

Holmgren *et al*

1 Freely-moving mice visually pursue prey using a retinal  
2 area with least optic flow

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Holmgren *et al*

15 **Abstract:**

16 Mice have a large visual field that is constantly stabilized by vestibular ocular reflex driven eye rotations  
17 that counter head-rotations. While maintaining their extensive visual coverage is advantageous for  
18 predator detection, mice also track and capture prey using vision. However, in the freely moving animal  
19 quantifying object location in the field of view is challenging. Here, we developed a method to digitally  
20 reconstruct and quantify the visual scene of freely moving mice performing a visually based prey capture  
21 task. By isolating the visual sense and combining a mouse eye optic model with the head and eye rotations,  
22 the detailed reconstruction of the digital environment and retinal features were projected onto the  
23 corneal surface for comparison, and updated throughout the behavior. By quantifying the spatial location  
24 of objects in the visual scene and their motion throughout the behavior, we show that the image of the  
25 prey is maintained within a small area, the functional focus, in the upper-temporal part of the retina. This  
26 functional focus coincides with a region of minimal optic flow in the visual field and consequently minimal  
27 motion-induced image blur during pursuit, as well as the reported high density-region of Alpha-ON  
28 sustained retinal ganglion cells.

29

Holmgren *et al*

30 **Introduction:**

31 The visual system of mice serves a variety of seemingly opposing functions that range from detection of  
32 predators, to finding shelter and selection of food and mates, and is required to do so in a diverse set of  
33 environments (Boursot, Auffray et al. 1993). For example, foraging in open areas where food is available  
34 involves object selection, and in the case of insect predation (Badan 1986, Tann, Singleton et al. 1991),  
35 involves prey tracking and capture (Langley 1983, Langley 1984, Langley 1988, Hoy, Yavorska et al. 2016),  
36 but the visual system can also simultaneously be relied on for avoidance of predation, particularly from  
37 airborne predators (Hughes 1977). Like with many ground-dwelling rodents (Johnson and Gadow 1901)  
38 predator detection in mice is served by a panoramic visual field which is achieved by the lateral placement  
39 of the eyes in the head (Drager 1978, Hughes 1979, Oommen and Stahl 2008) combined with monocular  
40 visual fields of around 200 degrees (Hughes 1979, Drager and Olsen 1980, Sterratt, Lyngholm et al. 2013).  
41 In mice, the panoramic visual field extends to cover regions above the animal's head, below the animals  
42 snout and laterally to cover ipsilaterally from behind the animals head to the contralateral side, with the  
43 overlapping visual fields from both eyes forming a large binocular region overhead and in front of the  
44 animal (Hughes 1977, Sabbah, Gemmer et al. 2017). In addition, eye movements in freely moving mice  
45 constantly stabilize the animal's visual field by counteracting head rotations through the vestibulo-ocular  
46 reflex (VOR) (Payne and Raymond 2017, Meyer, Poort et al. 2018, Meyer, O'Keefe et al. 2020, Michael,  
47 Abe et al. 2020) maintaining the large panoramic overhead view (Wallace, Greenberg et al. 2013) critical  
48 for predator detection (Yilmaz and Meister 2013).

49 Given the VOR stabilized panoramic field of view it is not clear what part of the visual field mice use to  
50 detect and track prey (but see: (Johnson, Fitzpatrick et al. 2021). Mouse retina contains retinal ganglion  
51 cells (RGCs), the output cells of the retina, with a broad diversity of functional classes (Zhang, Kim et al.  
52 2012, Bleckert, Schwartz et al. 2014, Baden, Berens et al. 2016, Franke, Berens et al. 2017). Given the  
53 lateral eye position, the highest overall density faces laterally (Drager and Olsen 1981, Salinas-Navarro,

Holmgren *et al*

54 Jimenez-Lopez et al. 2009, Sabbah, Gemmer et al. 2017, Stabio, Sondereker et al. 2018). Further, as the  
55 functionally defined ganglion cells (Zhang, Kim et al. 2012, Bleckert, Schwartz et al. 2014, Baden, Berens  
56 et al. 2016, Franke, Berens et al. 2017) and cone sub-types (Szel, Rohlich et al. 1992) are segregated into  
57 retinal subregions within the large stabilized field of view, recent studies suggest that retinal subregions  
58 are tuned for specific behavioral tasks depending on what part of the world they subtend (Hughes 1977,  
59 Zhang, Kim et al. 2012, Bleckert, Schwartz et al. 2014, Baden, Berens et al. 2016, Sabbah, Gemmer et al.  
60 2017, Szatko, Korympidou et al. 2020).

61 The challenge is to measure what part of the visual field the mouse is attending to during a visually based  
62 tracking task (Hoy, Yavorska et al. 2016) and the location of all objects within the field of view during the  
63 behavior. While recent studies have implied the relationship between prey and retina through tracking  
64 head position (Johnson, Fitzpatrick et al. 2021) or measured both the horizontal and vertical eye rotations  
65 (Meyer, Poort et al. 2018, Meyer, O'Keefe et al. 2020) during pursuit behavior (Michaiel, Abe et al. 2020)  
66 to uncover a large proportion of stabilizing eye-rotations, what is missing is the extent and location of the  
67 area used when detecting and pursuing prey, and the relationship to the retina (Bleckert, Schwartz et al.  
68 2014).

69 Here, we measured the position of a cricket in the visual fields of freely moving mice performing a prey  
70 pursuit behavior, using head and eye tracking in all three rotational axes, namely horizontal, vertical and  
71 torsional. Eye tracking included an anatomical calibration to accurately account for the anatomical  
72 positions of both eyes. To quantify object location in the animal's field of view and generate optic flow  
73 fields, head and eye rotations were combined with a high-resolution digital reconstruction of the arena  
74 to form a detailed visual map from the animal's eye perspective. Given that mice use multisensory  
75 strategies during prey pursuit (Langley 1983, Langley 1988, Gire, Kapoor et al. 2016) and can track prey  
76 using auditory, visual or olfactory cues (Langley 1983, Langley 1988), we developed a behavioral arena  
77 that isolated the visual aspect of the behavior by removing auditory and olfactory directional cues to

Holmgren *et al*

78 ensure that the behavior was visually guided. To transfer the retinal topography onto the corneal surface,  
79 we developed an eye model capturing the optical properties of the mouse eye. We show that during prey  
80 detection mice preferentially position prey objects in stable foci located in the binocular field and  
81 undertake direct pursuit. The stabilized functional foci are spatially distinct from the regions of highest  
82 total retinal ganglion cell density, which are directed laterally, but coincides with the regions of the visual  
83 field where there is minimal optic flow and therefore minimal motion-induced image disturbance during  
84 the behavior. Lastly, by building an optical model that allows corneal spatial locations to be projected onto  
85 the retina, we suggest that the functional foci correspond to retinal subregions containing a large density  
86 of Alpha-ON sustained RGCs that have center-surround receptive fields and project to both superior  
87 colliculus and dLGN (Huberman, Manu et al. 2008) and possess properties consistent with the  
88 requirements for tracking small and mobile targets (Krieger, Qiao et al. 2017).

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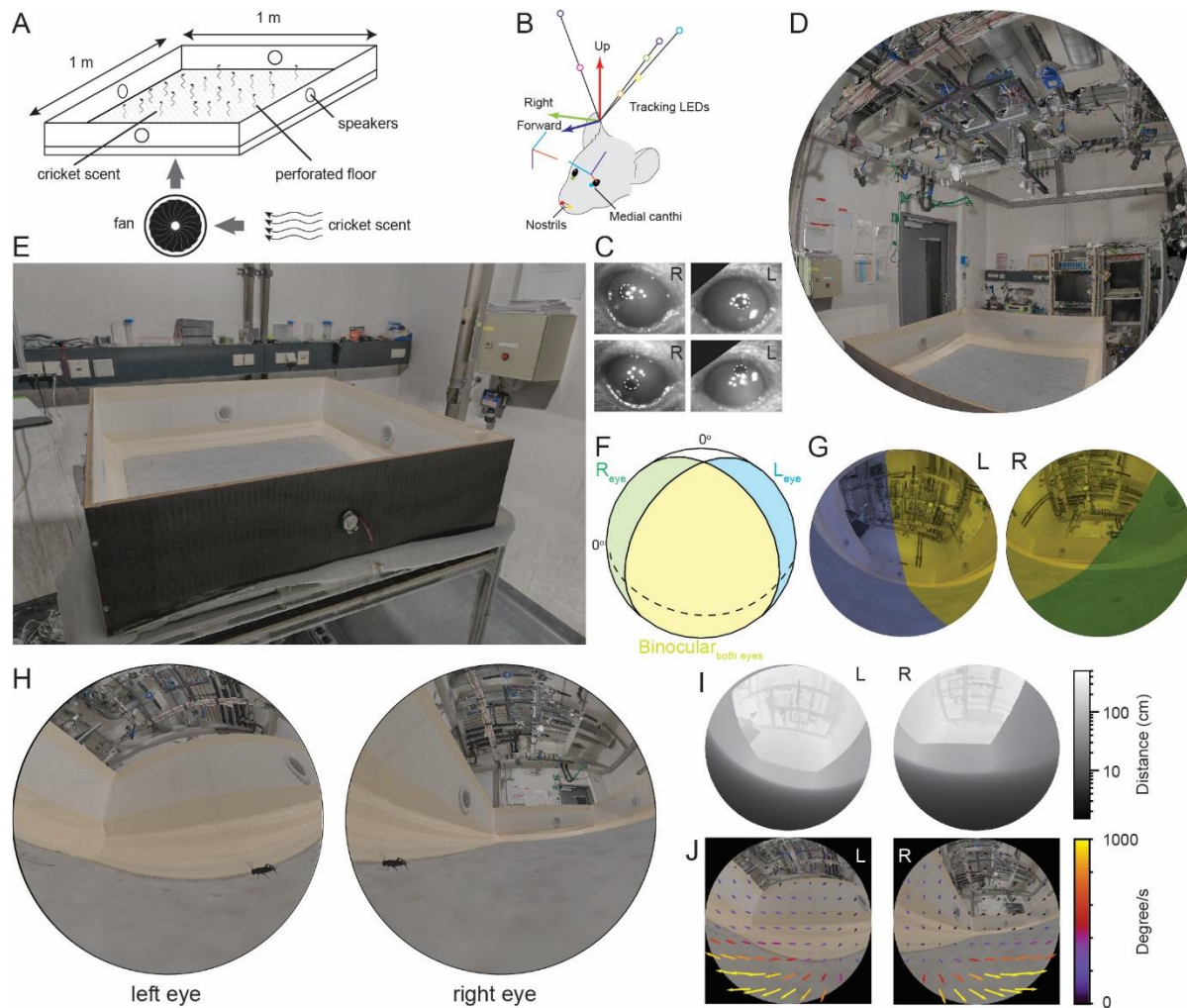
Holmgren *et al*

90 **Results:**

91 **Forming a view from the animal's point of view**

92 To measure what part of the visual field mice use during prey capture while also considering that mice  
93 can use multisensory strategies during prey pursuit (Langley 1983, Langley 1988, Gire, Kapoor et al. 2016),  
94 we first developed an arena which isolated the visual component of prey pursuit by masking olfactory and  
95 auditory spatial cues (Figure 1A, see Methods for details). By removing both olfactory and auditory cues,  
96 the average time to capture a cricket approximately doubled compared to removal of auditory cues alone  
97 (time to capture, median±SD, control 24.92±16.77s, olfactory & auditory cues removed, 43.51±27.82s,  
98  $p=0.0471$ , Wilcoxon rank sum test, N=13 control and 12 cue removed trials from N = 5 mice). To track  
99 mouse head and eye rotations during prey capture, we further developed a lightweight version of our  
100 head mounted oculo-videography and camera-based pose and position tracking system (Wallace,  
101 Greenberg et al. 2013) (Figure 1B and Methods, Figure 7 A and B). This approach allowed quantification  
102 of head rotations in all three axes of rotation (pitch, roll and yaw), as well as eye rotations in all three  
103 ocular rotation axes (torsion, horizontal and vertical, Figure 1C, Figure 1 – figure supplement 1 A and B).  
104 The same videography-based system was used to track and triangulate the position of the cricket (see  
105 Methods and Figure 1 – figure supplement 1C). To quantify the position and motion of the environment  
106 and cricket in the mouse field of view, we also developed a method that enabled a calibrated environment  
107 digitization to be projected onto the corneal surface. This approach utilized a combination of laser  
108 scanning and photogrammetry, giving a resolution for the reconstruction of the entire experimental room  
109 of 2 mm, as well as a detailed measurement of eye and head rotations (Figure 1D-E, and see methods).  
110 Mice, like rats (Wallace, Greenberg et al. 2013), have a large visual field of view which extends to also  
111 cover the region over the animal's head (Figure 1F). To ensure the entire visual fields of the mouse could  
112 be captured during behavior, we digitized the entire experimental room and contents (Figure 1E, Figure 1  
113 – figure supplement 1D-F, Movie 1). The coordinate systems of the environmental digitization and mouse

Holmgren *et al*



**Figure 1 with 1 supplement**

**Reconstruction of experimental arena and surrounds from the animal's perspective.**

(A) Schematic of experimental arena with olfactory and auditory noise. (B) Schematic of tracking, anatomical and eye camera calibration. Head position and orientation was tracked using seven IR-LEDs (colored circles). Nostrils (red, yellow filled circles), left (blue filled circle) and right (green filled circle) medial canthi were identified and triangulated in calibration images and used to define a common coordinate system (forward, blue arrow, right, green arrow, and up, red arrow), into which the calibrated eye camera location and orientation could also be placed (eye camera vertical, cyan, horizontal, purple, camera optical axis, red). (C) Example left- and right eye camera images with tracked pupil position (white dashed outlines). (D) Rendered digital reconstruction of the laboratory room and (E) experimental arena. (F) Schematic representation of mouse's left- (blue) and right (green) visual fields, showing also the region of binocular overlap (yellow) and un-seen region (white). (G) Reconstruction of the arena and room from the animal's left- and right eye perspective, with monocular and binocular regions colored as in (F). (H) Reconstruction of the animal's view of the prey (cricket - black) in the experiment arena. (I) Representation of left and right eye views of the arena and surrounding objects grayscale-coded by distance from the eye. (J) Rendered animal's eye views from the left- and right eyes with overlay of arrows representing optic flow during 10 ms during free motion.

Holmgren *et al*

116 and cricket tracking systems were registered using 16-20 fiducial markers identified in both the overhead  
117 camera images and the digitized environment. The average differences in position of fiducial points  
118 between the two coordinate systems were less than 1 mm (mean $\pm$ SD, x position, 0.18 $\pm$ 3.1mm, y position,  
119 0.07 $\pm$ 1.6mm, z position, 0.66 $\pm$ 1.8mm, N=54 fiducial points from 3 datasets). The next step was to re-  
120 create the view for each eye. First, and for each mouse, the positions of both eyes and nostrils were  
121 measured with respect to both the head-rotation tracking LEDs and head-mounted cameras, then  
122 calibrated into a common coordinate system (Figure 1B). Together, this enabled a rendered  
123 representation of the digitized field of view for each combination of head and eye rotations. This rendered  
124 image, from the animal's point of view, contained all the arena and lab objects (Figure 1G-H, Movie 2,  
125 Figure 1 – figure supplement 1G). In addition, to object position and distance (Figure 1I), the motion of  
126 the environment and each object in the field of view could be quantified as the mouse performed prey  
127 capture behaviors (Figure 1J, and Figure 1 – figure supplement 1H).

128

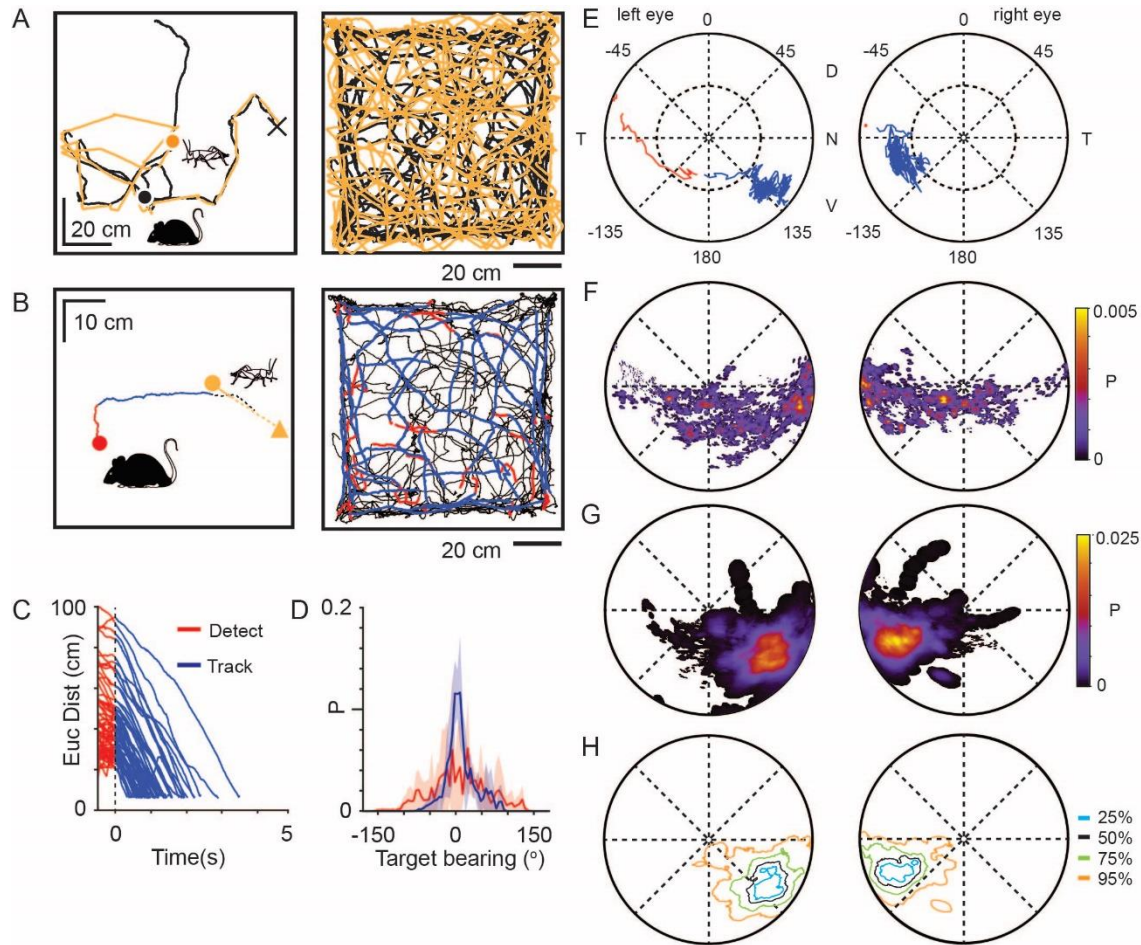
### 129 **Mice keep prey in a localized visual region during pursuit**

130 Crickets (*Acheta domesticus*), shown previously to be readily pursued and preyed upon by laboratory mice  
131 (Hoy, Yavorska et al. 2016), provided a prey target that could successfully evade capture for extended  
132 periods of time (total time for each cricket before capture: 64.4 $\pm$ 39.3 s, average time $\pm$ SD, N=21 crickets  
133 and 3 mice). To ensure that only data where the mouse was actively engaged in the detection and tracking  
134 of the cricket was used, we identified occasions where the mouse either captured the cricket, or contacted  
135 the cricket but the cricket escaped (see Methods for definitions), and then quantified the trajectories of  
136 both mouse and cricket leading up to the capture or capture-escape (Figure 2A). Within these chase  
137 sequences we defined three behavioral epochs (detect, track and capture, Figure 2B, see Methods for

138



Holmgren *et al*



**Figure 2 with 1 supplement**

**Mice use a focal region of their visual field to track prey.**

**(A)** Mouse (black) and cricket (orange) paths during a single pursuit sequence (left), and for all pursuit sequences in one session for one animal (right). Pursuit start denoted as filled circles and cricket capture as X. **(B)** Mouse (red and blue) and cricket (orange) paths during an individual pursuit sequence (left) and all pursuit sequences in one session from one animal (right), showing detect (red) and track (blue) epochs of the mouse path. Paths after a cricket escape shown dashed. Pursuit sequence start shown as filled circles, cricket landing point after a jump shown as a filled triangle. **(C)** Euclidean distance between mouse and cricket during detect (red) and track (blue) epochs ( $n=65$  trajectories,  $n=3$  mice). **(D)** Mean and SD bearing to cricket (angle between mouse's forward direction and cricket location) during detect (red), and track (blue) epochs for all sequences from all animals (detect: 57 epochs, 4406 frames; track: 65 epochs, 13624 frames,  $n=3$  animals, bin size =  $5^\circ$ ). **(E)** Trajectory of the projected cricket position in the left and right corneal views, during a single pursuit sequence. Color scheme as for D. The inner dashed circle is  $45^\circ$  from the optical axes. Dorsal (D), ventral (V), nasal (N) and temporal (T) directions indicated. **(F)** Average probability density maps for detect epochs (4628 frames from 3 animals). Orientation as in E. **(G)** Average probability density maps for track epochs (13641 frames from 3 animals). Orientation as in E. **(H)** Isodensity contours calculated from the average probability density maps for track epochs. (note that 50% means that this region contains 50% of the total density, and likewise for the other contours). Orientation as in E.

