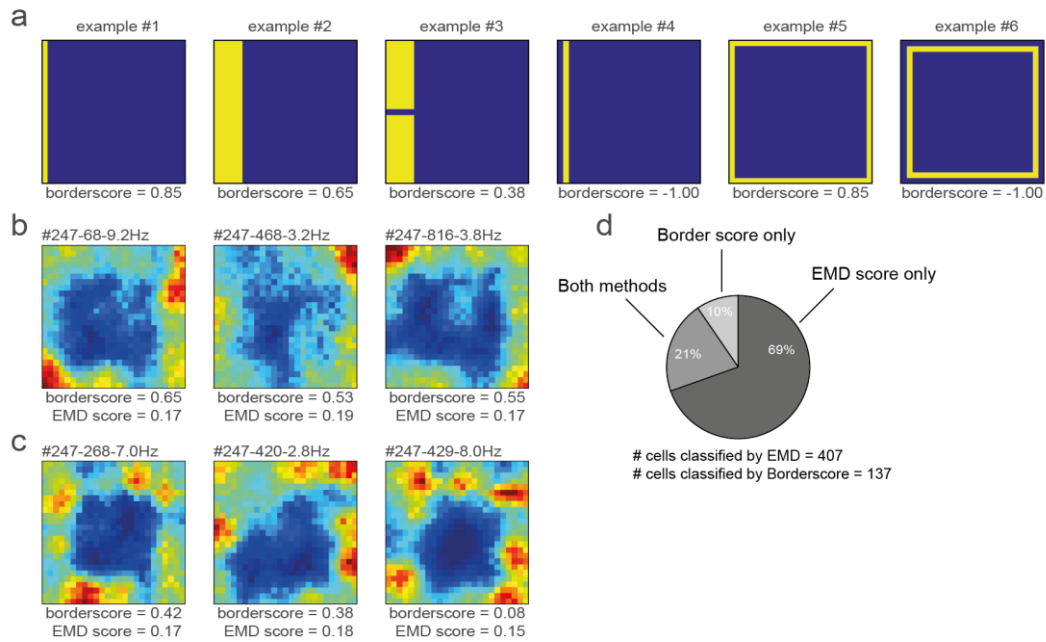




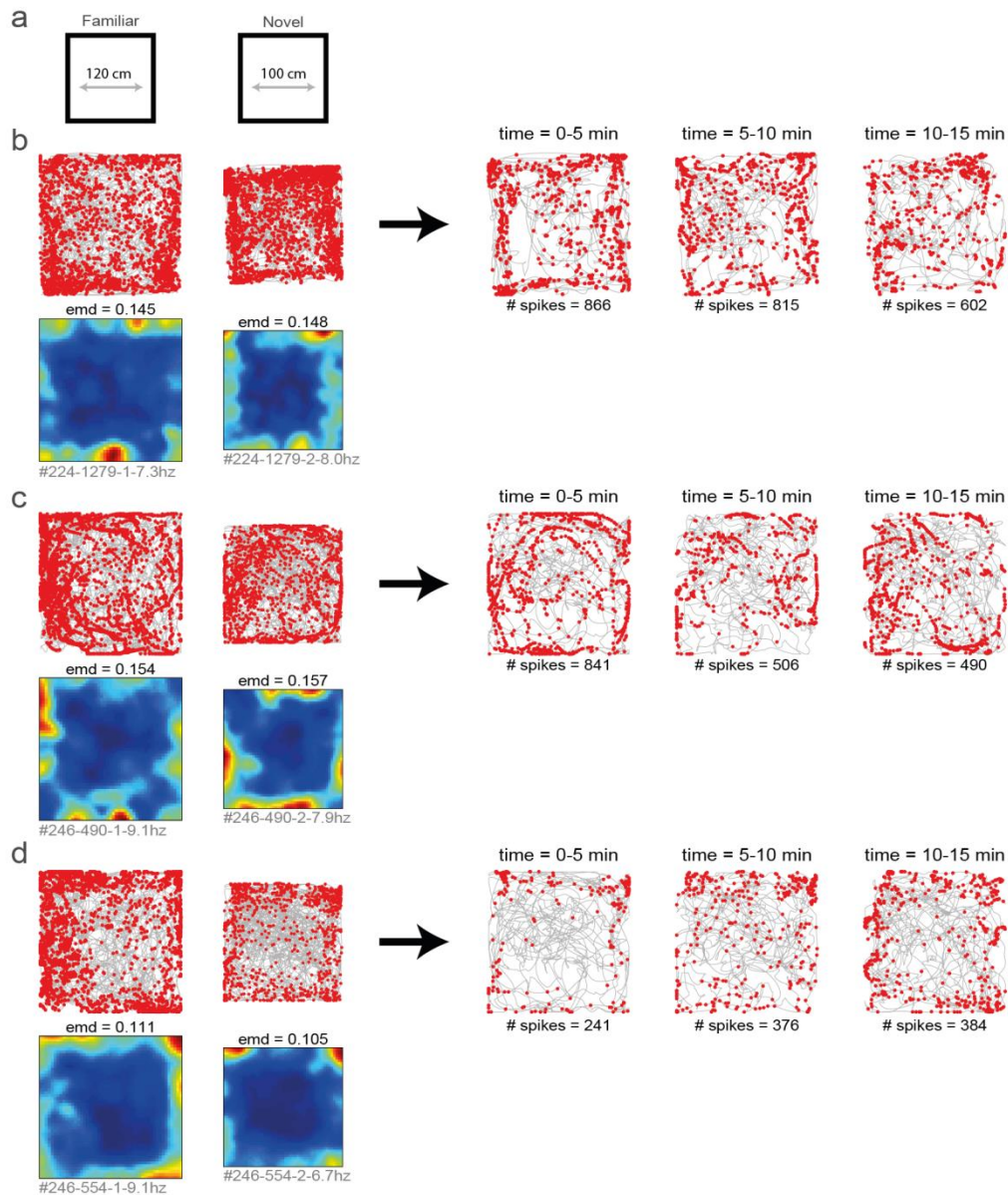
443 **Figure 5: RSC border cells provide local boundary information and receive input from**  
 444 **MEC. (a)** Spike trajectory plots and spatial ratemaps of two border cells recorded in RSC  
 445 and MEC. **(b)** Border cells in RSC and MEC have a similar distribution of peak firing rates.  
 446 **(c)** A decoder using a support vector machine classifier estimated the animal's distance to  
 447 the wall based on population spiking activity. Local distance information is present in both  
 448 regions, but extends further into the centre of the maze only in MEC. **(d)** Distributions of  
 449 firing rate as a function of distance to the preferred wall for border cells in both brain regions.  
 450 **(e)** An AAV encoding inhibitory DREADDs hM4Di was injected into MEC while tetrodes were  
 451 positioned into RSC. Scale bar, 1mm. **(f)** Four tetrodes were placed locally near the virus  
 452 injection site, showing affected neurons drastically decreased their activity 10-15 min after  
 453 subcutaneous administration of agonist-21 (DREADDs agonist). **(g)** An example RSC  
 454 border cell that is affected by MEC inhibition. **(h-i)** Border cells in RSC have decreased  
 455 border tuning and lower firing rates after inhibition of MEC. **(j)** Reversed experiment, with  
 456 electrophysiological recordings in MEC while the AAV was injected into RSC. Scale bar,  
 457 1mm. **(k)** Affected RSC neurons decrease their activity after administration of agonist-21. **(l)**  
 458 An example MEC border cell that is unaffected by inhibition of RSC. **(m-n)** Border cells in  
 459 MEC do not show any significant qualitative changes in border tuning or firing rates after  
 460 RSC inhibition. \*  $p < 0.05$ , Wilcoxon ranksum (b) or signed rank (h-i and m-n) test.



