1 Neural and sensory basis of homing behavior in the
2 invasive cane toad, *Rhinella marina*

3

4 Daniel A. Shaykevich[1]*, Daniela Pareja-Mejía[1,2], Chloe Golde[1], Andrius Pašukonis[3], and Lauren
5 A. O'Connell[1,4]*

6

7 1 Department of Biology, Stanford University, Stanford, CA, USA
8 2 Graduate Program in Zoology, Universidade Estadual de Santa Cruz, Bahía, Brazil
9 3 Institute of Biosciences, Vilnius University, Vilnius, Lithuania
10 4 Wu Tsai Institute for Neuroscience, Stanford University, Stanford CA, USA

11

12 Keywords: spatial cognition, navigation, amphibian, medial pallium, olfaction, magnetoreception

13

14 *To whom correspondence should be addressed:
15 Daniel Shaykevich
16 Department of Biology
17 Stanford University
18 371 Jane Stanford Way
19 Stanford, CA 94305
20 shaykeda@stanford.edu

21

22 Lauren O'Connell
23 Department of Biology
24 Stanford University
25 371 Jane Stanford Way
26 Stanford, CA 94305
27 loconnel@stanford.edu

28

29

30 **Word count:** Abstract: 195, Main text: 9116

Neural and sensory basis of homing behavior in the invasive cane toad, *Rhinella marina*

Abstract

The behavioral, sensory, and neural bases of vertebrate navigation are primarily described in mammals and birds. While many studies have explored amphibian navigation, none have characterized brain activity associated with navigation in the wild. To address this knowledge gap, we conducted a study on navigation in the cane toad, *Rhinella marina*. First, we performed a translocation experiment to describe how invasive cane toads in Hawaiʻi navigate home and observed homing following displacements of up to one kilometer. Next, we tested the effect of olfactory and magnetosensory manipulations on homing, as these senses are most commonly associated with amphibian navigation. We found that neither ablation alone prevents homing, further supporting that toad navigation is multimodal. Finally, we tested the hypothesis that the medial pallium, the amphibian homolog to the hippocampus, is involved in homing. Our comparisons of neural activity revealed evidence supporting a conservation of neural structures associated with navigation across vertebrates consistent with neural models of amphibian spatial cognition from recent laboratory studies. Our work furthers our evolutionary understanding of spatial behavior and cognition in vertebrates and lays a foundation for studying the behavioral, sensory, and neural bases of navigation in an invasive amphibian.

52 # Introduction

53   The ability to process spatial information is fundamental to survival for many animals,

54 who must navigate to locate shelter, forage, and mate. Much of our understanding of spatial

55 navigation comes from research in mammals, where the hippocampus is a key neural center for

56 spatial representations [1,2]. In mammals [3,4] and birds [5,6], the spatial function of the

57 hippocampus described in laboratory studies has been translated to its role in navigation in

58 nature. In fish [7,8], reptiles [9], and amphibians [10–13], laboratory studies suggest that neural

59 structures homologous to the hippocampus support spatial behaviors. The amphibian medial

60 pallium is homologous to the mammalian hippocampus [14,15], though with a more primitive

61 structure that may reflect the simplified architecture of ancestral tetrapods [16,17]. A series of

62 experiments in the toad *Rhinella arenarum* have implicated the medial pallium (in conjunction

63 with other brain regions) in the ability to complete a variety of spatial tasks using visual,

64 geometric, and auditory information [10–13]. However, how these findings translate to real-world

65 navigation in nature has not been explored.

66   Research across amphibians suggests a wide-spread ability to navigate to sites

67 important for reproduction. Many temperate-region amphibians migrate to breeding sites several

68 kilometers away, such as some salamanders [18–20] and pond breeding toads [21,22]. Some

69 Neotropical poison frogs (family Dendrobatidae) move with high precision within a few hundred

70 meters to transport tadpoles to water pools [23–25]. A variety of sensory modalities facilitate

71 these movements among amphibians, with olfaction [19,21,26] and magnetoreception [27–29]

72 most often implicated. Ability to navigate to home sites outside of the breeding context has also

73 been demonstrated in the cane toad, *Rhinella marina* [30]. However, the sensory modalities

74 used for navigation back to terrestrial home sites, rather than to water bodies related to

75 reproduction, has rarely been investigated.

76   The cane toad is native to Central and South America and was introduced globally,

77   including in Hawaiʻi and Australia. In Australia, the cane toad can disperse and travel long

78   distances [31,32], sometimes moving 200-meters per night to new locations [33], creating

79   difficulties for species management as the invasive front expands up to 60 km a year [32].

80   Elsewhere, cane toads rarely move more than 200 meters and often return to the same diurnal

81   shelters [30,34–36]. They do not migrate to breeding ponds like other bufonids in which

82   navigation has been studied [21,22] and their life-histories do not suggest a need for advanced

83   navigation. However, we previously showed that toads in French Guiana are able to return

84   home accurately from a full kilometer, a distance exceeding their routine movements [30].

85   These abilities and the toad's extreme prevalence make it an ideal model for studying homing

86   behavior and probing unanswered questions regarding the neural and sensory basis of homing.