Holmgren *et al*

140 definition details) based on the behavior of mouse and cricket, and similar to previous studies (Hoy,  
141 Yavorska et al. 2016).

142 Upon cricket detection, mice oriented and ran towards the cricket, resulting in a significant adjustment to  
143 their trajectory ( $\Delta$  target bearing:  $40.2 \pm 35.1^\circ$ ,  $P=6.20 \times 10^{-10}$ ,  $\Delta$  speed:  $10.2 \pm 7.4$  cm/s,  $P=1.91 \times 10^{-10}$ ;  $N=57$   
144 detect-track sequences  $N = 3$  mice; Paired Wilcoxon's signed rank test for both tests), and a rapid  
145 reduction in the Euclidean distance to the cricket (Figure 2C). During tracking, the cricket was kept in front  
146 of the mouse, resulting in a significant reduction in the spread of target bearings compared to during  
147 detect epochs (Figure 2D, Target bearing: detect  $6.2 \pm 62.1^\circ$ , track:  $2.5 \pm 25.6^\circ$ , mean  $\pm$  SD, Brown-Forsythe  
148 test  $p=0$ ,  $F$  statistic =  $7.05 \times 10^3$ ,  $N=4406$  detect and  $13624$  track frames,  $N=3$  mice), consistent with previous  
149 findings (Hoy, Yavorska et al. 2016). To avoid the closing phase of the pursuit being associated with  
150 whisker strikes (Shang, Liu et al. 2019, Zhao, Chen et al. 2019), tracking periods were only analyzed when  
151 the mouse was more than 3 cm from the cricket, based on whisker length (Ibrahim and Wright 1975).

152 Using the detailed digitization of the behavioral arena and surrounding laboratory method (Figure 1E,  
153 Movie 1), an image of the cricket and objects in the environment was calculated for each head and eye  
154 position during the predator-prey interaction (Movie 2). Using this approach, we addressed the question  
155 of what area of the visual field was the cricket located in during the various behavioral epochs. In the  
156 example pursuit sequence in Figure 2E the cricket was initially located in the peripheral visual field and  
157 then transitioned to the lower nasal binocular quadrant of the cornea-view during pursuit and capture  
158 (red trace in left eye to blue trace in both eyes). Correspondingly, an average probability density map  
159 calculated for all animals during the detect epoch showed a very broad distribution of cricket positions  
160 across the visual field (Figure 2F, Figure 2 – figure supplement 1A and B). Upon detection the mouse  
161 oriented towards the cricket, bringing it towards the lower nasal binocular visual field (Figure 2E). When  
162 averaged for all pursuit sequences from all animals, projected cricket positions formed a dense cluster on  
163 the cornea of both eyes (Figures 2G and 2H, Figure 2 – figure supplement 1A, C-D, 50% contour center for

Holmgren *et al*

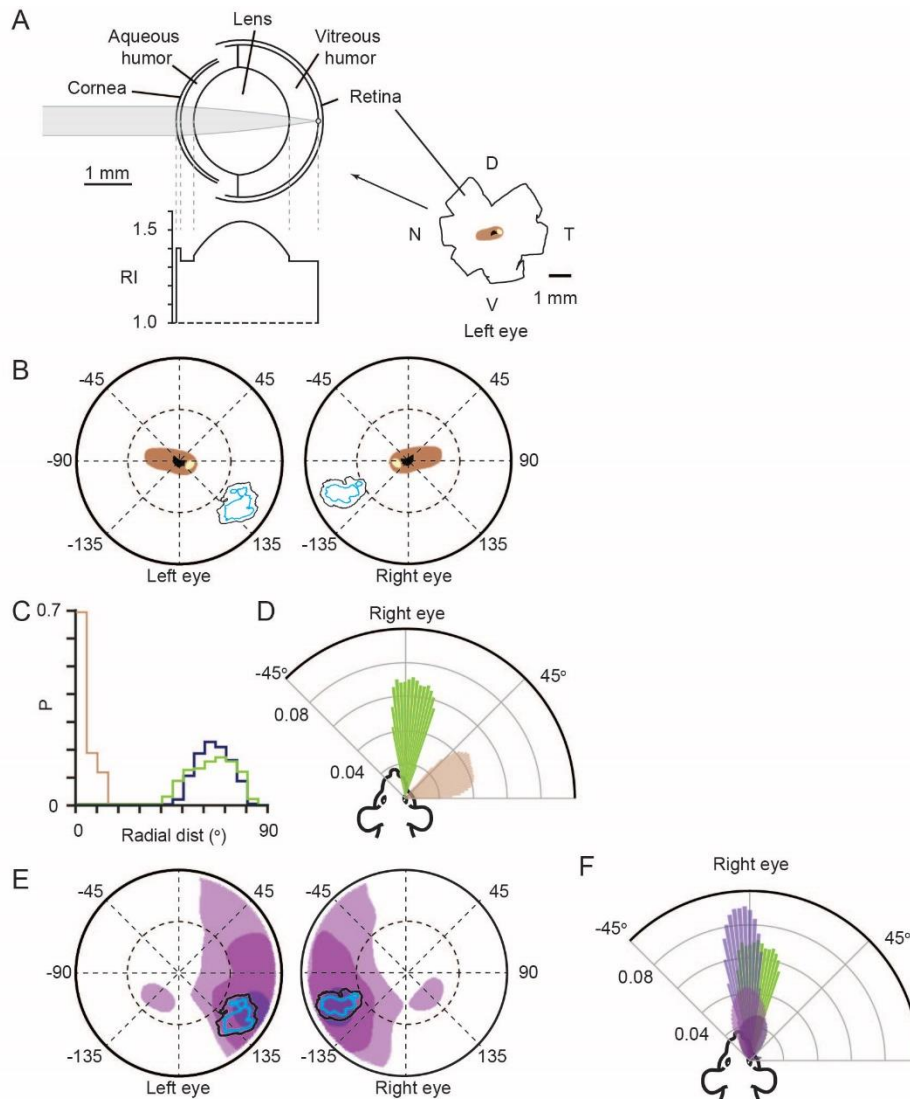
164 left and right eye respectively, radial displacement from optical axis  $64.3 \pm 7.5^\circ$  and  $63.3 \pm 9.9^\circ$ , rotational  
165 angle  $126.2 \pm 8.9^\circ$  and  $-115.7 \pm 6.1^\circ$ , mean  $\pm$  SD, N= 3 mice), which was significantly different from the  
166 cluster in the detect epoch (average histogram of the location of cricket image during tracking phase vs  
167 average histogram of the location of cricket during detect phase: Left eye  $P=3.54 \times 10^{-46}$ , Right eye  
168  $P=1.08 \times 10^{-81}$ , differences calculated by taking the Mean Absolute Difference with bootstrapping, N=57  
169 detect-track sequences, N = 3 mice). Thus, despite mice lacking a retinal fovea (Drager and Olsen 1981,  
170 Jeon, Strettoi et al. 1998), the image of the prey is kept on a local and specific retinal area during the  
171 tracking and pursuit behavior. The image of the prey was localized on a specific region of retina within the  
172 binocular field, while the region of elevated density of RGCs has been found to be located near the optical  
173 axis (Drager and Olsen 1981), which suggests that the location of the retinal specialization may not overlap  
174 with the functional focus.

#### 175 **Functional foci do not overlap with highest ganglion cell density**

176 To determine whether the identified functional focus spatially overlaps with the area of highest density  
177 of retinal ganglion cells we made a mouse eye-model (Figure 3A), modified from previous models (Barathi,  
178 Boopathi et al. 2008). Using the eye model, retinal spatial locations could be projected through the optics  
179 of the mouse eye to the corneal surface. We first reconstructed the isodensity contours of published RGCs  
180 (Drager and Olsen 1981) to define the retinal location of the mouse retinal specialization (Figure 3- figure  
181 supplement 1A-C, note that these contours are also in agreement with other recently published maps of  
182 total RGC density (Zhang, Kim et al. 2012, Bleckert, Schwartz et al. 2014)). The lens optical properties were  
183 based on a GRIN lens (present in both rats (Philipson 1969, Hughes 1979) and mice (Chakraborty, Lacy et  
184 al. 2014)). To determine the optical characteristics of this lens we developed a method which combined  
185 models of the lens surface and refractive index gradient (Figure 3A, Figure 3- figure supplement 1D and  
186 Tables 1 and 2, see methods for details). Using this model, the contours representing the retinal  
187 specializations were projected through the eye model onto the corneal surface to determine equivalent

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Holmgren *et al*



**Figure 3 with 1 supplement**

**Functional foci are not sampled by the highest density retinal ganglion cell region.**

(A) Schematic of mouse eye model (left upper) with profile of all refractive indices (RI, left lower). Reconstructions of the optic disc (black), highest (>8000 cells/mm<sup>2</sup>, beige) and second highest (>7000 cells/mm<sup>2</sup>, brown) retinal ganglion cell (RGC) density regions redrawn from Dräger & Olsen 1981, shown in lower right. (B) Position in corneal views of the high RGC density regions (brown and beige filled regions), and isodensity contours from Figure 2H after projection through the eye model. Orientation as in Figure 2E. (C) Horizontal axis histograms for the nasal half of the corneal view of the second highest RGC region (brown) and 50% isodensity contour for left (blue) and right (green) eyes. (D) Top-down view of the coverage regions for the right eye of the 50% isodensity contour (green, N = 7551 frames) and second highest RGC region (brown, N = 51007 frames) for a single animal. Bars represent the probability density function for the respective regions at that azimuth angle. (E) Position in corneal views of Alpha-ON sustained RGC densities (redrawn from Bleckert *et al.* 2014) after projection through the eye model. Colored regions show the 95% (dark purple), 75% (medium purple) and 50% (light purple) contour regions of the peak Alpha-ON sustained RGC density. Isodensity contours from Figure 2H. (F) Top-down view of the coverage regions for the right eye of the 95% (dark purple), 75% (medium purple) and 50% (light purple) Alpha-ON sustained RGC contour regions (same as in E, N = 51007 frames) and the 50% isodensity contour from D (green) for a single animal. For the Alpha-ON sustained RGC contour regions 50% means that this region contains all points which are at least 50% of the peak RGC density.

Holmgren *et al*

190 corneal locations (Figure 3B, Figure 3- figure supplement 1E). Comparing this corneal projected location  
191 to the functional focus location showed that the region with the highest RGC counts had no overlap with  
192 the functional focus (Figure 3B) and occupied non-overlapping peripheral locations (Figure 3C). Viewed  
193 from above the animal's head the functional foci were directed at the region in front of the animal's nose  
194 and within the region of stable binocular overlap (azimuth:  $1.4 \pm 8.8^\circ$  and  $-4.4 \pm 9.3^\circ$ , elevation  $5.7 \pm 2.1^\circ$  and  
195  $4.9 \pm 1.4^\circ$  for left and right eyes respectively, N = 13641 frames, N=3 mice), while the retinal specialization  
196 was directed laterally (azimuth:  $-66.2 \pm 6.7^\circ$  and  $70.3 \pm 4.7^\circ$ , elevation:  $30.8 \pm 12.2^\circ$  and  $41.0 \pm 13.5^\circ$  for left  
197 and right eyes respectively, N = 13641 frames N=3 mice. Figure 3D, Figure 3 – figure supplement 1F-G).  
198 As the projected location of the RGC high density region and the functional focus are both sensitive to  
199 torsional offsets and the location of the RGC region is also sensitive to the selected location for the optical  
200 axis of the eye model, we next measured what rotational transformations would be required for the RGC  
201 high density region and the functional focus to overlap. The size and locations of the two regions are such  
202 that there is no torsional rotation which would produce overlap (Figure 3 – figure supplement 1H-K). Any  
203 overlap of the regions would then require a large offset in the placement of the optical axis of the eye  
204 model on the redrawn retinal whole mount. Here we aligned the optical axis of the eye model with the  
205 center of mass of the redrawn optic disc, which has been measured as being  $3.7^\circ$  from the geometrical  
206 center of the retina (Sterratt, Lyngholm et al. 2013). As the spherical distance between the centers of the  
207 two regions was  $52^\circ$  (mean $\pm$ SD, Left,  $52.9 \pm 1.4^\circ$ ; Right,  $51.4 \pm 4.6^\circ$ , n=3 mice), no reasonable offsets or  
208 errors could result in overlap. Together this shows that that although mice maintain their prey within a  
209 focal visual region during the tracking phase of their pursuit behavior, this region does not overlap with  
210 the visual space represented by overall highest density RGC region of the retina (Drager and Olsen 1981,  
211 Jeon, Strettoi et al. 1998, Zhang, Kim et al. 2012). As a high-density of Alpha-ON sustained RGC's are  
212 spatially located on the dorso-temporal retina (Bleckert, Schwartz et al. 2014), consistent with projecting  
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Holmgren *et al*

214 **Table 1. Mouse eye model curvatures**

215 Radii of curvature of the optical components of the mouse eye model in Figure 3A.

216

217

<i>Ocular Component</i>	<i>Radius of curvature (<math>\mu\text{m}</math>)</i>
<i>Anterior Cornea</i>	-1408*
<i>Posterior Cornea</i>	-1372*
<i>Anterior Lens</i>	-1150*
<i>Posterior Lens</i>	1134*
<i>Retina</i>	1598*

224

225 \* -values from (Barathi, Boopathi et al. 2008)

226

227 **Table 2. Mouse eye model thicknesses and refractive indices**

228 Parameters for the mouse eye model in Figure 3A.

229

230

<i>Ocular Component</i>	<i>Thickness(<math>\mu\text{m}</math>)</i>	<i>Index of refraction</i>
<i>Cornea</i>	92*	1.402*
<i>Anterior chamber</i>	278*	1.334*
<i>Lens</i>	2004*	1.36 – 1.55 <sup>#</sup>
<i>Vitreous chamber</i>	609*	1.333*

235

236 \* - values from (Barathi, Boopathi et al. 2008)

237 <sup>#</sup> - minimum and maximum values after eye model optimization.

238

Holmgren *et al*

239 to the front of the animal, we next quantified whether this region overlapped with the functional focus  
240 observed here (Figure 3E).

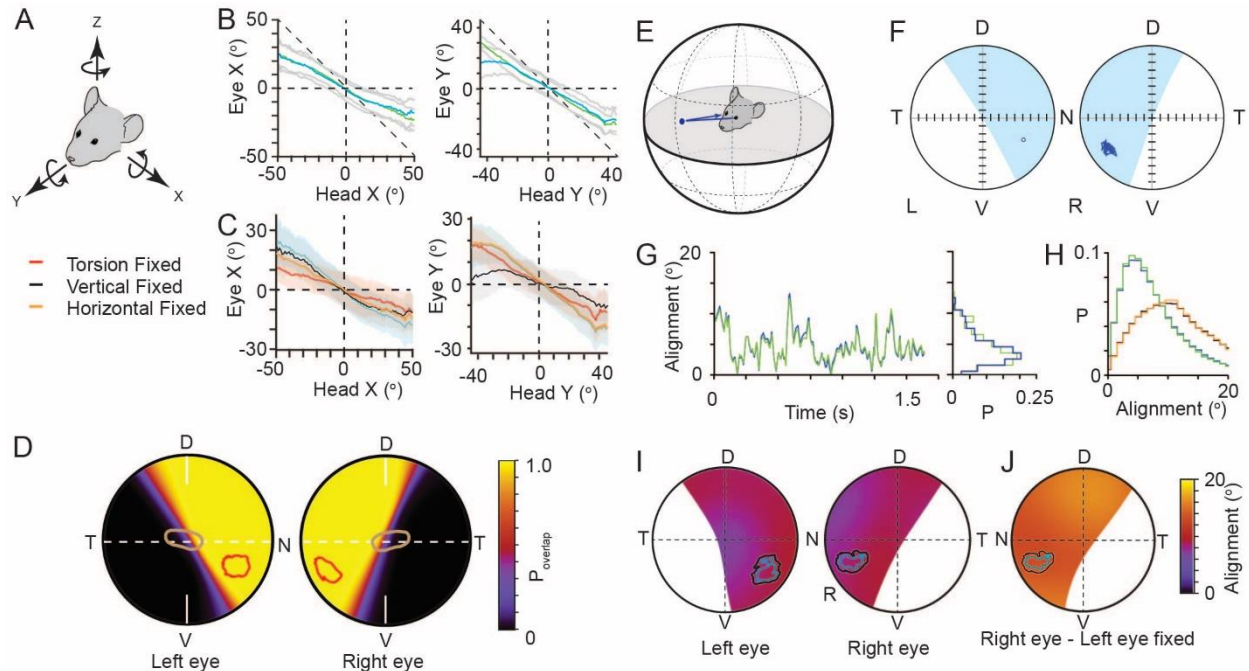
241 The average 50% contour of the functional focus was overlapped by the highest density of On Alpha-ON  
242 sustained RGC's by 35 and 67% for left and right eye respectively (Figure 3E, black, mean $\pm$ SD for left and  
243 right eye, 35.1 $\pm$ 19.8 %, 66.7 $\pm$ 0.09 %,  $p= 0.095$  &  $0.019$ , one-sided Student's t-test), and for the overlap  
244 with the second highest density was 83 and 95% (mean $\pm$ SD for left and right eye, 82.8 $\pm$ 20.1 %, 94.8 $\pm$ 24.7  
245 %,  $p= 0.042$  &  $0.003$ , one-sided Student's t-test), suggesting a high degree of correspondence between  
246 the highest density of Alpha-ON sustained RGC's and the functional focus during pursuit behavior. Viewed  
247 from above the animal's head the functional foci were directed at the region in front of the animal's nose  
248 azimuth: 1.4 $\pm$ 8.8 $^\circ$  and -4.4 $\pm$ 9.3 $^\circ$ , elevation: 5.7 $\pm$ 2.1 $^\circ$  and 4.9 $\pm$ 1.4 $^\circ$  for left and right eyes respectively,  $N =$   
249 13641 frames,  $N=3$  mice). The Alpha-ON sustained RGC's were also directed in front of the animal's nose  
250 (mean $\pm$ SD, elevation:16.0 $\pm$ 6.9 $^\circ$  and 10.8 $\pm$ 11.0 $^\circ$ , azimuth: -3.6 $\pm$ 0.7 $^\circ$  and 5.8 $\pm$ 7.9 $^\circ$  for left and right eyes  
251 respectively,  $N = 168400$  frames,  $N=3$  mice, Figure 3F). Together this suggests that objects falling within  
252 the functional foci are processed by Alpha-ON sustained RGC's.