461 **Supplementary Figure S1: Nissl-stained coronal sections showing recording locations**  
462 **and tetrode tracts for all recording experiments.** Shown are three typical coronal sections  
463 for each of the six animals included in the electrophysiological experiments. The top two  
464 rows include four rats (rats #50, #97, #167 and #224) with the electrode implanted in the  
465 right hemisphere, and the bottom row shows sections of two rats with a drive in the left  
466 hemisphere (rats #246 and #247). Recordings started at approximately 1mm below the  
467 surface of the cortex, and continued in a medioventral direction with a 25° angle until  
468 tetrodes reached either the midline or corpus callosum. Red triangles indicate the end of  
469 tetrode tracts. Scale bars, 500µm.

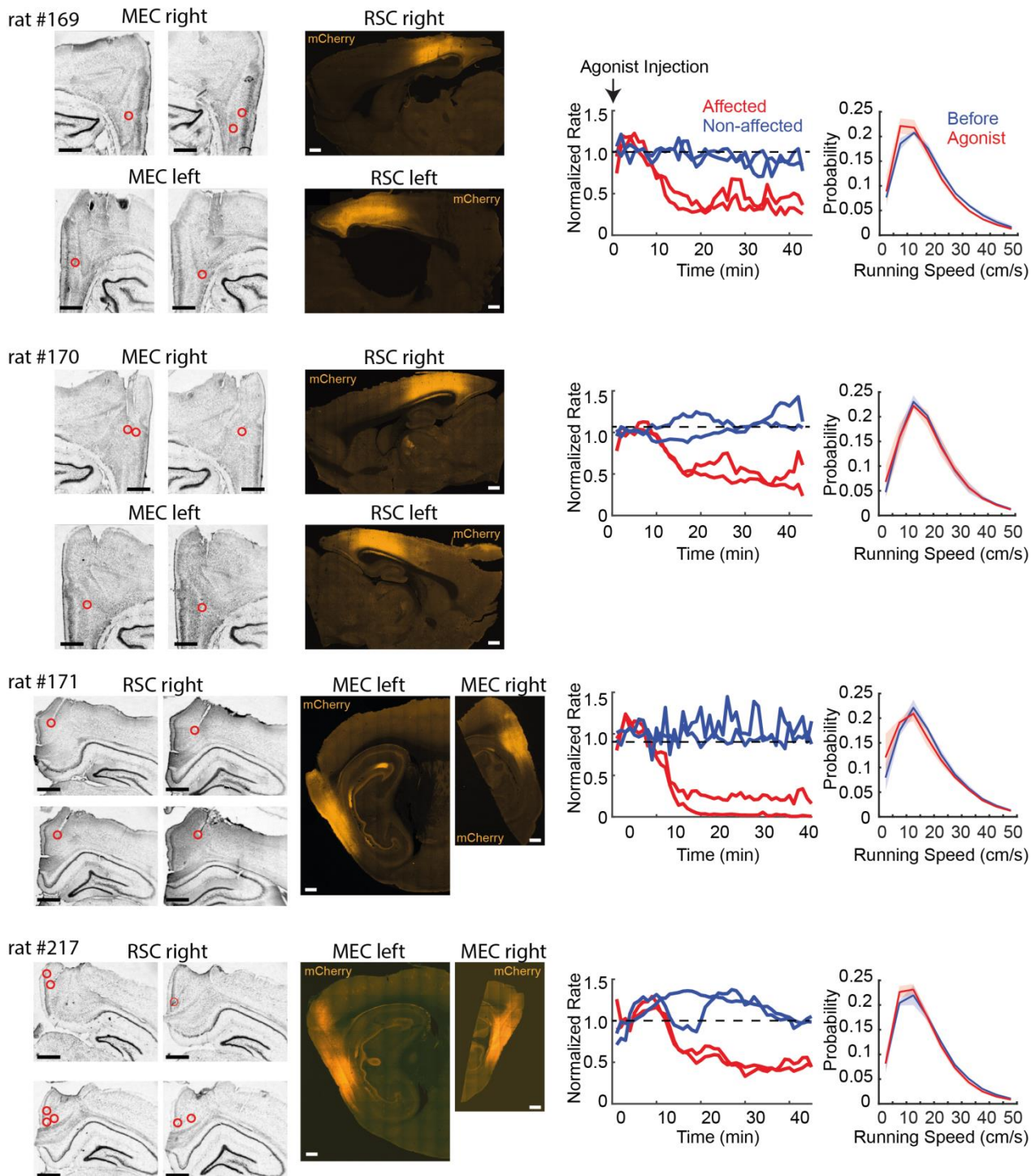


470 **Supplementary Figure S2: The original border score is unable to identify the majority**  
471 **of border cells in RSC. (a)** Shown are 6 examples of simulated ratemaps and their  
472 associated border scores. This metric is designed to capture the coverage of a firing field  
473 alongside a single wall, and a maximal score is reached when it occupies only bins that are  
474 directly connected to the wall (#1). Extension of the field towards the centre lowers the  
475 border score (#2), as does breaking the field into two or more sub fields (#3). The algorithm  
476 is unable to calculate a border score when the firing field does not directly touch the  
477 boundary (#4). The border score does not take into account symmetry, as the maximum  
478 score on any of the four walls is selected (#5-#6). **(b)** Shown are three example RSC border  
479 cells that were classified correctly by both the border score (values above 0.5) and our EMD  
480 template matching method (values below 0.1906). **(c)** By contrast are three similar RSC  
481 border cells that were identified only by the EMD method, as these cells had low, non-  
482 significant border scores. RSC border cells tend to form firing fields that are not necessarily  
483 connected to the wall, and are often not continuous due to additional directional tuning,  
484 hence leading to low border scores. **(d)** Distribution and overlap of border cell classification  
