

Fig. S1. Superimposition of ants' paths during training conditions and tests.

All paths in training trials just before a test and in tests 1-3 are shown superimposed without any filtering for consistency (See 'Exclusion of inconsistent ants' in Methods). **A:** paths recorded in pre-test training trials **B:** Paths in test 1 with bar shifted away from food. **C:** Paths in test 2 with bar shifted towards the food. **D:** Paths in test 3 with bar removed. In each panel, A gives the number of ants and P the number of paths.

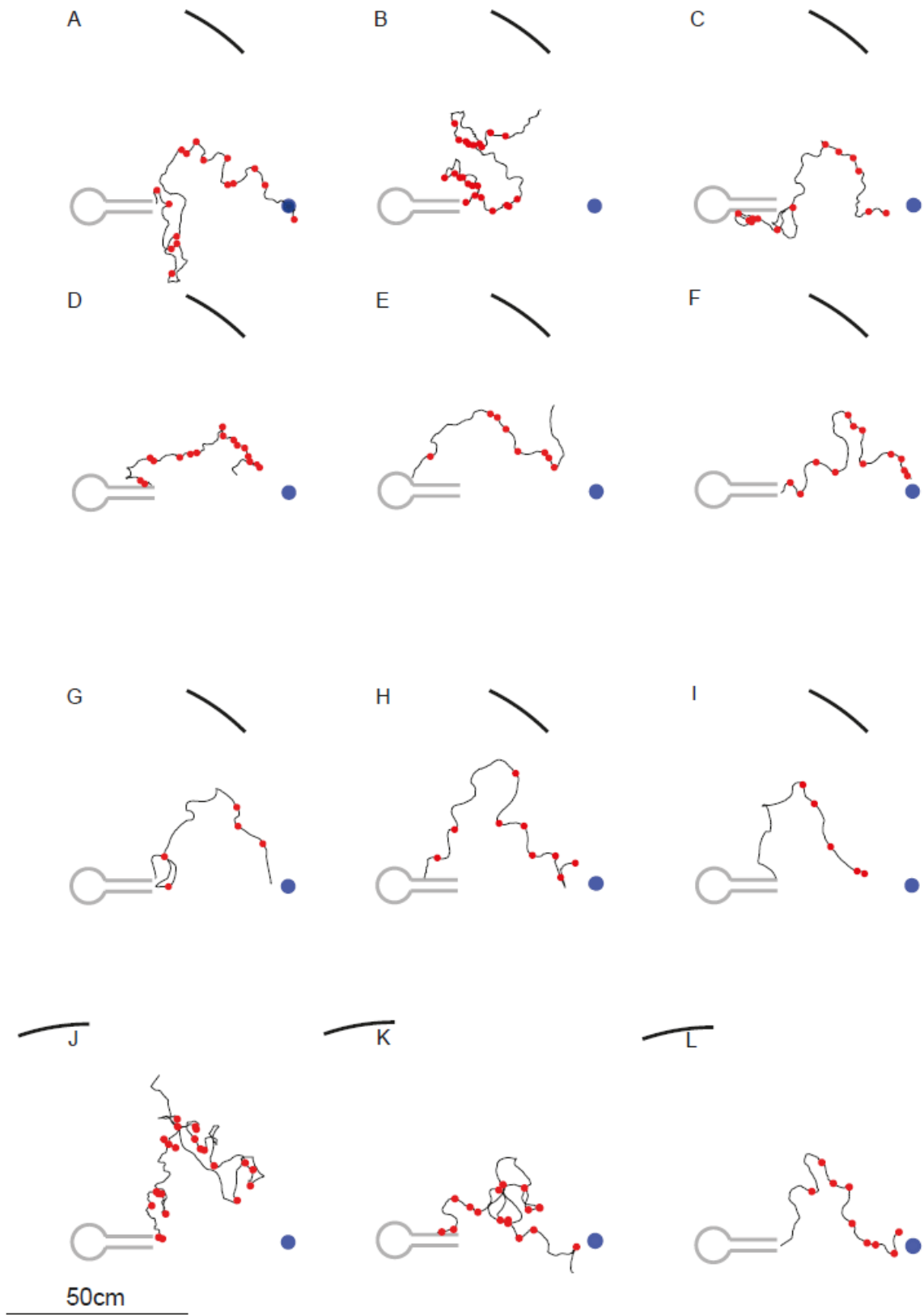


Fig. S2. Indications that ants can be guided by path integration to the food position.