### 253 **Combination of torsional, horizontal, and vertical eye rotations counter head rotations**

254 Eye movements in freely moving mice, like with rats (Wallace, Greenberg et al. 2013), can be large and  
255 rapid (Payne and Raymond 2017, Meyer, O'Keefe et al. 2020), and counter head rotations through the  
256 VOR (Figure 4 – figure supplement 1), enabling the large field of view around the animals head to be  
257 stabilized while the animal is moving. While the relationships between head rotations and both the  
258 horizontal and vertical eye rotations have been quantified, how these rotations combine with torsional  
259 rotations is not known. If mouse VOR operates similar to that observed in the rat (Wallace, Greenberg et  
260 al. 2013), torsional rotations in the mouse will play a significant role in stabilizing the visual field  
261 particularly during changes in head pitch. As with the vertical and horizontal rotations (Meyer, Poort et

262

Holmgren *et al*



**Figure 4 with 3 supplements**

**Functional foci are located within binocular regions in which motion is stabilized.**

**(A)** Schematic of the common head and eye rotational axes. **(B)** Relationship between head and eye rotations around the common X (left, 154500 frames from 3 animals) and Y (right, 165345 frames from 3 animals) rotational axes during pursuit and non-pursuit sequences. Plots show mean for left (blue) and right (green) eyes with standard deviation (gray). **(C)** Relationship between head and left eye rotations around the common X (left) and Y (right) rotational axes with; all eye rotations present (blue), torsional eye rotations frozen (red), vertical eye rotations frozen (black) or horizontal eye rotations frozen (orange). Plots show mean (lines) and standard deviations (colored filled regions). **(D)** Corneal view showing probability of overlap of left and right visual fields for one example animal (71995 frames), with overlay of isodensity contours (red) from functional foci (see Figure 2 – figure supplement 1D) and contour of second highest RGC region (brown) from Figure 3B. **(E)** Schematic of inter-ocular alignment. **(F)** Corneal view of alignment reference point in left eye (left) and variability in alignment of the re-projection of that point in the right eye (right) for a 1.6s data segment. **(G)** Kinetics (left) and associated distribution (right) of the variability in ocular alignment for left eye point projected to right eye (blue) and right eye point in left eye (green) for one example data segment (shown in G) from one animal. **(H)** Distributions of ocular alignment from all data segments (159318 frames, n=3 mice) with the measured eye movements for left into right eye (blue) and right into left eye (green) and alignment with eye movements frozen (left into right eye, black, right into left, orange). **(I)** Map of average inter-ocular alignment for all data segments (159318 frames, n=3 animals) with overlay of isodensity contours from Figure 2H. **(J)** Map of average inter-ocular alignment as in J with left eye movements frozen.

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Holmgren *et al*

265 al. 2018), torsional rotations in freely moving mice spanned a wide range of rotation angles (Figure 4 –  
266 figure supplement 2A-D), and were correlated with head pitch (Pearson’s correlation coefficient (r): detect  
267 -0.72, 0.58, track: -0.60 and 0.53 for left and right eyes respectively, N=4406 detect and 13624 track  
268 frames, N=3 mice, Figure 4 – figure supplement 2C-D) as well as head roll (Pearson’s correlation coefficient  
269 (r): detect: -0.46, -0.47 track: -0.45 and -0.48 for left and right eyes respectively, N=4406 detect and 13624  
270 track frames, N=3 mice, Figure 4 – figure supplement 2 L-M), as found previously for freely moving rats  
271 (Wallace, Greenberg et al. 2013). As with rats, the rotational relationship between the two eyes was  
272 dynamic with different forms of coordination (Figure 4 – figure supplement 2E-I), including episodes of in-  
273 and excyclovergence (torsional rotation of both eyes toward or away from the nose respectively) as well  
274 as dextro- and levocycloverision (torsional rotation of both eyes to the animal’s right or left  
275 respectively). We next analyzed how effectively rotations of the eye around multiple rotational axes  
276 combined to compensate the rotation of the head (Figure 4A, Figure 4 – figure supplement 3A-G). We  
277 compared movement of the head around its rotational axes and eye movements around the same  
278 rotational axes (Figure 4A), effectively defining alternative rotational axes for the eyes to match the axes  
279 of the head. Rotation of the eye around these re-defined axes would involve simultaneous rotations in  
280 multiple of the eye’s anatomical axes. The gain of this compensation was relatively linear and less than  
281 unity for both pitch- and roll-axes, indicating on average under-compensation of the head rotation (slope  
282 (gain) of relation for pitch axis,  $-0.45 \pm 0.12$  and  $-0.48 \pm 0.06$ ; roll axis  $-0.51 \pm 0.12$  and  $-0.62 \pm 0.05$  for left and  
283 right eye respectively, 168852 frames, N=3 mice). The relatively linear relationships between head and  
284 eye rotation around the head pitch and roll axes (Figure 4B) with a transition through the origin suggests  
285 that the horizontal, vertical and torsional eye movements are combined to effectively compensate pitch-  
286 and roll-related head movements. We next digitally froze each individual eye rotation axis (torsion,  
287 vertical and horizontal) and measured the effect on countering the head rotation (Figure 4C). For rotations  
288 around the head x-axis (head pitch changes) the gain of compensation was most affected by freezing

Holmgren *et al*

289 torsional rotations (Figure 4C, gain mean±SD, control:  $-0.45 \pm 0.12$  and  $-0.48 \pm 0.06$ ; torsion frozen  $-0.24 \pm 0.1$   
290 and  $-0.24 \pm 0.01$ , for left and right eyes respectively, N= 168852 frames, N=3 mice), while freezing vertical  
291 or horizontal rotations had more minor effects (Figure 4C, Table 3). The gain of compensation for rotations  
292 around the head y-axis (head roll changes) was dramatically affected by freezing vertical rotations (Figure  
293 4C, gain mean±SD, control:  $-0.51 \pm 0.12$  and  $-0.62 \pm 0.05$ , vertical frozen  $-0.16 \pm 0.14$  and  $-0.17 \pm 0.03$ , for left  
294 and right eyes respectively, N= 168852 frames, N=3 mice), with freezing torsion also reducing  
295 compensation gain but to a lesser extent (Figure 4C, Table 3). We next quantified the stability and  
296 alignment of the animal's binocular visual field during the pursuit sequences and determined the location  
297 of the functional foci within the stabilized region.

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Holmgren *et al*

299 **Table 3. Compensation gain of eye rotations for head X or Y-axis rotations**

300 Effect of digitally freezing torsional, vertical and horizontal eye rotations on the gain of compensation of  
 301 X and Y head rotations. Data taken from 168852 frames, from 3 animals

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Eye	Rotation direction	Rotation	All Rotations (mean ± SD)	Eye rotation frozen (mean ± SD)
Left	X	Torsion	-0.45±0.12	-0.24±0.1
		Horizontal	-0.45±0.12	-0.32±0.06
		Vertical	-0.45±0.12	-0.35 ±0.08
Right	X	Torsion	-0.48±0.06	-0.24±0.01
		Horizontal	-0.48±0.06	-0.36±0.08
		Vertical	-0.48±0.06	-0.34±0.03
Left	Y	Torsion	-0.51±0.12	-0.35±0.05
		Horizontal	-0.51±0.12	-0.51±0.11
		Vertical	-0.51±0.12	-0.16±0.14
Right	Y	Torsion	-0.62±0.05	-0.45±0.05
		Horizontal	-0.62±0.05	-0.62±0.02
		Vertical	-0.62±0.05	-0.17±0.03

320

Holmgren *et al*

321 **Functional foci are located in the motion-stabilized binocular visual field**

322 Similar to rats, left and right visual fields overlapped extensively (Hughes 1979, Drager and Olsen 1980),  
323 with eye movements creating variability in the extent of the overlap at the edges of the two visual fields,  
324 the transition from monocular to binocular (Figure 4D). The functional foci for both eyes were  
325 predominately contained within the region of continuous binocular overlap. A horizontal transect through  
326 the optical axis for all animals showed a gradual transition from continuous binocular coverage to zero  
327 binocular coverage commencing just nasal of the optical axis (Figure 4D, Figure 4 - figure supplement 3H  
328 and I), indicating that the region of highest RGC density spans this transitional region whereas the  
329 functional foci are, on average, contained within the binocular region (Figure 4D - figure supplement 3H).

330 We next quantified the variability of alignment of the left and right visual fields within the binocular  
331 region, and specifically in the functional focus location (Figure 4E) by using the center of mass (50%  
332 isodensity contour center) of the left eye functional focus as an initial reference point and projecting this  
333 point to the boundary of a hypothetical sphere surrounding the head. This contact point on the sphere  
334 was then re-projected into the right eye to identify the matching location of the left eye (Figure 4E). We  
335 then followed the trajectory of the re-projected point in the right eye to get a measurement of alignment  
336 variability (Figure 4F, for comparison with the locations in the right eye projected into the left eye see  
337 Figure 4 – figure supplement 3I-K). While pursuing crickets, alignment precision varied through time  
338 (Figure 4G) with the mean alignment of the reference point over all animals and data segments being ~8-  
339 9°, which is around the size of V1 cortical neuron receptive fields (~5-15° (Niell and Stryker 2008), Figure  
340 4H, mean±SD, left eye projected into right eye 8.8±6.9°; right eye projected into left eye 8.6±6.7°).

341 Repeating this analysis for all points within the region where the probability of binocular overlap was  
342 greater than 5% showed that there was a relatively uniform alignment over the entire region (Figure 4I),  
343 and that the average alignment error in the functional foci was 8-10°. Coordination of eye movements  
344 was important for alignment, as freezing the movements of one eye to its mean position resulted in a

Holmgren *et al*

345 significant increase in the alignment error when comparing individual cricket tracking sequences (left all  
346 rotations vs. left eye frozen  $P=1.78 \times 10^{-10}$ , right eye all rotations vs. right eye frozen  $P=7.12 \times 10^{-11}$ ,  $N=52$   
347 sequences, unpaired Student's t-test), and a ~54% increase in the mean alignment error over all frames  
348 for the reference location (Figure 4I, left eye projected into right eye (left eye frozen)  $13.4 \pm 8.3^\circ$ ; Right eye  
349 projected into left eye (right eye frozen)  $13.4 \pm 8.3^\circ$ , mean  $\pm$  SD, 159318 frames,  $N=3$  mice), which also  
350 resulted in a uniform increase in alignment error over the whole overlap region (Figure 4J and Figure 4 –  
351 figure supplement 3J-L). In summary, during pursuit behavior the functional foci are located in a stable  
352 binocular region of the mouse's visual field. However, in the absence of a mechanism for voluntarily  
353 directing its gaze towards a specific target, such as smooth pursuit, tight coupling of VOR evoked eye  
354 movements to head rotations would seem to be restrictive to an animal's ability to move the target into  
355 a specific part of their visual field during pursuit. We therefore next measured what mechanisms mice use  
356 to bring the prey into their functional focus.

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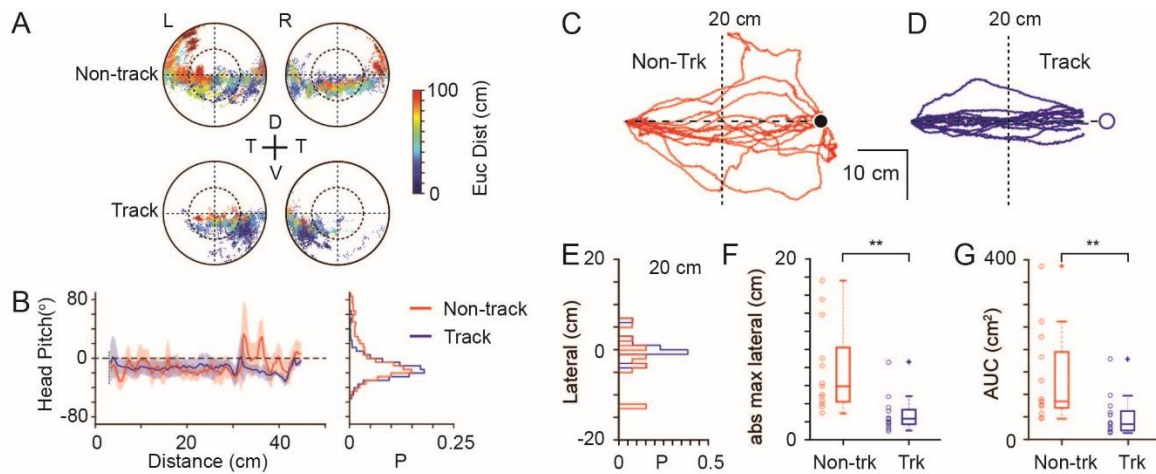
### 358 **Behavioral mechanisms for maintaining prey within functional foci**

359 At detection, mice orient towards their target, aligning their head with the prey and running towards it  
360 (Figure 2D), keeping the cricket within a narrow window around its forward direction. This provides a  
361 direct way for mice to hold their prey within their binocular visual fields (Figure 4D). We next measured  
362 whether additional head or eye movements are used to keep the target within the functional foci. If the  
363 mice were actively maintaining the prey within a fixed location of their visual fields the position of the  
364 cricket image should not change as the mouse approaches the cricket. The cricket image location could  
365 be maintained by either a head or eye rotation. If they were not actively maintaining the prey in a fixed  
366 location, the cricket images should shift downwards in the visual fields as the mouse approaches. To  
367 distinguish between these two possibilities we plotted the cricket positions in the eye views color-coded

Holmgren *et al*

368 by the distance between the mouse and cricket (Figure 5A). As the mouse approached the cricket during  
369 the track behavioral epoch, the projected cricket positions shifted lower in the visual field (Figure 5A  
370 lower). This suggests that the mice did not use additional head or eye movements (Figure 5 – figure  
371 supplement 1) to bring the cricket into the functional foci, but rather manipulated the cricket’s position  
372 in the eye view by orienting and moving towards the target. Consistent with this, head pitch remained  
373 stable as the mice approached the crickets (Figure 5B). Furthermore, there was no significant difference  
374 in head pitch as a function of distance to the cricket between non-tracking and tracking periods (non-  
375 tracking head pitch:  $-3.7 \pm 26.5^\circ$ , mean  $\pm$  SD, median =  $-11.3^\circ$ , tracking head pitch:  $-12.9 \pm 15.7^\circ$ , mean  $\pm$  SD,  
376 median =  $-14.6^\circ$ , Ks test,  $P=0.709$ , paired Student’s t-test  $P=0.197$ ,  $N=18$  detect-track sequences,  $N= 3$   
377 mice). In addition, and consistent with previous findings (Michaël, Abe et al. 2020), mice did not  
378 significantly converge their eyes as they approached the crickets (non-tracking head vergence:  $7.6 \pm 13.5^\circ$ ,  
379 mean  $\pm$  SD, median =  $8.6^\circ$ , tracking head vergence:  $2.5 \pm 16.7^\circ$ , mean  $\pm$  SD, median =  $3.2^\circ$ . Ks test,  $P=0.425$ ,  
380 paired Student’s t-test  $P=0.225$ ,  $N=18$  detect-track sequences  $N = 3$  mice, Figure 5 – figure supplement 1J  
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Holmgren *et al*



### Figure 5 with 1 supplement

#### Mechanisms used to maintain prey within a focal visual region.

(A) Corneal locations of cricket position color-coded by Euclidean distance to cricket for non-track (upper) and track (lower) epochs (18 data sequences, 15649 non-tracking and 8510 tracking frames,  $n=3$  animals). (B) Mean and SD head pitch with Euclidean distance to cricket (left) and distribution of head pitch angles (right) for non-track (red) and track (blue) epochs (datasets as in A). (C) Mouse trajectories during non-track epochs rotated and overlaid to show deviation from a direct path (13 trajectories from 3 animals). (D) Mouse trajectories as in D but during track epochs (13 trajectories from 3 animals). (E) Histogram of lateral deviations for non-track (red) and track (blue) data in C and D calculated 20 cm from the end of the trajectory. (F) Boxplots and individual data points of absolute maximal lateral deviation from a direct path between start and end points for non-track (red) and track (blue) epochs (datasets as in C & D), \*\*  $P=0.0006$ , Wilcoxon's Rank Sum Test. (G) Boxplots and individual data points of area under the curve (AUC) of mouse trajectories during non-track (red) and track (blue) epochs (datasets as in C & D), \*\*  $P=0.0029$ , Wilcoxon's Rank Sum Test.

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Holmgren *et al*

384 and Table 4). These observations suggest that the primary role for the eye movements is stabilizing the  
385 visual fields.

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387 **Table 4. Eye rotations during non-tracking and tracking periods**

388 Horizontal, vertical and torsional eye rotations during the non-tracking and tracking periods in Figure 5.  
389 Data taken from 18 non-track epochs and 18 track epochs, from 3 animals.