485 using the border score and EMD methods.



486 **Supplementary Figure S3: RSC border cells fire from the start in a completely novel**  
487 **environment. (a)** Several experimental sessions were performed under novel conditions;  
488 animals had never visited neither this maze nor the recording room before. **(b-d)** Shown are  
489 trajectory spike plots and spatial ratemaps of three example border cells in a familiar and  
490 novel room. Shown on the left is data of the entire recorded session. On the right a  
491 subdivision of only the novel session into blocks of 5 minutes each.





492 **Supplementary Figure S4: Tetrode locations and hM4Di expressions in the**  
 493 **experiments of DREADDs-mediated inactivation.** Left: Nissl stained sections and  
 494 fluorescent images from individual animals used for the DREADDs experiments. In rat #169  
 495 and #170, recordings were performed from bilateral MEC and AAV (AAV8-hSyn-hM4Di-  
 496 mCherry) was injected to the right RSC. Sagittal sections are shown for both Nissl-stained  
 497 and fluorescent images. Positions of tetrode tracks are indicated by red circles. In rat #171  
 498 and #217, recordings were performed from the right RSC, and the AAV was injected to  
 499 bilateral MEC. Coronal sections are shown for Nissl-stained images, and sagittal sections  
 500 are shown for fluorescent images. Right two columns: the left plots show normalized firing  
 501 rates of cells recorded from the virus injected site. The DREADDs agonist-21 was injected at



502 the beginning of the recording sessions. Two red traces show representative cells that  
503 exhibited a significant reduction of firing rates after the injection ( $p < 0.05$ , Wilcoxon ranksum  
504 test for rate changes between 0-10 min and 30-40 min), and blue traces are the cells that  
505 were not significantly affected by the drug. The right plots show the probability density of the  
506 animal's running speed during random foraging in the open-field arena, before and after the  
507 drug injection. DREADDs-mediated inactivation did not significantly affect the animal's  
508 running speed ( $p > 0.05$  in Friedman test). Each plot shows mean (solid lines) and s.e.m.  
509 (shaded).