A-L: Example paths in which ants turn to approach the food from directions other than the trained route. Path segments in which the ant's facing direction is within $\pm 10^\circ$ of the feeder position are marked with red dots. Suggestive evidence for a food vector monitored through PI comes from examples of ants that leave the direct route to the goal and turn and travel towards the food when they are some distance from the trained path. Deviations from the trained path happen when ants head towards the bar. These deviant paths can be interrupted by the ant turning towards the goal and travelling in its direction. When the distance towards the goal is longer and is not just a brief interruption to a path elsewhere (examples A and H), facing the goal tends to occur at the peaks and troughs of a zigzag approach, as it does in binocular ants (Lent et al., 2013). Panels A-I come from training trials with a drop of sucrose at the goal. Could the ants obtain guidance cues from the sucrose, itself? During experiments over many years in this set up, ants show no signs of detecting the food until they have almost reached it. These concerns do not arise during tests in which food is always absent (see panels J-L). During all tracks shown, the bar remains within the field of the capped left eye while the ant turns and moves towards the food.

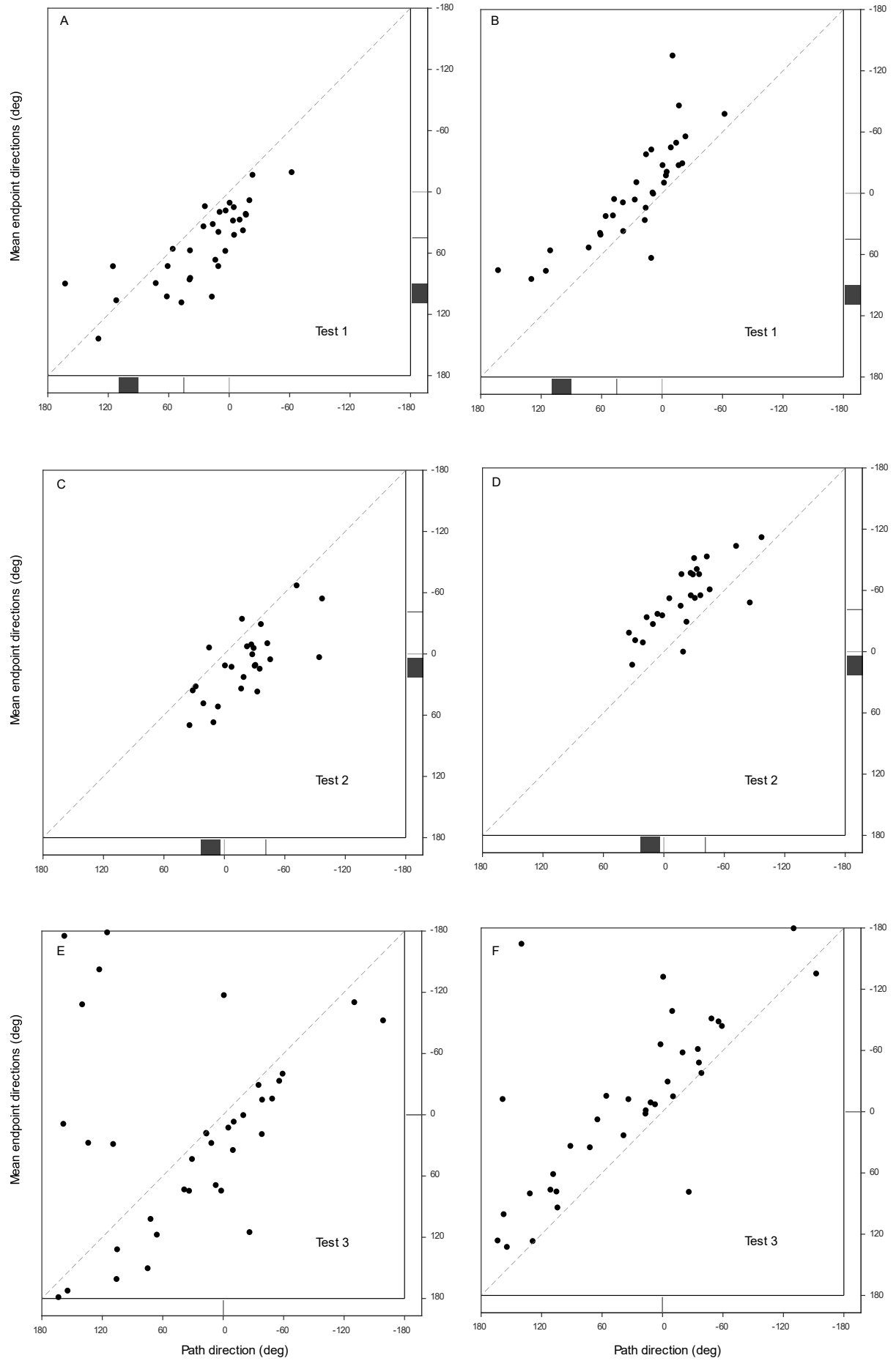