Ocular Rotation	Non-Trk (mean $\pm$ SD) (median)	Track (mean $\pm$ SD) (median)	p value (KS)	P value (Student T-test)
Lt Horizontal	-1.8 $\pm$ 9.9° (-1.7°)	-1.8 $\pm$ 14.9° (-3.5°)	3.9x10 <sup>-2</sup>	0.162
Lt Vertical	0.8 $\pm$ 11.2° (-0.4°)	4.5 $\pm$ 11.1° (4.9°)	0.425	0.616
Lt Torsional	2.9 $\pm$ 16.1° (0.0°)	1.3 $\pm$ 20.6° (0.0°)	0.945	0.610
Rt Horizontal	5.7 $\pm$ 10.9° (5.5°)	1.0 $\pm$ 9.9° (1.7°)	9.82x10 <sup>-2</sup>	1.08x10 <sup>-2</sup>
Rt Vertical	-3.6 $\pm$ 13.4° (-6.3°)	5.6 $\pm$ 12.7° (-7.1°)	0.945	0.804
Rt Torsional	0.32 $\pm$ 13.5° (0.0°)	0.7 $\pm$ 12.3° (0.0°)	0.425	0.366

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Holmgren *et al*

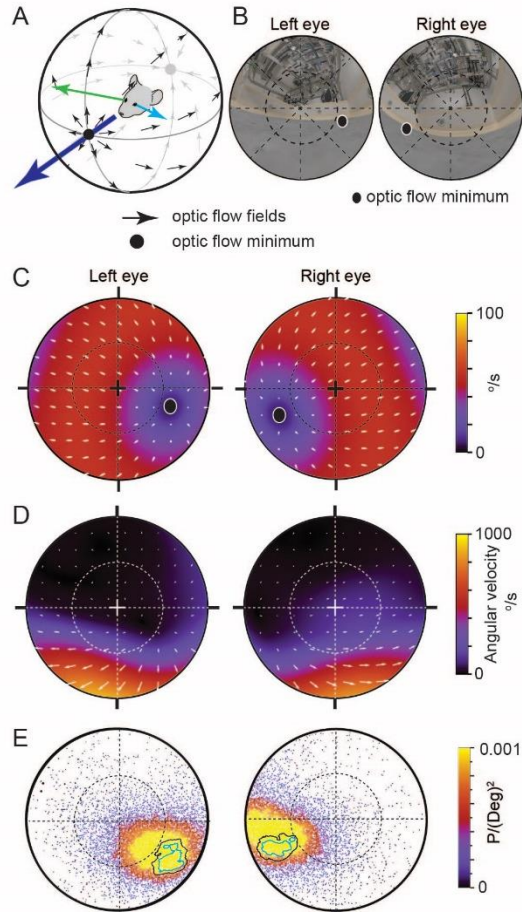
396 If mice successfully track and capture prey by retaining the target in front of them, then this should be  
397 reflected in the trajectories taken by the mice during the tracking epoch compared to the non-tracking  
398 behavioral epochs. During cricket tracking periods, mice ran directly towards the cricket, and their  
399 trajectories were significantly straighter than during equivalent non-tracking phases (Figures 5C-G).  
400 Lateral deviation at the half-way point (Figure 5E, non-tracking  $4.3 \pm 4.0$  cm, tracking  $1.4 \pm 2.0$  cm,  $P=0.009$ ),  
401 maximum lateral deviation (Figure 5F, non-tracking,  $7.7 \pm 4.9$  cm, tracking  $2.8 \pm 2.0$  cm,  $P=0.0006$ ) and the  
402 area between the trajectory and minimum distance path to the target (Figure 5G, area under the curve,  
403 non-tracking  $135.6 \pm 102.7$  cm<sup>2</sup>, tracking  $51.3 \pm 45.8$  cm<sup>2</sup>,  $P=0.0029$ ) were all significantly smaller in the  
404 tracking epochs (all comparisons mean $\pm$ SD, N=13 tracking and non-tracking sequences, N=3 mice,  
405 Wilcoxon's Rank Sum Test).

406 Together this suggests that mice do not make compensatory vertical head movements, tracking eye  
407 movements or vergence eye movements to keep prey within their functional foci but instead retain their  
408 target within a restricted bearing by running straight towards it. This raised the question of what  
409 advantage is this behavior to the mouse and what is unique about the functional focus position on the  
410 cornea?

#### 411 **Functional foci are located in region of minimized optic flow**

412 Optic flow is the pattern of object motion across the retina that can be self-induced, through eye, head or  
413 translational motion, or induced by motion of objects in the environment, or combinations thereof (for  
414 review see: (Angelaki and Hess 2005). In a freely moving animal in a still environment, translational motion  
415 results in a pattern of optic flow that consists of a radial flow-fields emanating from a point of zero-velocity  
416 (Figure 6A). While optic flow is used by many species for both navigation and the estimation of the motion

417



**Figure 6**  
**Functional foci are located in the regions of reduced optic flow during forward motion.** (A) Schematic of idealized optic flow (black arrows) as a mouse translates forwards (after (Sabbah, Gemmer et al. 2017)). Left (blue arrow) and right (green arrow) gaze vectors. (B) Location of optic flow minima in reconstructed mouse eye views of the cricket and experiment arena (from Figure 1H), circle represents 45°. (C) Optic flow map in corneal views, showing flow velocity (color coding) and direction (white arrows) calculated for the idealized spherical environment in 6A with forward motion of 50 cm/s. (D) Optic flow maps in corneal views during track epochs (5269 frames), from one animal. (E) Probability density map of optic flow poles in mouse corneal views during track epochs (data as in Figure 2G, 13641 frames), with overlay of isodensity contours from Figure 2H.

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Holmgren *et al*

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421 properties of moving objects, motion induced blur degrades image formation on the retina and decreases  
422 resolution depending on the animal's direction of travel (Land 1999). Optic flow is minimized in the  
423 direction of travel directly in front of the animal (Sabbah, Gemmer et al. 2017), with flow fields directed  
424 away from travel direction and forming a second minimum directly behind the animal's head (Figure 6A,  
425 see also (Angelaki and Hess 2005)). To measure the characteristics of optic flow in in both eyes of freely  
426 moving mice and to relate this flow pattern to the location of the functional foci, we next calculated  
427 average optic flow from freely moving data during pursuit behavior using the digitized environment and  
428 eye-views (Figure 6B). First, we calculated optic flow in the idealized case of forward translation motion  
429 when all surrounding surfaces were equidistant (Figure 6C). As mice have laterally facing eyes (optical axis  
430 =  $59.9 \pm 19.8^\circ$  and  $-62.3 \pm 14.7^\circ$  lateral of frontal for the left and right eyes respectively, N=3 mice), idealized  
431 forward motion resulted in the region of minimal optic flow in each eye being located off optical axis in  
432 the ventro-medial corneal region representing the animal's forward direction (radial displacement from  
433 optical axis  $36.64 \pm 0.92^\circ$  and  $-41.11 \pm 2.27^\circ$ , rotational angle  $122.95 \pm 17.05^\circ$  and  $-107.94 \pm 9.96^\circ$ , for the left  
434 and right eyes respectively, mean  $\pm$  SD, N=2 mice, Figure 6C). During free movement both the distance  
435 from the eyes to objects in the environment, as well as head and eye-rotations had a strong influence on  
436 the optic flow fields. We visualized the average flow fields during free motion by calculating the optic flow  
437 on the cornea during multiple pursuit trials (N=20 prey chases containing 52 tracking sequences, initial  
438 Euclidean distance mouse-cricket >20 cm). The resulting optic flow density maps were complex with a  
439 wide range of average speeds ( $133.44 \pm 221.42$  °/s, mean  $\pm$  1SD, median  $28.64$  °/s, interquartile range  $4.57$ –  
440  $137.18$  °/s, N=2 mice, Figure 6D). The area of lowest optic flow extended from nasal field of view to  
441 overhead (Figure 6D) but unlike the simulated case (Figure 6C) optic flow was not symmetric around the  
442 regions of minimal optic flow. Optic flow in the  $30 \times 30^\circ$  region surrounding the ventro-medial point of  
443 minimal optic flow was significantly lower than that in an equivalent region in the ventro-temporal region  
444 during free movement, but not in the simulated case (free movement: nasal  $46.3 \pm 9.8$  °/s, temporal:

Holmgren *et al*

445 199.4±29.0 °/s, P=0.0014, simulated: nasal 163.6±82.2 °/s, temporal: 833.0±416.5 °/s, P=0.0662,  
446 mean±SD, two-sided t-test, unequal variance, N=2 mice). Optic flow was higher in the lower visual field  
447 and considerably lower in the upper visual field (lower left eye visual field: 262.44±106.50 °/s, upper left  
448 eye visual field: 44.87±24.31 °/s, P=1.78x10<sup>-20</sup>, lower right eye visual field: 361.91±168.80 °/s, upper right  
449 eye visual field: 40.59±22.79 °/s, P=6.68x10<sup>-19</sup>, Two-sided t-test, unequal variance, N=2 mice), due to the  
450 greater distance between ceiling and mouse (distance to floor 2±1cm, distance to ceiling 308±107cm,  
451 9873 frames, N=3 mice). Given the advantage of low optic flow to mammalian vision, we next quantified  
452 the position of least optic flow during prey tracking. We calculated the location of the translational optic  
453 flow minimum in each frame for each eye, and created a probability map of this location over the visual  
454 field (Figure 6E). The region of highest likelihood for the presence of the optic flow minimum overlapped  
455 considerably with the functional foci in both eyes during the tracking epochs of the pursuit behavior  
456 (overlap of optic flow 95% minima and functional foci 50% regions: 100% and 99±1%, overlap of optic flow  
457 50% minima and functional foci 50 % regions: 61±14 % and 72±4 % in left and right eyes respectively, N=3  
458 mice, Figure 6E). Together this shows that mice preferentially maintain their prey in the region of reduced  
459 optic flow during pursuit, where the retinal image of their prey is least distorted due to motion induced  
460 image blur.

Holmgren *et al*

461 **Discussion:**

462 We developed a technique for reconstructing the visual fields in a freely moving mouse during prey pursuit  
463 to quantify the spatial relationship between the environment, cricket and the mouse. Using this approach,  
464 we show that mice, while pursuing crickets, preferentially maintain the prey in a localized region of their  
465 visual field, termed here the functional focus. The positional maintenance of the cricket was not achieved  
466 by active eye movements that followed the prey, but rather by the animal's change in behavior,  
467 specifically the head-movement and orientation towards the prey during pursuit. While eye rotations  
468 stabilized the visual field via the vestibulo-ocular reflex by countering head rotations, the rotations were  
469 not specific to either prey detection or prey tracking. This strongly suggested that eye-rotations in mice,  
470 like in rats, primarily stabilize their large field of view and that all three rotational axes, including ocular  
471 torsion, combine to counter head rotations. In addition, we also show that eye rotations cannot be  
472 predicted from head rotations in any one axis as has been suggested by recent studies of mouse eye  
473 motion (Meyer, Poort et al. 2018, Meyer, O'Keefe et al. 2020, Michael, Abe et al. 2020) but rather by a  
474 combination of all head rotations (Figure 4 – figure supplement 2). As the eye rotations were  
475 predominately associated with countering head-rotations, this raised the question of whether the mouse  
476 can use a large fraction of its stabilized visual field to pursuit crickets, or whether a specific region is  
477 utilized. To accurately determine the correspondence between the animal's visual field and the retinal  
478 image, we developed a quantitative model of the mouse eye and optics. Using this, we show that the  
479 location of the functional focus does not coincide with the retinal region with the highest total density of  
480 retinal ganglion cells that are laterally facing, but rather the highest density of Alpha-ON sustained RGCs,  
481 whose general properties have been previously proposed to be well suited for this purpose (Bleckert,  
482 Schwartz et al. 2014). Finally, we used the detailed, digitally rendered reconstruction of the arena and  
483 surrounding room to calculate the realistic optic flow in the visual fields (Gibson, Olum et al. 1955, Sabbah,  
484 Gemmer et al. 2017, Saleem 2020) of the mice as they pursued crickets, which showed that the functional

Holmgren *et al*

485 foci coincide with the region of the visual fields with minimal optic flow during the cricket pursuit, and  
486 presumably are thereby minimally distorted by motion-induced image blur (for review see (Angelaki and  
487 Hess 2005)). Critical to this finding was the ability to isolate the visual sense, generate both a detailed  
488 reconstruction of both the local environment and the animal's ocular anatomy and optical pathways, but  
489 also record eye motion in all three optical axes especially ocular torsion, something that has only been  
490 achieved in rats (Wallace, Greenberg et al. 2013). Lastly, by building an optical model and establishing the  
491 relationship between the retinal surface and the corneal surface we were able to relate the data  
492 generated from published studies on retinal anatomy (Drager and Olsen 1981, Sterratt, Lyngholm et al.  
493 2013, Bleckert, Schwartz et al. 2014) and physiology (Pang, Gao et al. 2003, Murphy and Rieke 2006, van  
494 Wyk, Wassle et al. 2009, Dhande, Stafford et al. 2015, Martersteck, Hirokawa et al. 2017, Sabbah, Gemmer  
495 et al. 2017) to our behavioral data.

496 Both estimates of the field of view of the mouse eye (Drager 1978) and electrophysiological  
497 measurements of receptive field locations of visually responsive neurons (Drager and Olsen 1980, Wagor,  
498 Mangini et al. 1980) have established that the binocular region of the visual field in mice is contained  
499 within the nasal visual field of each eye, and spans a region of 30-40° in front of the animal (Wagor,  
500 Mangini et al. 1980). We present here, that similar to the rat (Wallace, Greenberg et al. 2013), the  
501 overlapping monocular fields that make up the binocular overlap are not constantly maintained (Figure  
502 4H) but fluctuate at the margins as the eyes rotate to counter head rotations (Figure 4D), resulting in a  
503 region where there is a transition from one area with near continuous binocular coverage, through to a  
504 region that is invariably monocular. The functional focus described here lies within the region of high  
505 probability of maintained binocular overlap. This region of the visual field projects onto the temporal  
506 retina, which contains both ipsilaterally projecting (uncrossed) RGCs (Drager and Olsen 1980, Reese and  
507 Cowey 1986) and RGCs which form part of the callosal projection pathway (Olavarria and van Sluyters  
508 1983, Laing, Turecek et al. 2015, Ramachandra, Pawlak et al. 2020), both of which are considered central

Holmgren *et al*

509 to binocular visual processing. In addition, the current study adds to the significance to these previous  
510 findings and suggests that the functional focus location is well placed to support stereoscopic depth  
511 perception, assuming that this form of visual processing is available to and employed by the mouse (Scholl,  
512 Burge et al. 2013, Scholl, Pattadkal et al. 2015, La Chioma, Bonhoeffer et al. 2019, Samonds, Choi et al.  
513 2019, La Chioma, Bonhoeffer et al. 2020). In addition, while the overall highest density of retinal ganglion  
514 cells in mice is located in the region around the optical axis (Drager and Olsen 1981), a recent study  
515 examining the distributions of various different subclasses of RGCs has shown that the highest density of  
516 Alpha-ON sustained RGCs resides in the superior-temporal retina (Bleckert, Schwartz et al. 2014) in a  
517 region which would approximately coincide with the functional focus. These Alpha-ON sustained RGCs  
518 have center-surround receptive fields, a rapid response and fast conducting axon, and are thought to be  
519 “spot detectors” (for review see (Dhande, Stafford et al. 2015)). In addition, the Alpha-ON sustained RGCs  
520 in this particular retinal region differ from the same RGC-type in other regions of the retina as they have  
521 a significantly smaller dendritic tree radius and subtend a smaller area of physical space as well as have  
522 overlapping receptive fields (Bleckert, Schwartz et al. 2014). Taken together, the cellular properties as  
523 well as the region in-front of the animal which provides their input are consistent with the requirements  
524 for tracking small and mobile targets (Lettvin, Maturana et al. 1959, Dean, Redgrave et al. 1989, Bleckert,  
525 Schwartz et al. 2014, Procacci, Allen et al. 2020). A recent study has shown that both wide-field and  
526 narrow-field neuronal types in the mouse superior colliculus play central roles in the detection and pursuit  
527 phases of this pursuit task respectively (Hoy, Bishop et al. 2019), and consistent with this, Alpha-ON  
528 sustained RGCs having projections to the superior colliculus (Martersteck, Hirokawa et al. 2017). It is  
529 currently unclear how the primary visual cortex (V1) contributes to this behavior, but some role is possible  
530 if not probable, which would also be supported by the strong Alpha RGC projection to the dorsal lateral  
531 geniculate nucleus and thus V1 (Martersteck, Hirokawa et al. 2017). Additionally, an increased cortical

Holmgren *et al*

532 magnification factor occurs in the region corresponding to the nasal, binocular visual field (Schuett,  
533 Bonhoeffer et al. 2002, Garrett, Nauhaus et al. 2014).

534 Finally, we show that the region that contains these Alpha-ON sustained RGCs also coincides with the  
535 region of minimum optic flow and therefore reduced image blur during translation pursuit, a feature  
536 which would supports accurate localization of small targets by Alpha-ON sustained RGCs. Patterns of optic  
537 flow are thought to be an important component of perception of self-motion (Lappe, Bremmer et al.  
538 1999). Mechanistically supporting this, global alignment across the retina of the preferred orientation of  
539 direction-selective retinal ganglion cells with the cardinal directions of optic flow during idealized motion  
540 has been shown in mice (Sabbah, Gemmer et al. 2017). The average optic flow measured here was,  
541 perhaps not surprisingly, strikingly different from that observed with idealized motion, resulting in large  
542 part from the large differences to objects in the environment in which the behaviors were performed. For  
543 fast moving, ground dwelling animals like mice, considerable asymmetry in optic flow across the visual  
544 field may be the more normal case, considering that objects above the animal are, in general, likely to be  
545 more distant.

546 In freely moving rats it has been shown that ocular torsion is correlated with head pitch such that nose-  
547 up rotation of the head is counteracted by incyclotorsion (rotation towards the nose) of both eyes, with  
548 nose-down pitch counteracted by excyclotorsion (Wallace, Greenberg et al. 2013). These rotations have  
549 the effect of stabilizing the horizontal plane of the retina with respect to the horizon. The considerable  
550 radial separation between the optical axis of the eye and both the functional foci observed in the current  
551 study as well as the highest density region of Alpha-ON sustained RGCs (Bleckert, Schwartz et al. 2014)  
552 renders the direction in which these regions point highly sensitive to torsional rotation. Consequently,  
553 torsional rotation also has an important effect on alignment of the left and right visual fields in addition  
554 to its role in visual field stabilization. We show here that torsional rotation in freely moving mice is also  
555 dynamic, with episodes showing in- and excyclovergence as well as dextro- and levocycloverision. Further,



Holmgren *et al*

556 while the correlation between torsional rotation and head pitch observed in rats was measured, there  
557 was also an additional relation between ocular torsion and head roll consistent with VOR-evoked dextro-  
558 and levocycloverision. Consequently, prediction of torsion using a model based on head pitch alone  
559 resulted in an average error of around 7°, while an expanded model including roll as well performed better  
560 (Figure 4 – figure supplement 2J-O).

561 In summary, we show here that during pursuit mice preferentially keep their intended prey in a localized  
562 region of their visual fields, referred to here as the functional focus, but do so by orientating their head  
563 and body and running directly towards the prey rather than with specific eye movements. The location of  
564 the functional focus is within the binocular visual field, but in addition also coincides with the region of  
565 minimal optic flow during the pursuit, and presumably also minimally distorted by motion blur.