## 510 **Methods**

### 511 **Subjects**

512 All experiments were approved by the local authorities (RP Darmstadt, protocol F126/1009)  
513 in concordance with the European Convention for the Protection of Vertebrate Animals used  
514 for Experimental and Other Scientific Purposes. Subjects were 10 male Long-Evans rats  
515 weighing 400 to 550 g (aged 3-5 months) at the start of the experiment. Rats were housed  
516 individually in Plexiglass cages (45 x 35 x 40cm; Tecniplast GR1800) and maintained a  
517 reversed 12-h light-dark cycle, with behavioural experiments performed during the dark  
518 phase. Animals were mildly food restricted with unlimited access to water, and kept at 85 to  
519 90% of their free-feeding body-weight throughout the experiment. All rats had tetrodes  
520 located either unilaterally in RSC, of which six had a drive in the right hemisphere versus two  
521 animals in the left hemisphere, or bilaterally in MEC. Four rats were additionally injected  
522 bilaterally with an AAV encoding inhibitory DREADDs in either MEC or RSC. No statistical  
523 method was used to predetermine sample size, although the number of animals used here is  
524 similar to previous work.

### 525 **Surgery, virus injection and drive implantation**

526 Anesthesia was induced by isoflurane (5% induction concentration, 0.5-2% maintenance  
527 adjusted according to physiological monitoring). For analgesia Buprenovet (Buprenorphine,  
528 0.06 mg/mL; WdT) was administered by subcutaneous injection, followed by local  
529 intracutaneous application of either Bupivacain (Bupivacain hydrochloride, 0.5 mg/mL;  
530 Jenapharm) or Ropivacain (Ropivacain hydrochloride, 2 mg/mL; Fresenius Kabi) into the  
531 scalp. Rats were subsequently placed in a Kopf stereotaxic frame, and an incision was made  
532 in the scalp to expose the skull. After horizontal alignment several holes were drilled into the  
533 skull to place anchor screws, and craniotomies were made for microdrive implantation. The  
534 microdrive was fixed to the anchor screws with dental cement, while two screws above the  
535 cerebellum were connected to the electrode's ground. All tetrodes were then positioned at  
536 920  $\mu$ m depth from the cortical surface. All animals received analgesics (Metacam, 2 mg/mL  
537 Meloxicam; Boehringer Ingelheim) and antibiotics (Baytril, 25 mg/mL Enrofloxacin; Bayer) for  
538 at least 5 days post-surgery.

539 For tetrode recordings, rats were unilaterally implanted with a hyperdrive that contained 28  
540 individually adjustable tetrodes made from 17- $\mu$ m polyimide-coated platinum-iridium (90-  
541 10%; California Fine Wire; plated with gold to impedances below 150 k $\Omega$  at 1 kHz). The  
542 tetrode bundle consisted of 30-gauge stainless steel cannulae, soldered together in a 14x2  
543 rectangular shape for recordings of the entire RSC, 7x4 for anterior RSC, or two squared  
544 bundles for bilateral MEC. For RSC, tetrodes were implanted alongside the anteroposterior  
545 axis, starting at (AP) -2.5 mm posterior from bregma until -4 mm to -6.5 mm, (ML) 0.8 mm  
546 lateral from the midline, (DV) 1.0 mm below the dura, and at a 25° angle in a coronal plane  
547 pointing to the midline in order to get underneath the superior sagittal sinus. For MEC,  
548 tetrodes were implanted at 4.5 mm lateral of the midline, 0.2 mm anterior to the transverse  
549 sinus, at an angle of 15 degrees in a sagittal plane with the tips pointing to the anterior  
550 direction. Experiments began at least 1 week post-surgery to allow the animals to recover.

551 For DREADDs experiments, an AAV8-hSyn-hM4Di-mCherry (a gift from Bryan Roth;  
552 Addgene viral prep #44362-AAV8) was injected with an infusion rate of 100 nL/min using a

553 10  $\mu$ l NanoFil syringe and a 33-gauge bevelled metal needle (World Precision Instruments).  
554 After injection was completed the needle was left in place for 10 min. The virus was injected  
555 at two sites for each bilateral MEC (500 nL each at the depth of 2.5 mm and 3.5 mm from  
556 the cortical surface, 4.5 mm lateral to the midline, 0.2 mm anterior to the transverse sinus at  
557 an angle of 20° in a sagittal plane with the needle pointing to the anterior direction), or 4  
558 sites along the anteroposterior axis for each bilateral RSC (500 nL each at AP 2.5, 3.5, 4.5,  
559 5.5 mm, 0.8 mm lateral to the midline, at an angle of 25° in a coronal plane pointing to the  
560 midline). Flow was controlled with a Micro4 microsyringe pump controller. A small microdrive  
561 (Axona) connected to 4 wire tetrodes was additionally implanted nearby the injection site to  
562 evaluate the effects of the manipulation. Virus injection was performed in the same surgery  
563 as electrode implantation, and recordings began at least three weeks post-surgery to allow  
564 time for the virus to express.