Fig. S3. Correlations between the mean of all left or right endpoints made by each one-eyed ant and that ant's mean path direction during tests 1 to 3.

A, B: Test 1. A: Left turns: mean discrepancy between path heading and endpoint: 25.7° (CI: 16.8° to 25.5°; Likelihood ratio test: $\chi^2_1 = 18.8$, $P < 0.001$; circular correlation coefficient: $\rho = 0.82$, $P < 0.001$); B: right turns: mean discrepancy between path heading and endpoint: -28.0° (CI: -37.2° to -19.0°; Likelihood ratio test: $\chi^2_1 = 22.2$, $P < 0.001$; circular correlation coefficient: $\rho = 0.87$, $P < 0.001$). **C, D:** Test 2. C: Left turns: mean discrepancy between path heading and endpoint: 29.6° (CI: 19.7° to 39.1°; Likelihood ratio test: $\chi^2_1 = 22.4$, $P < 0.001$; circular correlation coefficient: $\rho = 0.70$, $P = 0.006$); D: right turns: mean discrepancy between path heading and endpoint: -32.6° (CI: -40.4° to -24.1°; Likelihood ratio test: $\chi^2_1 = 30.9$, $P < 0.001$; circular correlation coefficient: $\rho = 0.77$, $P = 0.002$). **E, F:** Test 3. E: Left turns: mean discrepancy between path heading and endpoint: 34.2° (CI: 20.8° to 47.5°; Likelihood ratio test: $\chi^2_1 = 12.4$, $P < 0.001$; circular correlation coefficient: $\rho = 0.55$, $P = 0.002$); F: right turns: mean discrepancy between path heading and endpoint: -32.3° (CI: -43.6° to -21.6°; Likelihood ratio test: $\chi^2_1 = 15.8$, $P < 0.001$; circular correlation coefficient: $\rho = 0.77$, $P < 0.001$). Conventions as in Fig. 4 B, C.