566  
567

Holmgren *et al*

568 **Methods:**

569 *Animal details*

570 Experiments were carried out in accordance with protocols approved by the local animal welfare  
571 authorities (Landesamt für Natur Umwelt und Verbraucherschutz, Nordrhein-Westfalen, Germany).  
572 Experiments were carried out using male C57/BL6JCrI mice (acquired from Charles River Laboratories).  
573 At the time of the cricket hunting experiments, mice (n=9) were between 2-8 months old, and weighed  
574 between 21-29g. Mice were maintained on a 12 hr light/dark cycle. Crickets (*Acheta domesticus*, Bugs-  
575 International, Germany) were housed in 480x375x210 cm cages with *ad lib* water and food (powdered  
576 mouse chow). Cricket body sizes ranged from 1 cm to 2 cm ( $1.8 \pm 0.3$  cm, mean  $\pm$  SD, n=25).

577

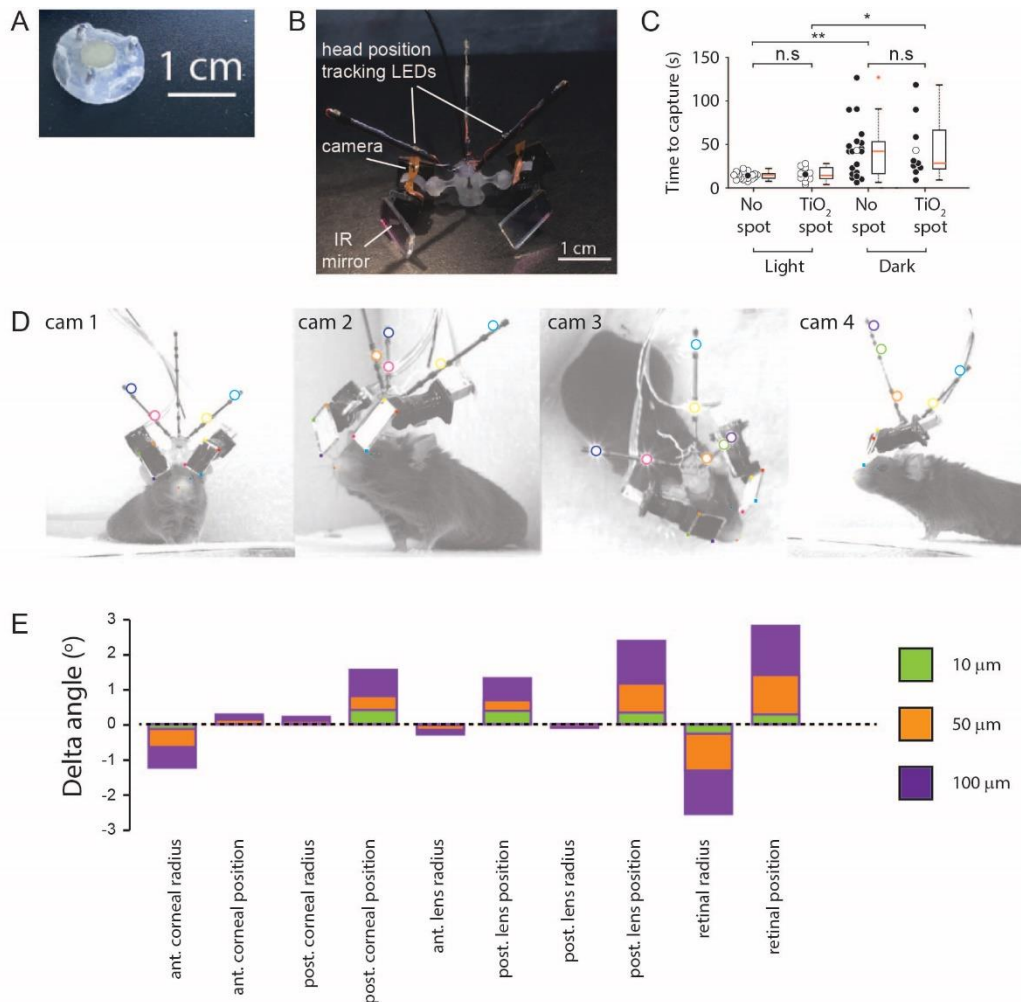
578 *Implant surgery*

579 Animals were anaesthetized using fentanyl, medetomidine and midazolam (respectively 50 $\mu$ g/kg, 5mg/kg  
580 and 0.5mg/kg, delivered i.p.), and analgesia was provided with carprofen (7mg/kg delivered s.c.). Body  
581 temperature was maintained using a thermostatically regulated heating pad. Respiration rate and depth  
582 of anesthesia was monitored throughout the procedure. Following opening of the skin and removal of  
583 connective tissue overlying the sagittal suture and parietal bones, the skull was cleaned with H<sub>2</sub>O<sub>2</sub> (3%). A  
584 custom-made implant, consisting of a flat circular attachment surface for attachment to the skull, and  
585 implant body with three anti-rotation pins and a magnet (Figure 7A-B), was fixed to the dried skull using  
586 a UV-curing dental adhesive (Optibond FL, Kerr Corporation, Orange, California, USA) and a UV-curing  
587 dental composite (Charisma, Kulzer GmbH, Hanau, Germany). The implant attachment surface and body  
588 were made from light-weight, bio-compatible dental resin (Dental SG, Formlabs, Germany). Skin margins  
589 were closed with 5/0 Vicryl sutures (Ethicon Inc, Somerville, NJ, USA) and a cyanoacrylate adhesive  
590 (Histoacryl, B.Braun, Melsungen, Germany). The injectable anesthetic combination was antagonized with

Holmgren *et al*

- 591 naloxone, atipamezole and flumazenil (respectively 1.2mg/kg, 0.5mg/kg and 0.75mg/kg, delivered i.p.),  
592 and the animal was allowed to recover.

Holmgren *et al*



**Figure 7. Methods.**

**(A)** Implanted baseplate with magnetic attachment point and restraining pin holes. **(B)** Miniaturized eye cameras and head position tracking system. Infrared illumination LEDs were mounted on the camera objective and reflected onto the eye using an IR-reflective mirror. Head position tracking IR-LEDs were mounted on three carbon-fiber struts attached to the head-mount. **(C)** Cricket capture times in lit or dark conditions in mice without ( $n=19$  pursuit sequences,  $n=6$  mice) or with ( $n=10$  pursuit sequences in lit conditions and  $n=9$  pursuit sequences in the dark,  $n=3$  mice) corneal TiO<sub>2</sub> torsion tracking spots, Lit vs Dark with no spot,  $P=0.0012$ , Lit vs Dark TiO<sub>2</sub> spot,  $P=0.0133$ , Lit without spot vs Lit with TiO<sub>2</sub> spot,  $P=0.69$ , Dark without spot vs Dark with TiO<sub>2</sub> spot,  $P=1$ . n.s. = non-significant,  $*P<0.05$ ,  $**P<0.01$ . Paired Wilcoxon's signed rank tests. For these experiments, pursuits were conducted in a smaller arena (480 x 375 x 210 cm). **(D)** Images of mouse with eye camera and head position tracking system for anatomical calibration. Head mount and anatomical features marked. Anatomical features: Left (blue filled circles) and right (green filled circle) medial canthi, left (orange filled circles) and right (red filled circle) nostril positions. Head mount features: position tracking LEDs (large colored circles), IR mirror corner positions (small colored filled squares). **(E)** Sensitivity of the radial elevation on the retina in the mouse eye model to changes in the radii of curvature and thicknesses of the model optical components.

Holmgren *et al*

594 *Positioning of the head-mounted cameras*

595 The eye cameras for oculo-videography were mounted on mounting arms which were attached to a  
596 baseplate with complementary holes to the anti-rotation pins on the implant and fitted with a magnet of  
597 complementary polarity. During positioning of the head-camera, mice were anaesthetized with isoflurane  
598 (induction: 3-5% isoflurane, maintenance: 2.0% isoflurane in air). Anesthetic depth and body temperature  
599 were monitored as above. The cameras were positioned to have a sharp image of the entire eye, with the  
600 mounting arms adjusted such that the cameras and mounting system caused minimal disruption to the  
601 mouse's lateral and frontal field of view. Mounting arms were secured with cyanoacrylate adhesive glue  
602 (Histoacryl, B.Braun, Melsungen, Germany). The eye-camera system was then removed and the animal  
603 allowed to recover.

604

605 *Training procedure*

606 Mice were acclimated to cricket capture in their home cage. Individual crickets were placed in the mouse's  
607 home cage overnight, in addition to their standard *ad lib* mouse food. Mice were handled and habituated  
608 to the experimenter, the head cameras, and the head tracking mounts. The ability of each mouse to  
609 visually track the crickets was assessed using the protocol of Hoy et al.(Hoy, Yavorska et al. 2016). Briefly,  
610 the ability of the mice to track and capture crickets in a white walled, 480 x 375 x 210 cm arena was  
611 assessed in lit and dark conditions (Figure 7C). Mice were given 2 minutes in which to capture the crickets.  
612 Prior to the assessment mice were food deprived overnight before the trial.

613 *Placement of torsion tracking marks*

614 Crenellations along the iridial-pupil border were less distinct in mice than those previous described in rats  
615 (Wallace, Greenberg et al. 2013). Ocular torsion changes were therefore measured by tracking the

Holmgren *et al*

616 rotations of small spots of titanium dioxide (TiO<sub>2</sub>) paste dots (~ 300 μm) applied to ventral and/or  
617 temporal locations on the cornea as described in (van Alphen, Winkelman et al. 2010). The TiO<sub>2</sub> paste  
618 consisted of TiO<sub>2</sub> powder (Kronos Titan GmbH, Leverkusen, Germany) mixed with a small quantity of  
619 sterile Ringer's solution. Application of the TiO<sub>2</sub> spots was performed with the animal anaesthetized with  
620 isoflurane (induction: 5% isoflurane, maintenance: 0.5-1.0% isoflurane in air, total time anesthetized 5-  
621 10mins). Anaesthetic depth and body temperature was monitored as above. Following application of TiO<sub>2</sub>  
622 spots, mice were allowed to recover for >45 minutes prior to a cricket hunt. The presence of the TiO<sub>2</sub>  
623 marks did not significantly change the animal's cricket hunting performance as assessed by the average  
624 time taken to capture crickets (Figure 7C).

625

#### 626 *Experiment procedure*

627 Initially, mice were allowed to explore the experimental arena (1x1x0.26 m) without head camera mounts.  
628 During subsequent training sessions mice were acclimated to cricket hunting, with the head cameras on,  
629 in the experiment arena. Auditory white noise (60-65 dB, NCH-Tone generator v 3.26, NCH Software, Inc.  
630 Greenwood Village, USA) was provided through 4 speakers (Visaton, Germany), one on each wall of the  
631 arena. Olfactory noise was provided by ventilating the arena (TD-1000/200 Silent fan, S&P, Barcelona,  
632 Spain) through a perforated floor (5cm perforation spacing) with air blown through a cage containing live  
633 crickets (cricket cage dimensions 480x375x210cm). During experiments the arena was lit by a single lamp  
634 (4000 K, 9W, Osram, Munich, Germany) positioned ~1m above the arena. During each experiment the  
635 mouse was given 5 minutes to explore the arena without head cameras. After this period the mouse was  
636 removed from the arena and the head cameras were mounted. At the commencement of each hunt the  
637 cricket was released at a variable location into the central region of the arena.

638

Holmgren *et al*

639 *Eye camera and head position tracking system*

640 Head and eye tracking was performed as described in (Wallace, Greenberg et al. 2013), with modifications  
641 as described below. The eye camera mount and implant were re-designed to be smaller, lighter and  
642 stronger (Figure 7A-B). The camera system body, camera holders and mounting arms were produced using  
643 a Formlabs Form2 SLA 3D printer (Formlabs Inc., USA), with Dental SG Resin (Formlabs Inc., USA) as the  
644 primary construction material. The cable used for data transfer and camera and position tracking LEDs  
645 power inputs was a flat flexible printed circuit (Axon Kabel GmbH, Leonberg, Germany). Eye movements  
646 were recorded at 60 Hz (camera resolution 752x480 pixels), with illumination provided by a ring of three  
647 IR-LEDs ( $\lambda=850$  nm, OSRAM SFH4050 or SFH4053 @ 70mA, RS Components, Germany) surrounding the  
648 camera lens. The mouse's head position and head rotations were tracked using seven IR-LEDs ( $\lambda = 950$   
649 nm, OSRAM SFH4043 @ 70mA, RS Components, Germany) mounted on three struts of carbon fiber that  
650 projected from the body of the camera system. The resultant total system weight was ~3g, including  
651 effective cable weight.

652

653 *Mouse head and cricket position tracking*

654 The positions of the cricket within the arena were recorded using 4 cameras (488 x 648 px, recorded at  
655 200 Hz, piA640-210gm, Basler cameras, Basler Ahrensburg, Germany) fitted with NIR-blocking filters  
656 (Calflex X, Qioptiq, Germany). Cameras were located ~1.5 m above the arena, and were positioned so that  
657 the arena was covered at all points by 2 or more cameras from differing vantage points. Mouse IR-head  
658 tracking LEDs were recorded at 200 Hz using 4 cameras (piA640-210gm, Basler cameras, Basler  
659 Ahrensburg, Germany). Image acquisition, synchronization and mouse head rotation calculations were  
660 performed as described previously (Wallace, Greenberg et al. 2013).

661

Holmgren *et al*

662 *Anatomical model*

663 Head mount features and mouse anatomical features (medial canthi and nostril positions) were recorded  
664 at 50 Hz using four synchronized cameras (acA2040-90 um, Basler cameras, Basler Ahrensburg, Germany)  
665 fitted with 25 mm focal length objectives (CCTV lens, Kowa Optical Products Co. Ltd, Japan) calibrated as  
666 described for the overhead cameras in (Wallace, Greenberg et al. 2013). Cameras were positioned to  
667 provide images of the animal and headset from different angles to allow triangulation of the anatomical  
668 features (Figure 7D). During acquisition of the calibration images, the animal was illuminated with 12 IR-  
669 LED modules, ( $\lambda = 850$ , Oslon Black PowerStar IR-LED module, ILH-IO01-85ML-SC201\_WIR200, i-led.co.uk,  
670 Berkshire, UK) run at 1A. Position tracking LED, medial canthi, nares, mirror corner and camera chip corner  
671 positions were marked in 2 or more camera views, in multiple synchronized frames. Based on the  
672 triangulated positions of anatomical features, head cameras and position tracking LEDs the mouse's eye  
673 position could be placed a common coordinate system.

674 To establish the animal's horizontal plane from the head tracking LEDs, a position for the animal's nose  
675 was first defined by averaging to 3D positions of the marked nostrils. A pre-forward vector was calculated  
676 using the direction between mean of eyes and nose and a pre-up vector as vector orthogonal to the pre-  
677 forward and vector between the eyes. Next, the left vector was defined as orthogonal to pre-forward and  
678 pre-up. Finally, the system was rotated by  $40^\circ$  around the left vector such that forward vector was  
679 elevated. This established a head-fixed forward-left-up coordinate system that was based on the bregma-  
680 lambda sagittal plane by tilting the eyes-nose plane by an angle of  $40^\circ$ .

681 *Interpolation*

682 Head tracking frame rates were 200Hz, while eye tracking cameras recorded at 60 Hz. Eye positions  
683 were consequently interpolated as follows: Let

684 
$$R_{t1}, R_{t2} \in SO(3)$$



Holmgren *et al*

685 be two rotations that transform the vector  $(0,0,-1)^t$  into the gaze vectors  $v_{t1}, v_{t2}$  in head fixed  
686 coordinates at times  $t1, t2$ . Then for a time  $t'$  with

687 
$$t' = t1 + s * (t2 - t1), 0 < s < 1$$

688 the corresponding rotation  $R_{t'}$  is interpolated such that  $v_{t'}$  is placed on the geodesic defined by  $v_{t1}, v_{t2}$   
689 with an angle of  $s * \angle (v_{t1}, v_{t2})$  to  $v_{t1}$ , and the rotation of a vector perpendicular to  $(0,0,-1)^t$  is  
690 continuous and uniform between  $t1$  and  $t2$ .

691

692 *Camera calibration*

693 Overhead cameras for animal position and pose tracking, tracking of crickets and the cameras used for  
694 generation of the anatomical model were calibrated as described for the overhead cameras in (Wallace,  
695 Greenberg et al. 2013) and the eye camera calibration performed as described in (Wallace, Greenberg et  
696 al. 2013).

697

698 *Pupil position and pupil torsion tracking*

699 Pupil boundary tracking, compensation for eye image displacement, and gaze vector calculation was  
700 performed as described previously in (Wallace, Greenberg et al. 2013). Where contrast between pupil and  
701 iris was insufficient to allow automated pupil position tracking, pupil positions were manually tracked.

702 The  $TiO_2$  spots for tracking ocular torsion were tracked manually in each image frame. Torsional rotations  
703 were determined based on the tracked  $TiO_2$  spot positions as follows. Total rotation of the eye was defined  
704 as previously described in (Wallace, Greenberg et al. 2013), as:

705 
$$R_{eye} = R_{\phi}R_{\theta}R_{\psi}$$

Holmgren *et al*

$$706 \quad = \begin{bmatrix} 1 & 0 & 0 \\ 0 & \cos \phi & -\sin \phi \\ 0 & \sin \phi & \cos \phi \end{bmatrix} \begin{bmatrix} \cos \theta & 0 & -\sin \theta \\ 0 & 1 & 0 \\ \sin \theta & 0 & \cos \theta \end{bmatrix} \begin{bmatrix} \cos \psi & -\sin \psi & 0 \\ \sin \psi & \cos \psi & 0 \\ 0 & 0 & 1 \end{bmatrix}$$

707 where  $\phi$ =vertical,  $\theta$ =horizontal and  $\psi$ =torsional rotations. The mouse's gaze vector has the coordinates  
708  $[0 \ 0 \ -1]^T$  for the reference position of the eye, and in each frame:

$$709 \quad v^{gaze} = R_{eye} \begin{bmatrix} 0 \\ 0 \\ -1 \end{bmatrix}$$

710

711 With the eye in its reference position, we assume that the marked TiO<sub>2</sub> spot is located in the x-y plane of  
712 the eye camera (Wallace, Greenberg et al. 2013). The anatomical location of this marked spot can then  
713 be described by two unknown parameters  $r$  (where  $r>1$  is the 3D distance of the eyeball surface to the  
714 eyeball center, and a distance of 1 describes the rotation radius of the pupil) and  $\alpha$  is the fixed angle  
715 between the TiO<sub>2</sub> mark and the gaze vector. After eye rotation the 3D location of the TiO<sub>2</sub> is:

$$716 \quad v^{mark} = R_{eye} \begin{bmatrix} r \sin \alpha \\ 0 \\ -r \cos \alpha \end{bmatrix}$$

717 and the predicted pixel coordinates of the spot in the image are:

$$718 \quad \hat{p}^{mark} = \begin{bmatrix} a_{EC} \\ b_{EC} \end{bmatrix} + \frac{f}{z_0} \begin{bmatrix} v_1^{mark} \\ v_2^{mark} \end{bmatrix}$$

719 where  $a_{EC}$  and  $b_{EC}$  are the location in the image of the center of the eye ball and  $\frac{f}{z_0}$  a scaling  
720 factor, both of which are determined in the calibration procedure for pupil boundary tracking, described  
721 in full in (Wallace, Greenberg et al. 2013).