### 565 **Spike sorting and cell classification**

566 All main analyses and data processing steps were performed in MatLab (MathWorks).  
567 Neural signals were acquired and amplified using two 64-channel RHD2164 headstages  
568 (Intan technologies), combined with an OpenEphys acquisition system, sampling data at 15  
569 kHz. Neuronal spikes were detected by passing a digitally band-pass filtered LFP (0.6-6  
570 kHz) through the 'Kilosort' algorithm to isolate individual spikes and assign them to separate  
571 clusters based on waveform properties (<https://github.com/cortex-lab/KiloSort>) (Pachitariu,  
572 Steinmetz, Kadir, Carandini, & Harris, 2016). Clusters were manually checked and adjusted  
573 in autocorrelograms and for waveform characteristics in principal component space to obtain  
574 well-isolated single units, discarding any multi-unit or noise clusters.

575 **RSC border cells.** We applied a novel template-matching procedure to classify RSC  
576 neurons as border cells using the Earth Mover's Distance (EMD), a distance metric from the  
577 mathematical theory of optimal transport (Hitchcock, 1941; Rubner et al., 1998). First, the  
578 animal's spatial position occupancy was divided into 4x4 cm spatial bins, and the firing rate  
579 in each position bin was calculated by dividing the number of spikes with the amount of time  
580 spent there. The resulting ratemap was smoothed by applying a 2D Gaussian filter (width of  
581 1 bin), and converted to a probability distribution by taking unit weight. We then calculated  
582 the Earth Mover's Distance relative to a "border template" using a MatLab implementation of  
583 the fastEMD algorithm (<https://github.com/dkoslicki/EMDeBruijn>) (Pele & Werman, 2008,  
584 2009). This border template consisted of a 25x25 matrix with each bin's value set to 0,  
585 except the outer ring bins with a value of 1, smoothed with the same Gaussian kernel and  
586 converted to unit weight. Several additional templates were constructed to assess the effects  
587 of behavioural and neural manipulations (see **Fig. 2, 3**), adding additional weight in the  
588 location of placed objects/walls, or removing it in the absence of an outer wall. The EMD  
589 distance between a ratemap and a template represents the minimal cost that must be paid to  
590 transform one distribution into another, and is thus a normalized metric of dissimilarity  
591 (Grossberger et al., 2018).

592 To assess whether a cell's ratemap was significantly similar to the border template, we  
593 computed a null distribution to compare against using Monte Carlo simulations. We  
594 performed 32.000 permutations of a shuffling procedure, and for each iteration we randomly  
595 sampled a spike-train from the data, time-shifted this vector along the animal's recorded  
596 trajectory by a random interval of at least 4 seconds and less than the total trial length,  
597 wrapping any excess at the end back to the beginning. We then used this shifted data to

598 compute a ratemap and calculated the EMD distance relative to the border template. Criteria  
599 for border cell classification was an EMD dissimilarity score below the 1<sup>st</sup> percentile of this  
600 null distribution in all regular sessions, and an average firing rate of at least 0.5 Hz (see **Fig.**  
601 **1d, 1e**).