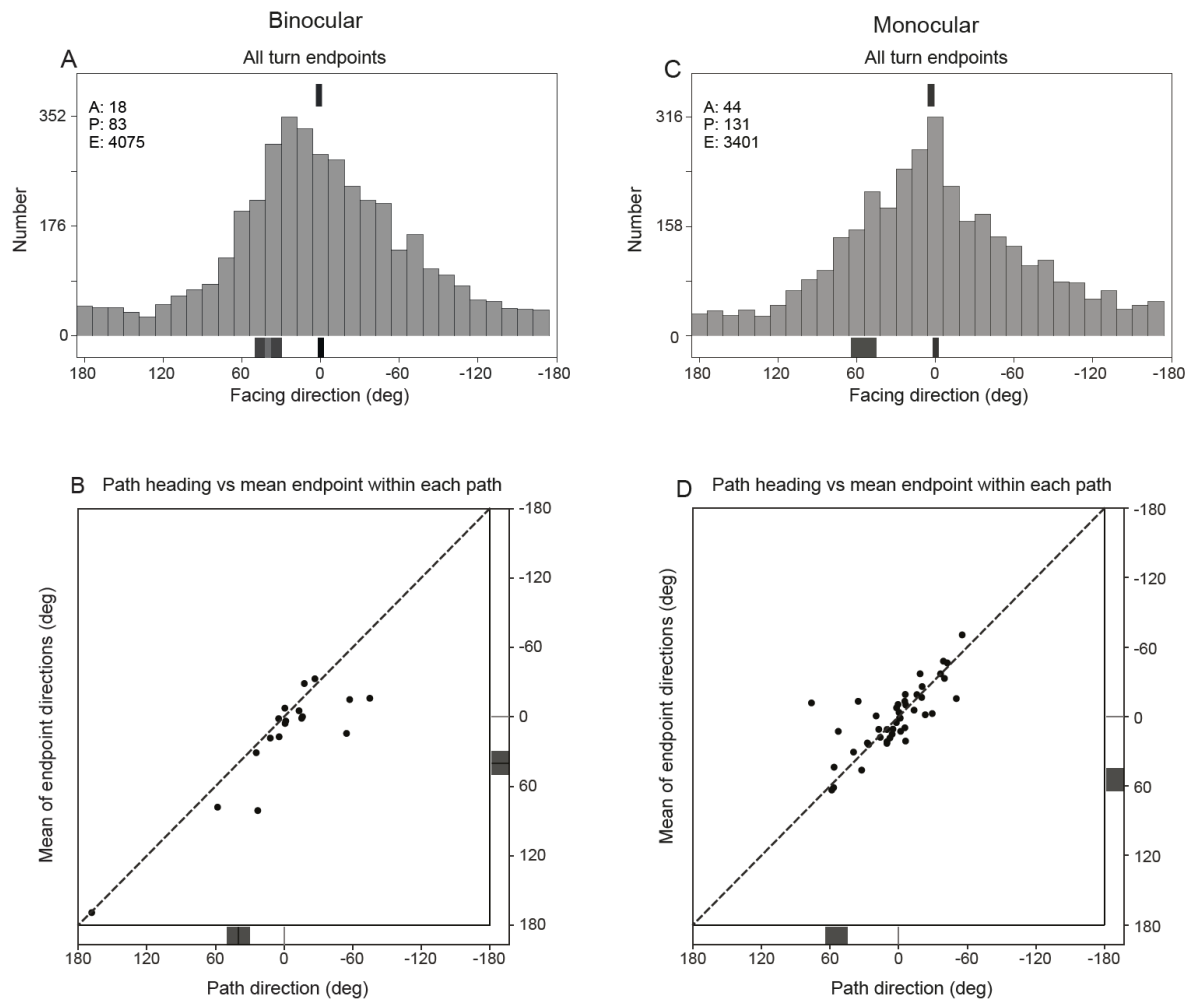


Fig. S4. Comparison between monocular and binocular ants of the endpoints of left and right turns combined during pre-test training.

A: Facing directions of binocular ants. Mean of the mean of all endpoints made by each ant does not differ from 0° (mean: 8.1° , CI = -6.6° to 24.4° ; Likelihood ratio test: $\chi^2_1 = 0.59$, $P = 0.444$). **B:** Correlation between the mean of all endpoints made by each binocular ant and that ant's mean path heading (circular correlation coefficient: $\rho = 0.71$, $P = 0.026$). Mean endpoints were to the left of path headings (mean discrepancy = 16.0° , 95% CI = 5.6° to 27.1° ; Likelihood ratio test: $\chi^2_1 = 6.41$, $P = 0.011$). **C:** Facing directions of monocular ants. Mean of the mean of all endpoints made by each ant does not differ from 0° (mean 0.99° (CI= -7.3° to 8.8° ; Likelihood ratio test: $\chi^2_1 = 0.81$, $P = 0.814$). **D:** Correlation between the mean of all endpoints made by each monocular ant and that ant's mean path heading (circular correlation coefficient: $\rho = 0.77$, $P < 0.001$). The mean discrepancy between endpoints and path headings is consistent with zero (mean discrepancy = 1.4° , 95% CI = -6.7° to 3.8° , Likelihood ratio test: $\chi^2_1 = 0.20$, $P = 0.652$). Details as in Fig. 4.