722 When  $r$  and  $\alpha$  are known the value  $\psi$  can be determined. Using the Matlab function **fminbnd** the squared  
723 2D distance

Holmgren *et al*

724 
$$|\hat{p}^{mark} - p^{mark}|_2^2$$

725 between the predicted and marked locations of the TiO<sub>2</sub> mark is minimized.

726 This method is used to determine the ocular torsion based on the TiO<sub>2</sub> spot location, both during and after  
727 calibration. Calibration was performed as follows:

728 For a given  $r$  and  $\alpha$  choice,  $\psi$  can be calculated as above. The sum of square errors in pixel locations is  
729 then calculated over all frames. We optimized over  $r$  and  $\alpha$  using the Matlab function **fminsearch**. To  
730 initialize  $r$ , we make use of the fact that the pupil model,  $p^{mark}$  and  $r$  together determine the 3D location  
731 of the mark  $v^{mark}$  in each image. For each frame we first calculated:

732

733 
$$\Delta a = \frac{p_1^{mark} - a_{EC}}{f/z_0}$$

734

735 
$$\Delta b = \frac{p_2^{mark} - b_{EC}}{f/z_0}$$

736

737 
$$m = \min\left(1, \frac{r}{\sqrt{\Delta a^2 + \Delta b^2}}\right)$$

738

739 
$$v_{init}^{mark} = \begin{bmatrix} m\Delta a \\ m\Delta b \\ -\sqrt{r^2 - m^2(\Delta a^2 + \Delta b^2)} \end{bmatrix}$$

740

Holmgren *et al*

741 
$$\alpha_{init} = \cos^{-1}(v^{gaze} \cdot v_{init}^{mark} / r)$$

742 Using this method  $\alpha_{init}$  is estimated separately for each frame, and if the choice of  $r$  is correct then these  
743 values should agree. We can use **fminbind** to minimize the following with respect to  $r$ :

744

745 
$$\text{Var}(\alpha_{init}) = \overline{(\alpha_{init} - \overline{\alpha_{init}})^2}$$

746 After  $r$  is initialized,  $\alpha_{init}$  is calculated, with  $\alpha$  initialized using the mean over frames.

747 Torsional rotations were normalized by calculating a mean torsion value for the 0.01 % of frames that  
748 were closest to both median pitch and roll of the head. Torsional values in other tracked frames were then  
749 normalized to this mean torsion value.

750

### 751 *Cricket Position Tracking*

752 Cricket body positions were automatically tracked using the method and algorithm described for tracking  
753 eye corners, as described in the section “*Compensation for lateral eyeball displacement – tracking of*  
754 *anatomical landmarks around the eye*” in (Wallace, Greenberg et al. 2013). To increase the contrast  
755 between the region around the cricket in the image and the cricket, ~100 background image frames (in  
756 which neither mouse nor cricket was present) were averaged and subtracted from frames in which the  
757 cricket was present. In frames where automated cricket position tracking was not possible, frames were  
758 tracked manually. As the cameras used for cricket tracking had been calibrated along with the animal  
759 position tracking cameras (see above), the 3-dimensional location of the cricket could be triangulated in  
760 a common coordinate system with the animal’s position.

761

Holmgren *et al*

762 *Classification of behavioral periods*

763 To decrease the effects of tracking noise and rapid head rotations, mouse velocity, target bearing and  
764 inter-animal Euclidean distances were first filtered using a 50ms sliding window Gaussian filter.

765 The criteria used to classify the different hunt phases were based on those described in (Hoy, Yavorska et  
766 al. 2016). In an initial step, behavioral end points ( $t_{end}$ ) for capture periods were identified by manual  
767 inspection of the tracking movies. Further identification of the behavioral start points ( $t_{start}$ ) and  $t_{end}$  points  
768 for the different hunt sequence epochs were then identified as described below.

769 The  $t_{end}$  points were defined as:

770 A. The  $t_{end}$  point for a detect period was defined as the last frame before (1) Mouse head velocity  
771 in the direction of the cricket was  $\geq 20$  cm/s, (2) The mouse's bearing towards the cricket was constantly  
772 below  $90^\circ$  and (3) the Euclidean distance between the mouse and cricket was continuously decreasing.

773 B. The  $t_{end}$  point for a tracking period was identified by locating local minima in the mouse-cricket  
774 Euclidean distance time plots, where local minima were defined as points at which the mouse came within  
775 a contact distance of 6 cm (measured from the tracked point on the mouse's head, giving a  $> 3$  cm  
776 separation between the mouse's nose and the cricket). These were followed either by a capture period  
777 (see below) or were followed by a  $\geq 5$ cm increase in inter-animal Euclidean distance, which were defined  
778 as cricket escapes. In cases where the absolute value of the target bearing was  $> 90^\circ$  before the mouse  
779 turned towards the prey, the start of the tracking period was taken as the first frame in which the bearing  
780 to the target was  $< 90^\circ$ . Only tracking periods, in which the initial Euclidean distance between the mouse  
781 and cricket was  $> 20$  cm were analyzed.

782 C. The  $t_{end}$  point for the capture period was taken to be the point 6 cm away from the cricket,  
783 following which a cricket captured and consumed.

Holmgren *et al*

784 The start points of the hunt epochs were defined as follows:

785 A. The  $t_{\text{start}}$  for the detect period was the frame 500 ms prior to the detect  $t_{\text{end}}$  point.

786 B. The  $t_{\text{start}}$  for the tracking period was the first frame after the  $t_{\text{end}}$  detect frame.

787 C. The  $t_{\text{start}}$  for the capture period was either; (1) the first period in which the mouse approached  
788 the cricket and directly caught it, or (2) the first frame in which the mouse approached the cricket and all  
789 subsequent cricket escapes (prior to the final cricket capture) were less than 5cm outside the contact zone  
790 (11 cm inter-animal Euclidean distance).

791 Cases in which the eye cameras were dislodged by the animal during the chase ( $n=4$  hunt sequences) were  
792 included in the dataset up until the point where the cameras were dislodged.

793

794 *Target bearing*

795 Target bearing was defined as the angle between the cricket position and the mouse's forward head  
796 direction in the horizontal plane.

797

798 *Digital reconstruction of arena*

799 For the digital reconstruction, the company 3dScanlab (Cologne, Germany) was engaged to create a  
800 complete scan, photo series and 3D mesh model of the arena and room, which they performed using an  
801 RTC 360 3D laser scanner (Leica, Germany). The 3D point cloud produced by the laser scanner was  
802 converted to a 3D mesh model, to which textures of the experiment arena obtained from photographs  
803 (Nikon D810, 36 mpx) were baked.

804 The camera tracking coordinate system, in which the mouse and cricket positions were tracked, and the  
805 scanned coordinate system of the 3D mesh model were aligned based on 16 fiducial points which could

Holmgren *et al*

806 be clearly identified in both tracking camera images and the scan. Crickets were modelled as 2cm  
807 diameter, 1 cm thick disks centered on their tracked position with the disk's axis oriented parallel to  
808 gravity.

809

#### 810 *Generation of animal's eye view*

811 Each eye was modelled as a hemisphere with a 180° field of view whose equator was perpendicular to the  
812 animal's gaze vector. For the projection of the environment onto the cornea, frame-wise animal's eye  
813 views for both eyes were created with custom written software in C++ (g++ 7.5.0, QMake 3.1, Qt 5.9.5,  
814 libopenexr 2.2.0, libpng 1.6.34 and OpenGL-core-profile 4.6.0) on a GeForce RTX 2070 (NVIDIA driver  
815 450.66), using first cube mapping followed by a transformation into a spherical coordinate system. To do  
816 this, individual frame-wise coordinate transformations were made using the eye locations and  
817 orientations determined as described above to transform the mesh model of the arena and cricket to a  
818 static eye coordinate system using custom written vertex shaders to perform the coordinate  
819 transformation and the fragment shaders to texture the mesh. A cube-map (1024 x 1024 pixels per face)  
820 was created by performing such coordinate transformations for a 90 degree view in the direction of the  
821 optical axis of the eye and four mutually orthogonal directions. Custom written code was then used to  
822 transform the cube-map into a spherical coordinate system, with a 180 degree opening angle, using vertex  
823 shaders, resulting in a 1024 x 1024 pixel frame exported as png and OpenEXR files. In addition to the color  
824 map, maps of depth (pixel-wise object intersection distance), object identification and 3D position of the  
825 object intersection point in the contralateral eye's coordinate system were also generated.

826

#### 827 *Prey image probability density maps*

Holmgren *et al*

828 For generation of the prey image probability density maps, animal's eye views were rendered that  
829 contained the cricket only (i.e. without inclusion of arena and room). Density maps from multiple detect-  
830 track sequences, and multiple animals, were made by averaging.

831

### 832 *Ocular Alignment*

833 Ocular alignment was defined as the consistency of the projection of a given point in the eye view of one  
834 eye into the other in an infinitely distant environment. This is equivalent to a projection in an idealized  
835 finite-distant spherical environment while assuming a distance between the animal's eyes of 0. For  
836 calculation, the radius of the sphere can then be set to 1 (without loss of generality). A point, located at  
837 the center of mass of the functional focus in each eye, was chosen from which to calculate the degree of  
838 inter-ocular alignment. This point was projected from one eye to the sphere surface and into the  
839 contralateral eye. The degree of alignment between the two eyes was calculated as follows:

840 Let

$$841 \quad R_i, L_i: \mathbb{R}^3 \rightarrow \mathbb{R}^3$$

842 be the affine transformations for the left and right eye, and let

843

$$844 \quad E \subseteq \mathbb{R}^3$$

845 be the idealized environment. For a given direction  $u \in S^2$  we calculate the projection into the right eye

846  $p_i \in \mathbb{R}^3$  by:

$$847 \quad p_i = L_i^{-1} \cdot R_i \cdot u$$

848 The average alignment is then calculated using the formula:



Holmgren *et al*

849 
$$\bar{\Sigma} 2 \cdot \arcsin(|p_i - \langle \bar{\Sigma} p_i \rangle|/2)$$

850 where  $\bar{\Sigma} p_i$  denotes mean and  $\langle \rangle$  denotes normalization.

851

852 *Visual field overlap*

853 Visual field overlap was analyzed in the idealized finite-distant spherical environment described above for  
854 ocular alignment. Visual overlap was calculated from the frame-wise maps of 3D object intersection points  
855 in the contralateral eye (see above section “*Generation of animal’s eye view*”) generated for the ocular  
856 alignment analysis: pixels whose 3D object intersection points had an angle of less than 90° to the optical  
857 axis were considered part of the overlapping field of view. Probability maps of overlap were calculated by  
858 averaging.

859 For analyses of the effect of freezing eye movements, eye rotations (horizontal, vertical and torsional)  
860 were set to the mean rotation in one eye, and the effect quantified in the other eye view.

861

862 *Optic flow*

863 To calculate the optic flow in a given pixel for a given eye, we consider the difference vector between the  
864 3D positions in the static eye coordinate system of the object intersection point for this pixel one frame  
865 before and after the frame of interest, divided by  $2 \cdot dt$  and mapped to unit distance by dividing by the  
866 distance between eye and interception point. This yields a 3D motion vector which is independent of  
867 influences of the frame rate. The spherical projection used in the rendering process described above is a  
868 non-conformal, locally non-isometric map, meaning that angles between lines and distances between  
869 points are not preserved. This makes it necessary to evaluate the flow in each point in a local, orthonormal  
870 3D coordinate system defined by the direction vector between the eye position and the object

Holmgren *et al*

871 intersection point and derivative vectors along the angular coordinates  $v\theta$  and  $v\varphi$  at that point. Thus, we  
872 define the 2D flow at a given point as the orthogonal projection of the 3D flow vector onto the local plane  
873 spanned by  $v\theta$  and  $v\varphi$ . In this study, we only use the first two components of the vector, while the third  
874 component contains the motion in radial direction to the eye.

875 In Figure 5C optic flow was calculated for the animal in the idealized spherical environment described  
876 above, meaning the animal's head was equidistant to the surrounding at all points. This simplified scene  
877 was characterized as follows. Let

878 
$$h \in \mathbb{R}^3$$

879 be the coordinate of the center of the mouse's head, then the scene around it was defined as

880 
$$\{p \in \mathbb{R}^3 \mid p_z = 0 \cup |p - h| = r\}$$

881

882 with  $r = 50$  cm. For optic flow calculations the sphere is considered fixed in global coordinates, and the  
883 flow is evaluated at the point where the mouse is in the center of the sphere translating forward at a  
884 speed of 1 cm/s.

885 In Figure 5E optic flow was calculated with the animal in the digitally reconstructed environment (see  
886 above).

887

888 *Coloring of optic flow poles in mouse corneal views*

889 The points in the scatter plot of optic flow poles in mouse corneal views were color-coded for the  
890 density of neighboring points using a two-dimensional Gaussian smoother with standard deviation

Holmgren *et al*

891 
$$\sigma = \frac{2\pi}{180}$$

892 For a given point, the density was calculated as:

893 
$$s_i = \sum_{j \in F} \frac{1}{2\pi\sigma^2} \exp\left(\frac{-|x_i - x_j|^2}{2 \cdot \sigma^2}\right) / |F|$$

894 where  $F$  is the set of all considered frame indices, and

895 
$$x_i = \frac{\partial_h [p]_i}{|\partial_h [p]_i|}$$

896 where  $\partial_h [p]_i$  is the discrete central difference quotient of the mouse's eye trajectory  $p$  in frame  $i$ , in the  
897 coordinate system of the respective eye, evaluated over  $h=4$  frames.

898

### 899 *Mouse Eye Model*

900 When constructing the eye model, we took experimentally determined values from (Barathi, Boopathi et  
901 al. 2008) (see Table 1). While we recognize that this study employed a different strain of mice to the one  
902 used here, the methodology used provides estimates of physical and optical parameters measured under  
903 conditions closest to those relevant for the current study. Further, variation of these parameters was not  
904 found to change the model to an extent that would influence the conclusions drawn from analyses  
905 involving the eye model (see below). These values distinctly define the spatial shapes and positions of the  
906 refractive components of the model eye (Figure 3A), as well as refractive indices for all but the lens,  $n_{lens}$ .  
907 We further assume a pupil radius of 594  $\mu\text{m}$ , which is the mean of constricted and dilated mouse pupil  
908 sizes from (Pennesi, Lyubarsky et al. 1998). We define the focal point of a bundle of rays as the point with  
909 minimal least squares distance to the rays. To optimize the missing refractive index  $n_{lens} : \Omega \rightarrow R^+$   
910 inside the lens body  $\Omega \subset \mathbb{R}^3$ , we first calculated two lens models and optimized them such that the focal  
911 point of 10000 rays emitted from an object at 10 cm distance on the optical axis lay on the retina. The  
912 first model, for optimization of the lens surface, was derived with optimal constant refractive index  $n_c \in$

Holmgren *et al*

913  $R^+$  over the volume. The second model, for lens gradient optimization, was derived with a smooth  
914 transition of refractive index to the anterior and posterior lens boundary, ie.  $n_b = 1.333$  on  $\partial\Omega$ . We then  
915 used Poisson's equation  $\Delta n_g = c$ , and optimized the strength of the gradient  $c \in R^+$ . We assumed the  
916 final lens model as a linear combination of these two models:

917 
$$n_{lens} = \alpha \cdot n_c + (1 - \alpha) \cdot n_g$$

918 with  $\alpha \in [0,1]$ , where we optimized  $\alpha$  as described for the above models, but from a point 10 cm away  
919 and 45° off optical axis. The derived refractive indices (Table 2) were within the range measured in (Cheng,  
920 Parreno et al. 2019).

921 To test the sensitivity of the model to changes in assumed physical parameters, we systematically changed  
922 the radius of curvatures listed in Table 1, and the thickness listed in Table 2 by 10, 50 and 100  $\mu\text{m}$  (several  
923 different values were used, to check the linearity of the dependence). We calculated the propagation of  
924 uncertainty through the eye model by analyzing the variation of radial elevation on the retina of the 45  
925 rays (above), taking the numerical differentiation of each input variable that was used in the model. Lens  
926 optimization was performed for each newly generated eye model (as described above). The maximum  
927 deviations were 0.4, 1.38, 2.76 degrees respectively for the 10, 50 and 100  $\mu\text{m}$  changes (Figure 7E), and  
928 overall none of the observed effects on the model would influence the conclusions drawn from the  
929 analyses performed using the eye model.

930

931

932 *Projection from retina to cornea*

933 The refractive elements in the rodent eye do not behave like ideally corrected optical elements, with the  
934 result that there is a distribution of incident rays with slightly varying angles of incidence on the cornea

Holmgren *et al*

935 which converge on any given point on the retina. Projection from retina to cornea therefore requires an  
936 estimate of the distribution of outside world angles of incidence for any point of interest on the retina. To  
937 do this, we used a Monte-Carlo simulation to back-trace through the optics a set of randomly chosen rays  
938 emerging from the point of interest on the retina. Since the intensity of light on a surface with an incoming  
939 angle of  $\theta$  is proportional to  $\cos(\theta)$ , this function was also chosen for the probability density distribution  
940 of ray exit angles. The rays were then traced until they either hit any opaque surface, resulting in the  
941 affected ray being discarded, or passed through the anterior cornea, in which case the ray was accepted  
942 and its angle added to the distribution of passing exit angles for the respective point on the retina.  
943 Refraction on boundary layers between different indices of refraction was performed analytically  
944 according to Snell's law. In volumes with a continuous variable refractive index (i.e. gradient-index (GRIN)  
945 optics), we used a finite-elements model. We first discretized the lens as a 40x40x40 lattice of side length  
946 2.4 mm. We then started from initial conditions where  $s(0)$  is the point of incidence and  $v(0)$  is the vector  
947 of incidence multiplied by the speed of light  $c$ . The subsequent discrete trajectory and direction of  
948 propagation is then calculated step-wise according to

$$949 \quad s(t_{i+1}) := s(t_i) + v(t_i) \cdot (t_{i+1} - t_i)$$

950

$$951 \quad \tilde{v}(t_{i+1}) := \tilde{v}(t_i) + \nabla \log n(s(t_{i+1})) \cdot (t_{i+1} - t_i)$$

952

$$953 \quad v(t_i) := \frac{\tilde{v}(t_i)}{|\tilde{v}(t_i)|^2}$$

954 The gradient is calculated in the lens lattice as the three-dimensional difference quotient, and then  
955 linearly interpolated to the exact position  $s(t_i)$  of the ray.