602 **MEC border cells.** To compare classification results with a related metric we computed the  
603 original border score for each cell (Solstad et al., 2008). We first estimated a cell's firing field  
604 by isolating a continuous region of at least 200 cm<sup>2</sup> and a maximum of 70% of the arena  
605 surface where the firing rate was above 30% of the peak firing rate. This was an iterative  
606 search until all fields with the above criteria were identified. We next computed the border  
607 score,  $b$ , for each wall separately:

$$b = \frac{c_M - d_M}{c_M + d_M}$$

608 where  $c_m$  was defined as the maximum coverage of any single field over the wall and  $d_m$  the  
609 mean firing distance, calculated as the average distance to the nearest wall over all bins  
610 covered by the field. This was done separately for each of the four walls out of which the  
611 maximum score was selected. Cells recorded in MEC were classified as border cells  
612 whenever their border score was above the threshold of 0.5 (corresponding to the 99.3  
613 percentile of scores generated from randomly time-shifted spikes) for either of the two  
614 recorded sessions, and had an average firing rate of at least 0.5 Hz.

615 **Head direction cells.** The rat's head direction was calculated based on the relative x/y-  
616 position of two light emitting diodes (LEDs), corrected for an off-set in placement of the  
617 LED's relative to the animal's true head direction. For each cell the mean vector length  
618 (MVL) and direction (MVD) was calculated by computing the circular mean and direction  
619 from a vector that contained the head direction of the animal at spike timings in unit space. A  
620 cell was classified as a head direction cell when its MVL was greater than the 95<sup>th</sup> percentile  
621 of a null distribution obtained by 1000-fold Monte Carlo simulations with randomly time-  
622 shifted spike trains.

### 623 **Border rate maps**

624 Locations of walls were estimated based on the most extreme values of the position of the  
625 animal. The animal's distance to the wall was computed for each of the four walls separately  
626 by taking the difference between the wall's location and the animal's position in the  
627 respective x or y- dimension, and selecting the lowest value at each time point. The direction  
628 of this wall relative to the animal's direction was computed by calculating the angle  
629 difference between the animal's true heading direction and a vector pointing directly towards  
630 the wall (e.g. relative to an angle of 0° for the east wall, 90° for the north wall etc.). Because  
631 0° corresponds with the 'east' side in angular polar plots, this data was further shifted by 90°  
632 to align the front of the animal with the 'north' part in border maps (see **Fig. 4c**) to improve  
633 visual interpretation of the results.

634 Firing rate in these body-centric border coordinates was calculated by dividing the animal's  
635 occupancy in these coordinates into 4 cm distance bins and 20° angle bins. The number of  
636 spikes in each bin was then divided by the time spent there, further smoothed using a 2-D  
637 Gaussian kernel (1 bin width), similar to how spatial rate maps are computed. A cell's  
638 preferred direction and distance was obtained by finding the bin with maximal firing rate, and

639 selecting the bin's corresponding distance and angle values. For visualization purposes only  
640 this matrix was transformed into a circular diagram shown in Fig 4.

### 641 **Decoding analysis**

642 For decoding of wall distance from the activity of border cells in RSC and MEC, the optimal  
643 wall with maximum coverage by firing fields was chosen for individual cells (the same  
644 procedure as used in border-score calculations (Solstad et al., 2008)). To determine the  
645 optimal head direction to the selected wall for individual border cells, we searched for a  
646 range of head directions (60-degree range in 5-degree steps) that gave the maximum mean  
647 firing rate of the cell when the animal was within 20 cm of the wall. We then focused on  
648 neural activity when the animal was at this optimal head direction and in the range of wall  
649 distances from 0 to 50 cm at 10 cm steps (5 ranges in total), but excluding timepoints where  
650 the animal was within 25 cm of other walls to avoid their potential influence. All of the  
651 incidents when the animal was in each of the 5 wall-distance ranges were equally divided  
652 into 20 segments in time, and mean firing rates of individual border cells in the 20 segments  
653 were assembled together across recording sessions. To implement a decoding analysis, 20  
654 cells were randomly chosen, and the order of 20 segments was randomly shuffled for each  
655 cell, such that the data in each segment is a collection of firing rates from 20 border cells  
656 across various time points of behaviours when the animal was in a particular distance range  
657 to the wall. Ensemble firing rates of border cells in one of the segments were selected as a  
658 test dataset, and the rest of the data were used to train a support vector machine (using a  
659 MATLAB package LibSVM with a linear function (Chang & Lin, 2011)). Trained weights were  
660 then applied to the activity of border cells in the test dataset to estimate the animal's  
661 distance to the wall, which was repeated for all segments to be tested (leave-one-out cross-  
662 validation), giving a representative decoding performance for the selected population of  
663 cells. This procedure was repeated for different cell pairs for 1000 times to estimate a  
664 statistical distribution of decoding performance (bootstrap resampling method).