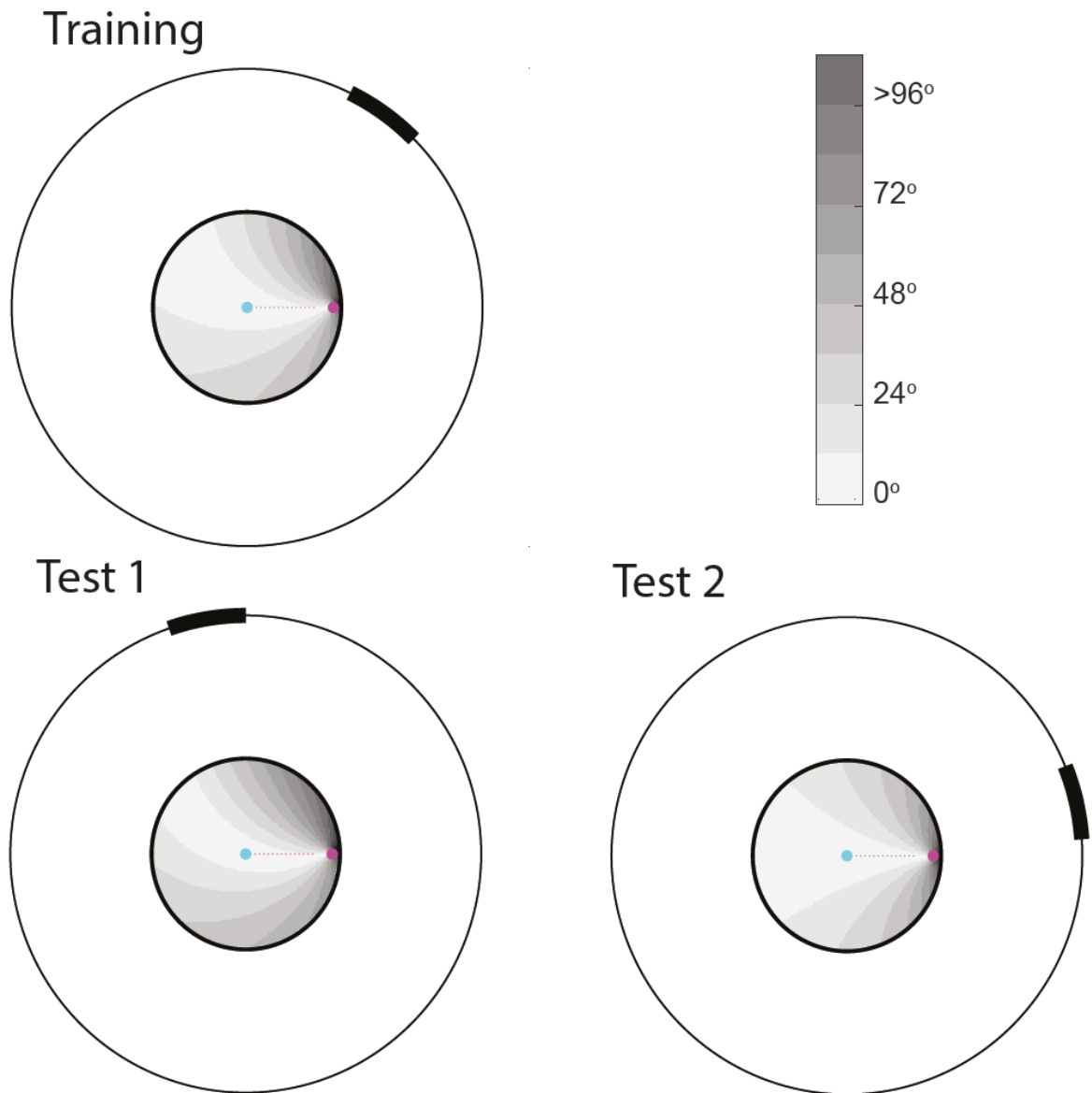


Fig. S5. Difference between the position of bar on retina of an ant in the centre of the arena and in other locations within the arena.

Outer ring: 180 cm diameter cylinder with vertical bar; inner ring 60 cm diameter arena. Differences are shown in 12° steps shown in scale bar. Differences are small within the arena in which ants follow routes (Figure S1).