956

957 *Projection of retinal ganglion cell density contours onto the model eye cornea*

Holmgren *et al*

958 To determine the corneal location corresponding to the histologically identified retinal specialization in  
959 the mouse, isodensity lines were redrawn from (Drager and Olsen 1980) in Illustrator and digitized using  
960 Matlab. Isodensity lines enclosing regions containing the highest and second highest density of retinal  
961 ganglion cells, as well as the optic disc and outline of the retinal whole mount, were redrawn directly from  
962 Figure 3A in (Drager and Olsen 1981), with horizontal being taken as horizontal (nasal-temporal) in the  
963 figure. The isodensity lines were scaled to match the eye diameter used for model eye, then placed into  
964 the model eye such that the center of mass of the optic disc reconstructed with the retinal ganglion cell  
965 contours was coincident with the intersection of the optic axis and retina in the eye model (Supplementary  
966 Figure 2A-C). As the eye model was rotationally symmetrical, no further alignment between the histology  
967 and eye model was necessary. The high retinal ganglion cell density regions were then back-projected  
968 from retina to cornea as described above (Supplementary Figure 2D-E).

969

970 *Eye in head coordinates*

971 To quantify the effect of head rotations on VOR evoked eye movements in a common coordinate system,  
972 head rotations were normalized such that the average pitch and roll were 0. Axes were labeled X and Y  
973 respectively and eye rotations were represented using this horizon-aligned X-Y coordinate system.  
974 Positive head X values indicate head pitched up, while negative head X values indicate head pitched down.  
975 Negative head Y values indicate roll left, while positive Y values indicate roll right. Comparisons of the  
976 relationship between head and eye rotations were carried out using differential rotations between frame  
977 and average pose, defined in the following way:

978  $l': L \rightarrow G, r': R \rightarrow G, h: H \rightarrow G$  are the affine transformations between Cartesian global coordinate system  
979 G, head-fixed coordinate system H and left/right-eye coordinate systems L/R.

980

Holmgren *et al*

981 The transformations from L/R respectively to H are:

982 
$$l = h^{-1} \cdot l'$$

983 
$$r = h^{-1} \cdot r'$$

984 We calculate the left and right eye differential rotations as:

985 
$$l_{delta} = l \cdot \bar{l}^{-1}$$

986 
$$r_{delta} = r \cdot \bar{r}^{-1}$$

987 where  $\bar{l}$  and  $\bar{r}$  denote the average transformations over all frames (chordal L2 mean, implementation  
988 from SciPy 1.4.1).

989

### 990 *Statistical Analysis*

991 Within one experimental trial, the experimentally measured variables of interest are highly correlated  
992 with each other. This fact prevents us from using standard statistical tests on the whole time-trace to  
993 establish if any difference we observed in the data across different experimental conditions are significant  
994 or not, as one requirement of these kind of tests is that the samples from the populations being compared  
995 are independent of each other. However, we realized that trial-to-trial variability is the dominant source  
996 of variability in the data, whereas within-trial variability explains a smaller fraction of the total variance  
997 observed (a more detailed report is found in Table 4). For this reason, we decided to represent each  
998 temporal trace by its median value. We used the median and not the mean, because the former is more  
999 resistant to the presence of outliers and it is better suited to represent the "average" value of a variable  
1000 in this context. This operation reduced the size of the dataset to one data points per trial, which we can  
1001 reasonably assume to be independent of each other.

1002

Holmgren *et al*

1003 **Movie 1.** Digitized and rendered view of the experiment arena and surrounding environment. Laser  
1004 scanned and digitally reconstructed experiment environment, providing distance and positional  
1005 information of objects within the mouse's environment. When combined with the tracked 3D cricket  
1006 positions and the tracked mouse head and eye positions and rotations this allowed the generation of a  
1007 frame-by-frame mouse eye view of the prey and the surroundings.  
1008

1009 **Movie 2.** Mouse eye views during cricket detection and tracking. Upper panels: Digitally rendered mouse  
1010 left and right eye view's of its prey (cricket - red) and the surrounding environment during prey detection,  
1011 tracking and prey escape. Lower panel: recorded pursuit sequence. Green points indicate the tracked  
1012 cricket body center. Note the transition from a peripheral monocular to a binocular lower nasal location  
1013 within the visual fields. Note also the large overhead visual field.

1014



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Holmgren *et al*

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Holmgren *et al*

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Holmgren *et al*

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Holmgren *et al*

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Holmgren *et al*

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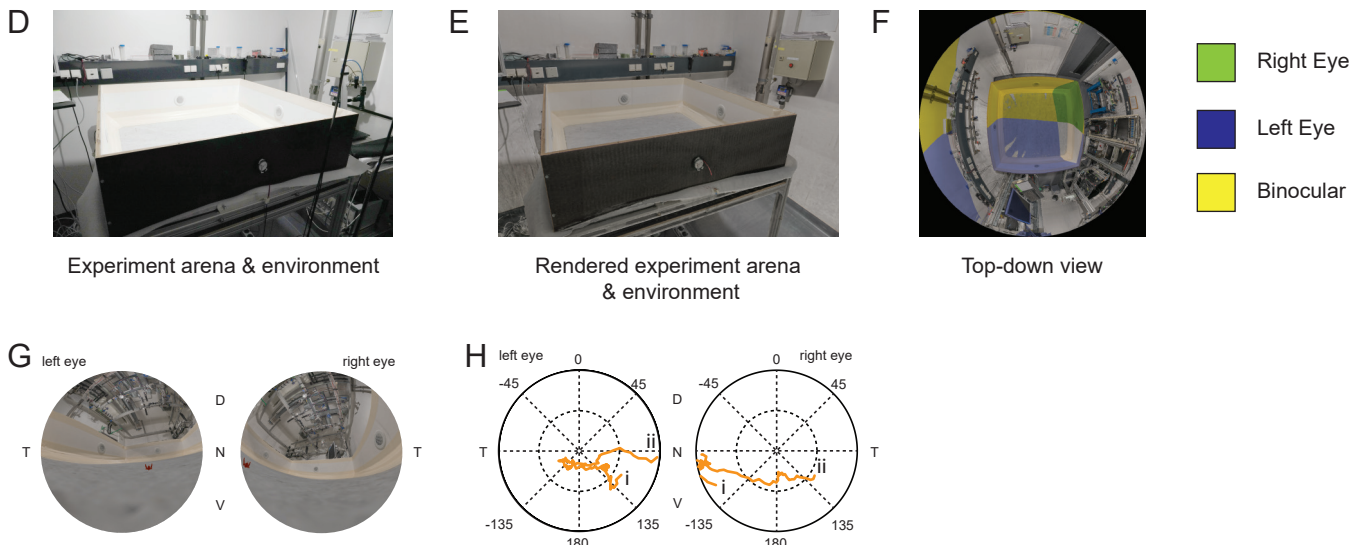
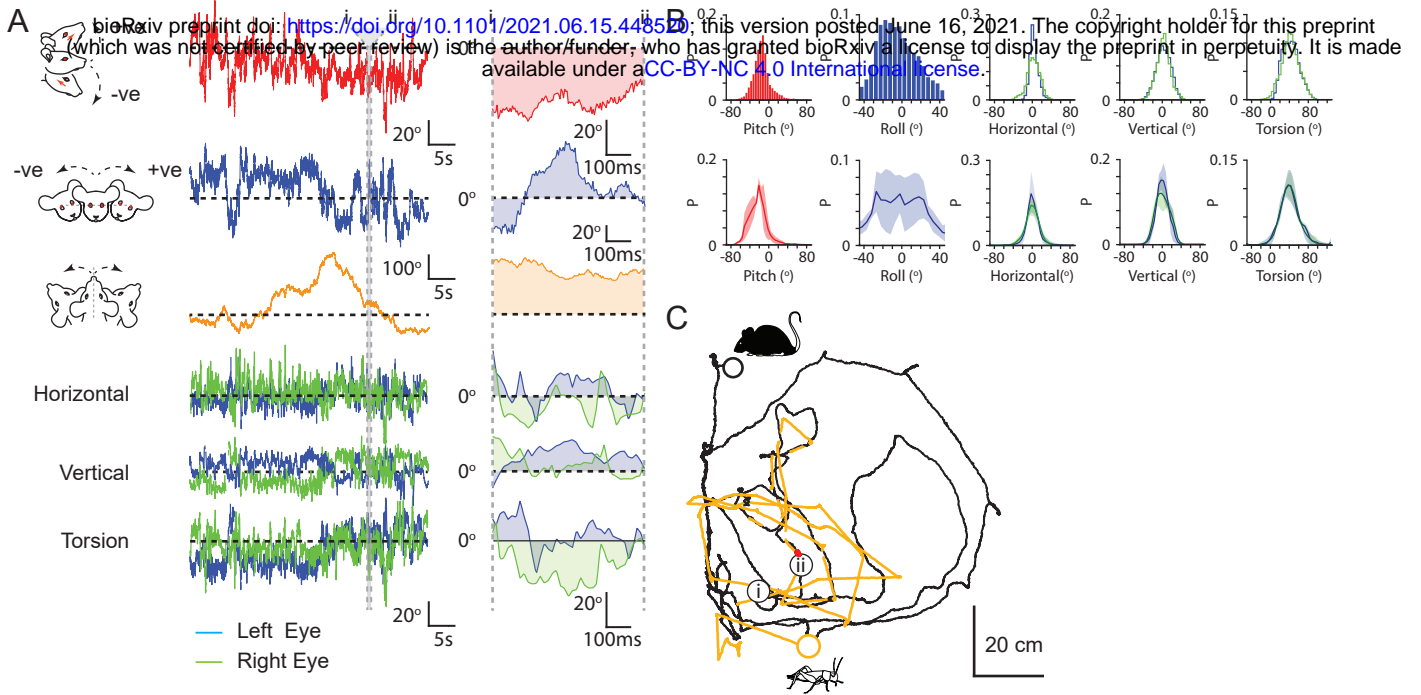
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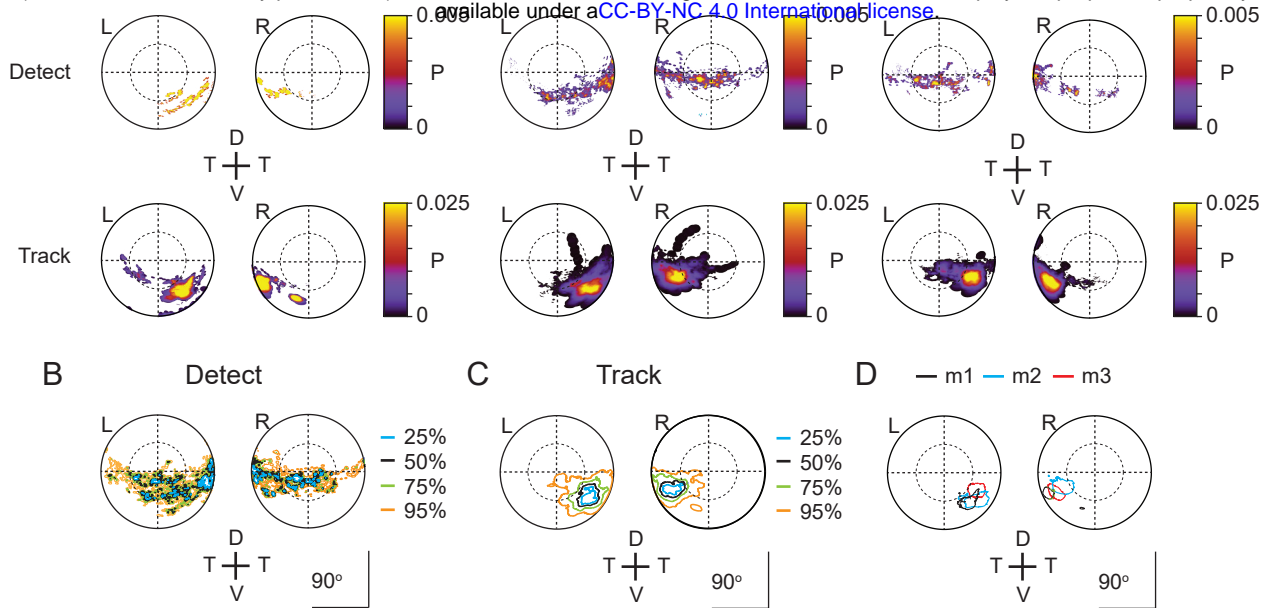


## Figure 1 – figure supplement 1

### Generation of mouse eye views during cricket pursuit.

(A) Head pitch (red), roll (blue) and yaw (orange) and associated left (blue) and right (green) horizontal, vertical and torsional eye movements during the 46.2s, example cricket pursuit sequence shown in C. (Right) Head and eye rotations during the 0.65s region between i and ii in the cricket pursuit sequence in C. (B) Example (upper rows) head pitch (547118 frames), roll (547118 frames), and horizontal (612161 frames), vertical (547118 frames) and torsional (612161 frames), eye rotations ( $n=1$  animal). Lower rows: head and eye rotations from 3 mice. Data for B (lower), from 1436204 frames, from 3 animals. (C) Mouse (black) and cricket (orange) paths during a 46.2s segment of a single pursuit sequence for one animal. (D) Photograph of experiment arena and surrounding environment. (E) Digital rendering of the same experiment arena and surrounding environment. (F) Top-down view of the mouse's left and right monocular and binocular fields of view (mouse's head would be centered at the intersection point of monocular and binocular fields of view). (G) Cricket (red) position in the rendered left and right eye corneal fields of view of the experiment arena and surrounding environment during the pursuit sequence in C. (H) Trajectory of the projected cricket position in the left and right corneal views, during the pursuit sequence in C.

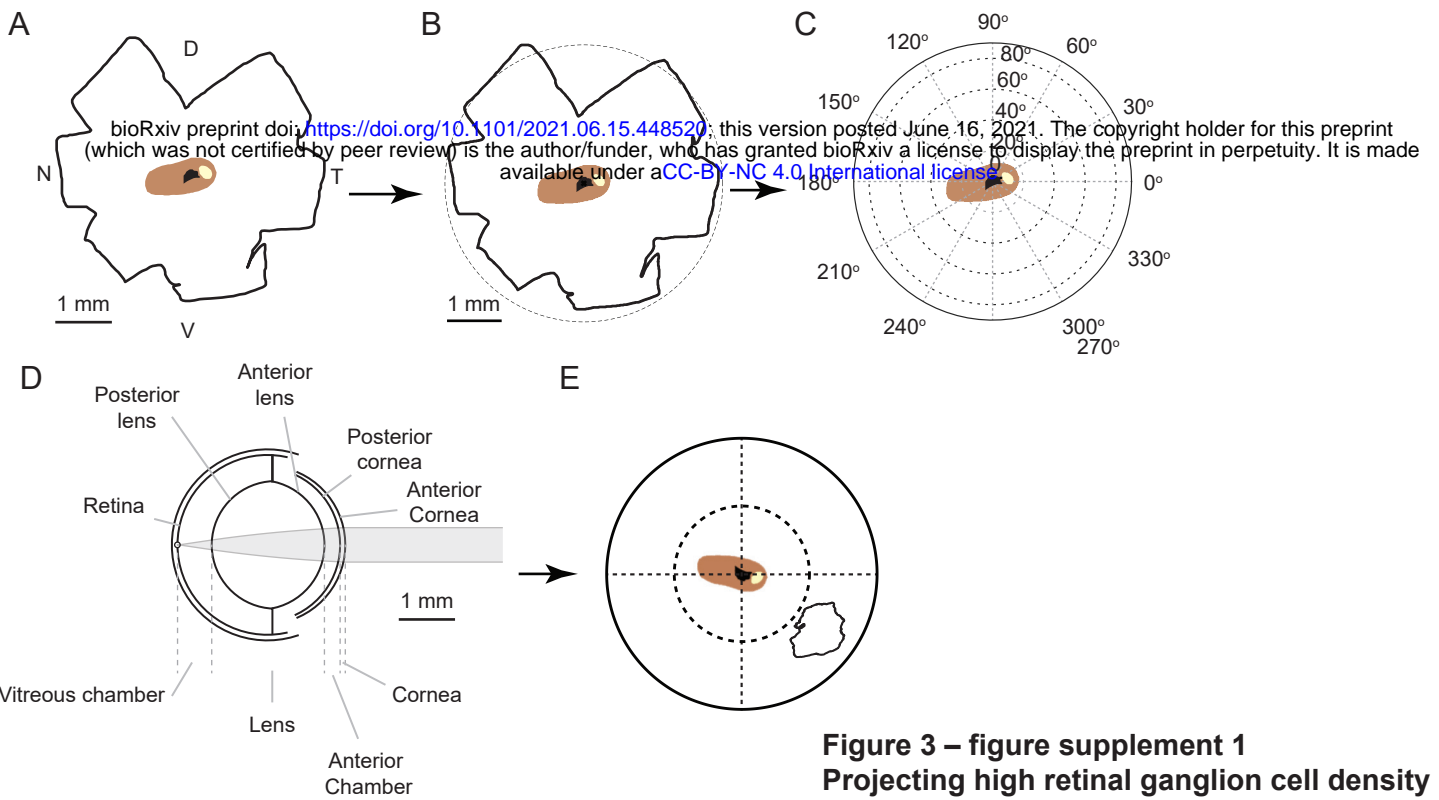




**Figure 2 – figure supplement 1.**

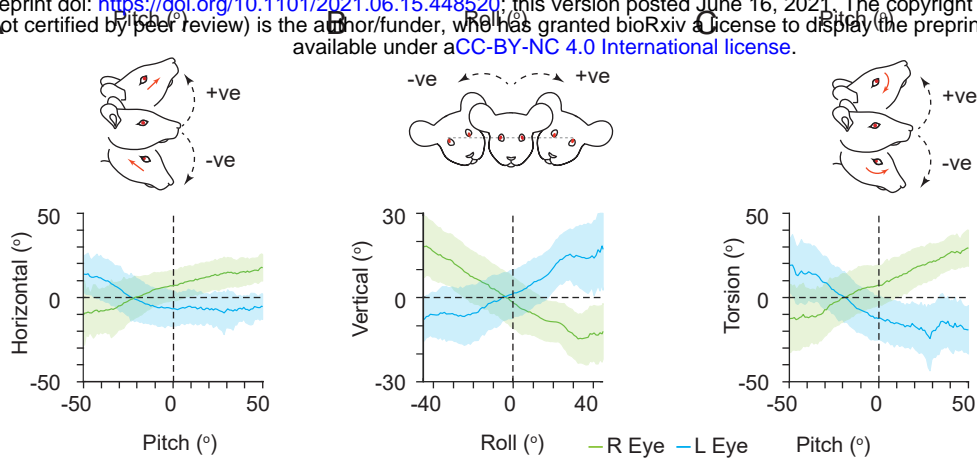
**Individual corneal prey image heatmaps.**

(A) Probability density maps for detect (upper row) and track (lower row) epochs for each of the three animals individually. Data from 4 detect and 5 track sequences, 27 detect and 28 track sequences and 17 detect and 19 track sequences for mouse 1, 2 and 3 respectively. (B) Isodensity contours calculated from the average probability density maps for all detect epochs from all 3 animals. (C) Isodensity contours for all track epochs from all 3 animals. (D) 50% isodensity contour (defined as in Figure 2H) during track epochs for each of the three mice (m1-m3) individually.