### 665 **Behavioural methods**

666 Data was collected over a total of 30-120 min per day while rats foraged for food (chocolate  
667 cereal) in a squared open field arena, either 100x100 cm or 120x120 cm in size. Each  
668 session consisted of 10-15 min of free exploration in the arena, separated by 5-10 min of  
669 resting time on a pedestal. No curtains surrounded the recording arena, with the exception of  
670 the rotation and darkness experiments where all distal cues were blocked completely. The  
671 surface of the arena was elevated 50 cm above the ground, and was enclosed by three  
672 black and one white wall with a 50 cm height that were positioned with consistent orientation  
673 in the room for all animals. The experimental set-up was extensively cleaned with a 70%  
674 ethanol solution in between every recording session to eliminate any odours.

675 Behavioural manipulation experiments always followed the same protocol of A-B-B-A', where  
676 A is a regular session, and the manipulation was performed in B. This allowed for a recovery  
677 phase after the manipulation in the final session A'. The only exception was the drop-edge  
678 experiment (**Fig. 3e**) where the animal had limited motivation; so to ensure good coverage of  
679 the arena we reduced the protocol to A-B-A'. All changes to the maze were made in between  
680 the first and second session while the animal was resting on a pedestal. For the added wall  
681 manipulation (**Fig. 2a**), an additional black wall (50 cm length x 50 cm height x 1 cm width)  
682 was placed in the maze, protruding from one outer wall at half-length towards the centre. For



683 the added object manipulation (**Fig. 2f**) a circular, non-climbable aluminium object (10 cm  
684 diameter x 50 cm height) was placed off-centre 40 cm away from the north and west walls.  
685 For the DREADDs-mediated manipulation experiments, animals were injected with agonist-  
686 21 (DREADDs agonist 21 dihydrochloride, 3.52 mg/mL [10 mM]; Hellobio) subcutaneously  
687 after the first recording session, followed by at least 30 min waiting time to allow the drug to  
688 reach the brain and take effect before starting the next recording session.

689 The animal's position and head direction were obtained by tracking two LEDs on the  
690 headstage at 25 Hz and recording under dimly lit conditions. For darkness sessions, we  
691 switched to an infra-red OptiTrack camera system (Natural Points Inc.). Six Flex 3 cameras  
692 were placed around the experimental set-up that recorded the location of three reflective  
693 markers in an asymmetric frame attached to the headstage. Position and direction data were  
694 acquired and processed using Motive 2.0 software.

### 695 **Histological procedures**

696 Once the experiment was completed, animals were deeply anesthetized by sodium  
697 pentobarbital and perfused intracardially with saline, followed by 10% formalin solution.  
698 Brains were extracted and fixed in formalin for at least 72 hours at 6° C temperature. Frozen  
699 coronal sections were cut (50 µm) and stained using cresyl violet and mounted on glass  
700 slides. Electrode tips were identified by comparison across adjacent sections, with the  
701 location of recorded cells estimated by backward measurement from the most ventral tip of  
702 the tetrode tracks.

### 703 **Statistical procedures**

704 All statistical tests were two-sided and non-parametric, unless stated otherwise. Error bars in  
705 all figures represent standard error of the mean (S.E.M.). All values mentioned in text are  
706 medians ± S.E.M.

707

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714

### 715 **Author Contributions**

716 J.B.G.v.W. and H.T.I. designed the experiment. J.B.G.v.W. performed all experiments and  
717 analyses, except for the cue-rotation experiment and DREADDs experiment, which were  
718 performed by S.S.B. and H.T.I. respectively. J.B.G.v.W. and H.T.I. wrote the manuscript.

## 719 **Competing Interests statement**

720 The authors declare no competing interests.

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