**Figure 3 – figure supplement 1**  
**Projecting high retinal ganglion cell density region from retina to cornea.**

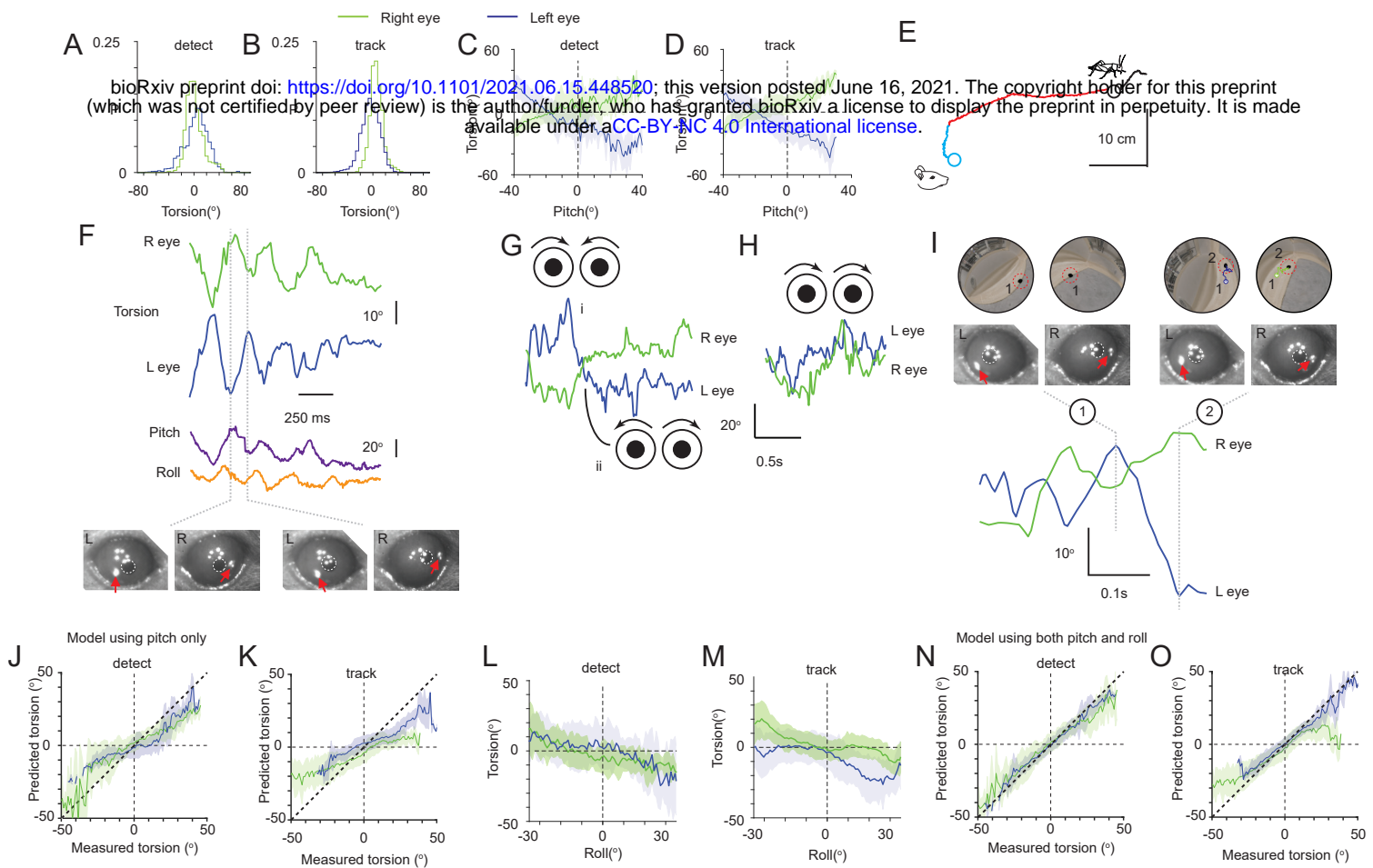
**(A)** Retinal whole mount redrawn from (Dräger and Olsen 1981) including whole mount outline (black), and outlines of the optic disc (black) and highest (>8000 cells/mm<sup>2</sup>, beige) and second highest (>7000 cells/mm<sup>2</sup>, brown) retinal ganglion cell density isodensity lines. **(B)** Overlay of the redrawn retinal whole mount from A and a representation of the mouse eye equatorial diameter (dashed) from (Tkachenko 2010). The center of the equatorial diameter was overlaid with the center of mass of the outline of the optic disc of the redrawn whole mount (black cross). Color coding as in A. **(C)** Retinal isodensity lines represented in spherical coordinates. Color coding as in A. **(D)** Schematic of mouse eye model (from Figure 3A). **(E)** Regions within the isodensity contours from A and the 50% isodensity contour from the track epochs from Figure 2H projected through the mouse eye model into the corneal view from the left eye (from Figure 3B). **(F)** Top-down view of the coverage region for the left eye of the 50% isodensity contour (blue) and second highest RGC region (brown). Bars represent the probability density function for the respective regions at that azimuth angle. Mouse's forward direction directed to 0°, and mouse's right directed to 90°. **(G)** Top-down view of the coverage region for the right eye of the 50% isodensity contour (green) and second highest RGC region (brown). Conventions as in F. **(H & I)** left and **(J & K)** right eye corneal views, showing the effect on the orientation and location of RGC regions and isodensity contours of ± 25° torsional offsets. Original position of RGC region, beige; position after offset brown; color-coding of isodensity contours as in Figure 2H.



#### Figure 4 – figure supplement 1

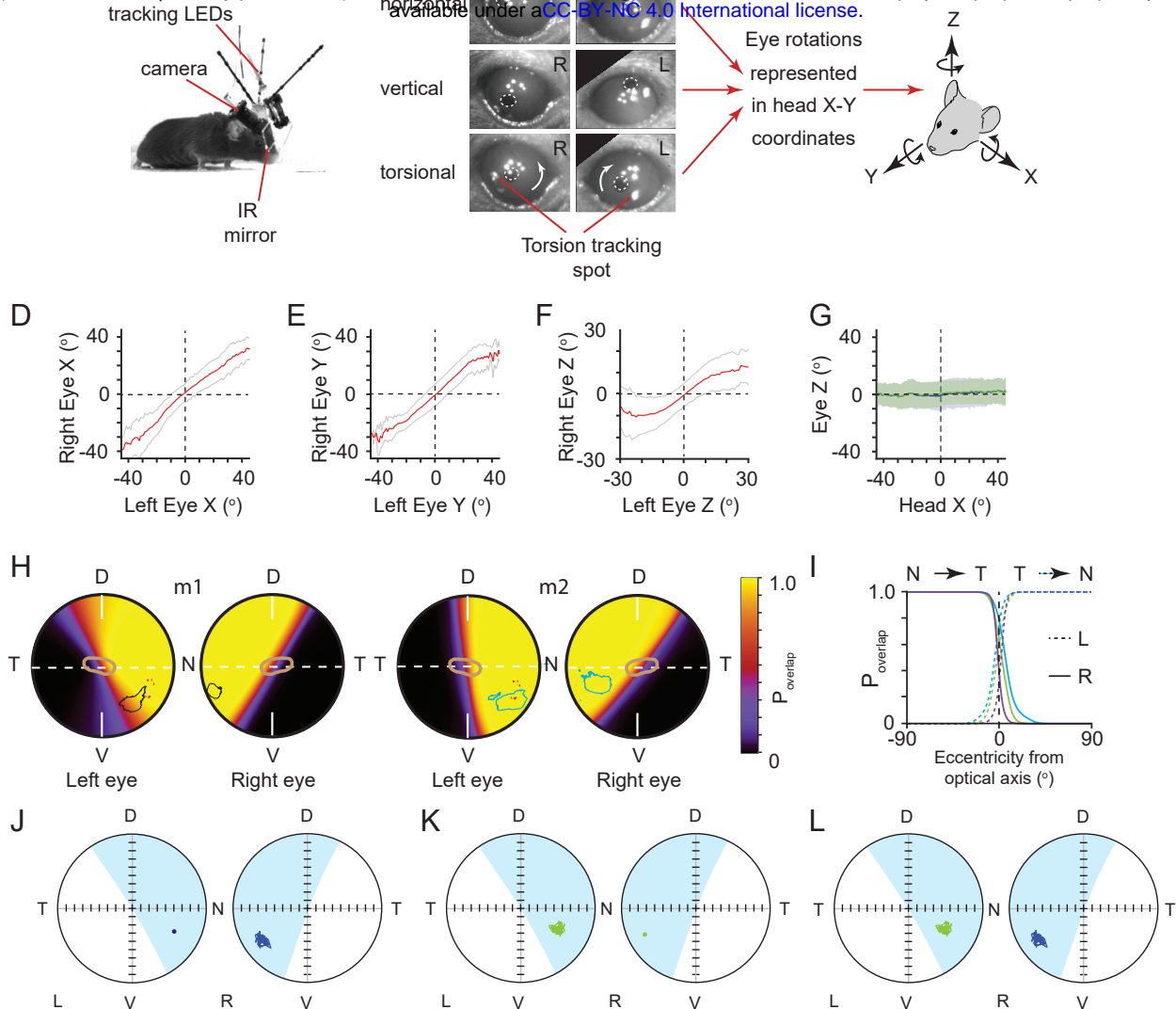
#### VOR relationships between head and eye rotations and alignment of left and right eyes.

**(A)** Relationship between mouse head pitch and horizontal eye rotations (left eye, blue; right eye, green; mean $\pm$ SD). **(B)** Relationship between head roll and vertical eye rotations. Plot conventions as in A. **(C)** Relationship between head pitch and torsional eye rotations. Plot conventions as in A. Data for A-C, from 1436204 frames, from 3 animals.



## Figure 4 – figure supplement 2 Ocular torsion during cricket pursuit.

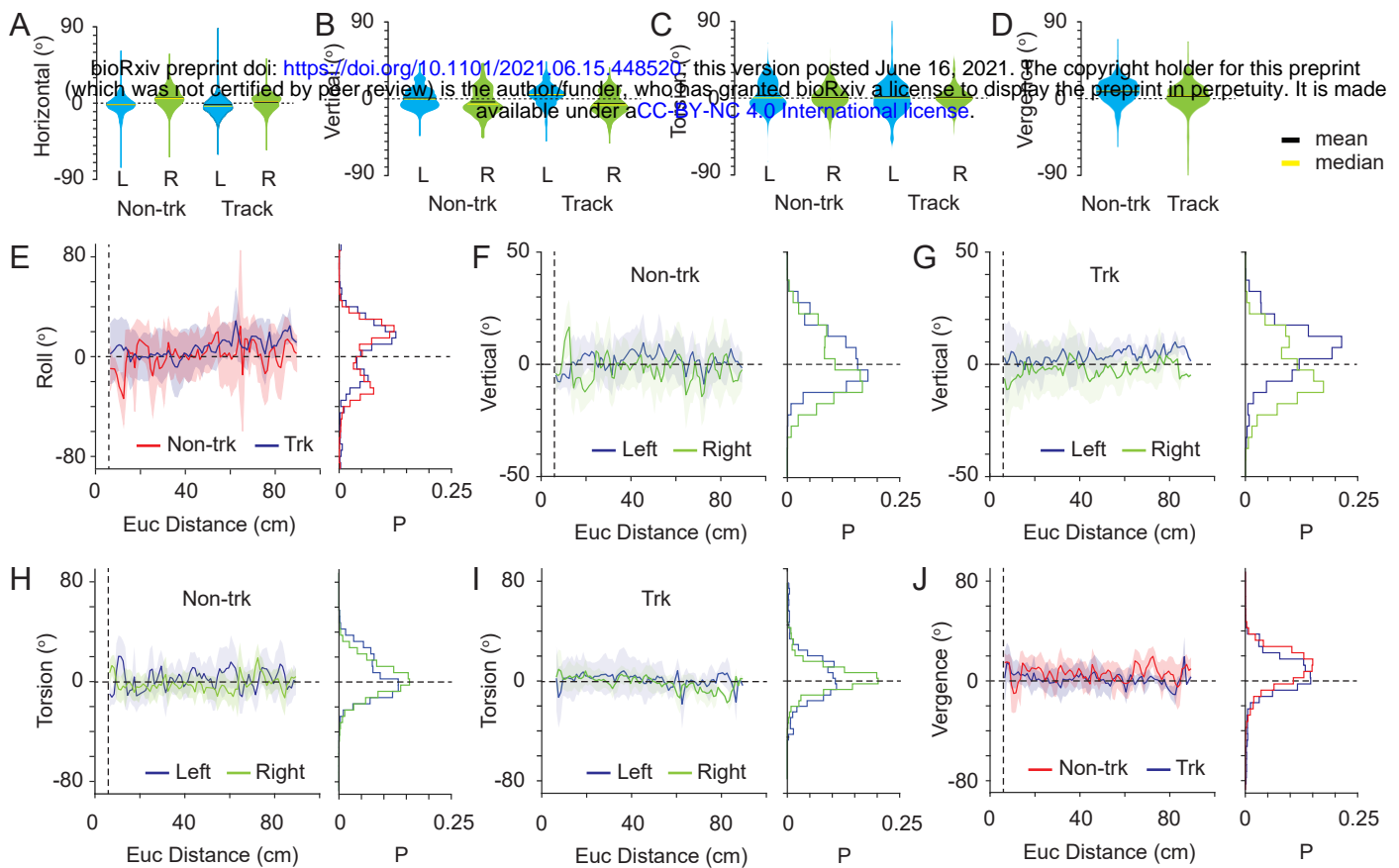
**(A)** Distribution of left (blue) and right (green) eye torsional rotations during detect epochs. Data from 57 epochs (4406 frames) from 3 animals. **(B)** Distribution of ocular torsion during track epochs. Conventions as in A. Data from 65 epochs (13624 frames) from 3 animals. **(C)** Average relationship (mean $\pm$ SD) between head pitch and torsional eye rotations during detect epochs for left (blue) and right (green) eyes. Data from 57 epochs (4406 frames) from 3 animals. **(D)** Average head pitch and torsional eye rotations relationships during track epochs. Conventions as in C. Data from 65 epochs (13624 frames) from 3 animals. **(E)** Mouse (detect epoch, blue; track epoch, red) and cricket (black) trajectories during one example pursuit sequence. **(F)** Torsional rotations of the left (blue) and right (green) eyes, and head pitch (purple) and roll (orange), during the pursuit sequence in E. Lower panels show example eye images from the indicated time points in the kinetic traces. Red arrows indicate tracked TiO<sub>2</sub> spots. **(G)** Example sequences showing torsional rotation kinetic traces for left (blue) and right (green) eyes during in- (i) and excyclovergence (ii) from one pursuit sequence. Schematics show the ocular rotations in the left and right eyes. **(H)** Example sequence showing dextrocyclovergence in one pursuit sequence. Conventions as in G. **(I)** Example of the effect of torsional rotations on prey image location. Corneal eye views of the cricket (black ellipse in red dashed circle) and arena (upper) and associated eye images (middle) at the time points indicated in the torsion kinetic traces (lower) for the left (blue) and right (green) eyes. Note cricket trajectories in left and right corneal eye views, which show the trajectory of the cricket in the corneal views between time points 1 and 2. Red arrows in eye images show TiO<sub>2</sub> torsion tracking spots. **(J)** Performance of a model predicting torsion based on head pitch alone for left (blue) and right (green) eyes during detect and **(K)** track epochs. **(L)** Average (mean $\pm$ SD) relationship between head roll and torsional eye rotations during detect epochs for left (blue) and right (green) eyes. Data from 57 epochs (4406 frames) from 3 animals. **(M)** Average head roll and torsional eye rotation relationship during track epochs. Conventions as in L. Data from 65 epochs (13624 frames) from 3 animals. **(N)** Performance of a model predicting torsion based on both head pitch and roll. Conventions as in J. For both J and N, data taken from 57 detect epochs (4406 frames), from 3 animals. **(O)** Performance of a model predicting torsion based on both head pitch and roll during tracking phases. For both K and O, data taken from 65 prey tracking epochs (13624 frames), from 3 animals.



**Figure 4 – figure supplement 3**

**VOR relationships between head and eye rotations and alignment of left and right eyes.**

**(A)** Image of mouse with detachable miniaturized eye cameras and head position tracking system. **(B)** Example eye images showing horizontal, vertical and torsional eye rotations. Note TiO<sub>2</sub> spots on the corneal surface for tracking torsion highlighted in lower panels. **(C)** Schematic of the common head and eye rotational axes. Relationship between **(D)** left and right eye X-rotations, **(E)** Y-rotations and **(F)** Z-rotations in common rotational axes. **(G)** Relationship between head X rotations and eye Z rotations for left eye (blue) and right eye (green). Data for D-G are represented as mean±SD, and are from 154500 frames from 3 animals. **(H)** Corneal view showing probability of overlap of left and right visual fields for two example animals m1 (left, 36449 frames) and m2 (right, 50874 frames), with overlay of isodensity contours (m1 -black, m2 - blue) from functional foci (see Figure 2 – figure supplement 1D) and contour of second highest RGC region (brown) from Figure 3B. **(I)** Profile of probability of overlap for left (dotted) and right (solid) eyes as a function of angular distance from optical axis for all three animals. Profile taken from horizontal axis through optical axis as shown in Figure 4D (dotted line in 4D, N = 3 animals, green = 36449 frames, blue = 50874 frames, purple = 71995 frames, respectively). **(J)** example of ocular alignment for the reference spot in the left eye projected into the right eye. **(K)** reference spot in the right eye projected into the left eye. **(L)** alignment over time for both reference spots.



**Figure 5 – figure supplement 1.**

**Eye movements during non-tracking and tracking periods.**

**(A)** Violin plots showing the variability in horizontal eye rotations for left (blue) and right (green) eyes during non-tracking (Non-trk) and track (Track) epochs. **(B)** Variability in vertical eye rotations during non-tracking and track epochs. Conventions as in A. **(C)** Variability in torsional eye rotations during non-tracking and track epochs. Conventions as in A. **(D)** Variability in ocular vergence during non-tracking and track epochs. Conventions as in A. **(E)** Average relationship (mean±SD) between head roll and Euclidean distance from mouse to cricket during non-track (red) and track (blue) epochs. Data histogram shown at right. **(F)** Average relationship (mean±SD) between vertical eye rotations of left (blue) and right (green) eyes and Euclidean distance between mouse and cricket during non-track epochs. Data histogram shown at right. **(G)** Average relationship between vertical eye rotations and mouse-cricket Euclidean distance during track epochs. Conventions as in F. **(H)** Average relationship between torsional eye rotations and mouse-cricket Euclidean distance during non-track epochs. Conventions as in F. **(I)** Average relationship between torsional eye rotations and mouse-cricket Euclidean distance during non-track epochs. Conventions as in F. **(J)** Average relationship between ocular vergence and mouse-cricket Euclidean distance during non-tracking and tracking epochs. Conventions as in E. For all panels, data taken from 18 non-track epochs (15649 frames) and 18 track epochs (8510 frames), from 3 animals.