87   In this study, we conduct a field experiment to examine cane toad navigational ability, as

88   well as associated sensory systems and neural activity. We hypothesized that the medial

89   pallium is involved in navigation and predicted that homing toads would have higher neural

90   activity in this brain region compared to non-homing and non-translocated toads. We next

91   hypothesized that olfaction and magnetoreception guide homing behavior, given their function in

92   other bufonids [21,22], and predicted that olfactory or magnetoreception ablations would disrupt

93   the homing ability or accuracy.

Materials and Methods

94

(a)   Study sites

95

96   Experiments occurred at four sites on the island of Oʻahu in Hawaiʻi. We sampled at two

97   sites on the south of the island (West Loch Golf Course and Hawaiʻi Prince Golf Course) and

98   two sites on the north shore (Waimea Valley and Turtle Bay Golf Course) (Figure S1). Waimea

99   Valley is forested, intercut with paved paths, and has a stream that ran approximately 2-km from

100   a waterfall at the eastern terminus of the field site to a pond at the western terminus with

101   approximately 60-m decline in elevation. All other sites are golf courses with open greens and

102   negligible changes in elevation, interrupted by ponds and forested borders. Turtle Bay was the

103   only site directly bordering the ocean and had wider forested borders than other golf courses.

104   Surface area over which tracking occurred varied between sites (Figure S1). Toads were

105   distributed throughout most of the surface area of each site, suggesting a widely suitable habitat

106   at each location.

107   We collected daily weather data from the National Oceanic and Atmospheric

108   Administration online database for every day of translocation experiments. The amount of

109   precipitation was significantly different between field sites (Kruskal-Wallis, $\chi^2_{(3)}$= 15.14, p =

110   0.0017), with the northern sites (Turtle Bay and Waimea Valley) receiving more precipitation

111   than the southern sites. Both northern sites also had lower maximum temperatures (Kruskal-

112   Wallis, $\chi^2_{(3)}$= 39, p = 1.74e-8).

113   ## (b)   Animals

114   Toads were located visually at night (sunset was approximately 19:00 and sunrise

115   approximately 06:30), as cane toads are nocturnal and generally do not exhibit diurnal

116   movements. We photographed the dorsum for re-identification, measured snout-vent-length

117   (SVL) with a tape measure, and measured mass with a portable scale (KUBEI-790, KUBEI,

118   China). Eighty-two toads (40 males, 42 females) were tagged and tracked for at least three days

119   between 18 February and 12 May 2022. Their mean mass was 233.8 g (sd +/- 98.6) and mean

120   SVL was 12.4 cm (+/- 1.6). Toad size was not evenly distributed between sites (Mass: Kruskal-

121   Wallis, $\chi^2_{(3)}$= 19.687, p = 0.0002; SVL: ANOVA, $F_{(3)}$ = 14.67 , p = 1.15e-07). Sex was

122   determined by size and male release call and confirmed by gonad identification upon dissection.

123   Females were larger than males (Mass: Wilcoxon, W = 1315.5, p = 1.05e-05; SVL: t-test, $t_{(77.41)}$

124   = 4.44, p = 2.99e-05). Unless otherwise stated, "size" refers to SVL hereafter.

125   (c)   Tagging and baseline tracking

126   Radio tagging and tracking was executed similarly to methodology we previously

127   described [30]. Radio tags (BD2, Holohil Systems Ltd, Carp, ON, Canada) were attached to

128   toads' waists using silicone tubing and thread (Figure 1A). The tagging process lasted 14.2 min

129   on average (+/- 7.8) after which toads were released at the exact point where they were

130   collected. A GPS point was taken in the ArcGIS Field Maps app (ESRI, Redlands, CA, USA)

131   using a Bad Elf GPS Pro+ gps device (Bad Elf, West Hartford, CT USA) connected by Bluetooth

132   to an iPhone (Apple, Cupertino, CA, USA). Following release, toads were located once in the

133   evening prior to their active period and at least twice each night. Toads were tracked to describe

134   their daily movements and to identify their home areas (termed 'baseline' hereafter) between

135   three and 10 days (4.978 +/- 1.48 days) prior to translocation or euthanasia. Any reference to a

136   "baseline point" indicates a coordinate point a toad occupied prior to translocation (i.e. during

137   natural movements). We removed three toads from the analysis of baseline activity because we

138   could not properly observe their movements after they entered burrows.

139   (d)   Translocation

140   Translocations generally occurred between 20:00 and 22:00. Sixty-two toads (32

141   females and 30 males) were translocated. Toads were caught, their waists examined to ensure

142   no abrasion from the tag belt and placed into an enclosed net cage and walked in an indirect

143   route for 30-60 minutes to a distance approximately 500- or 1000-meters from the point

144   recorded during the baseline tracking period that lay furthest in the direction of translocation.

145   Therefore, the minimum distance to any location of the toad observed prior to translocation

146   matched the translocation distance. Toads gathered in similar locations were translocated in

147   diverging directions as allowed by the geography of each site. A Raleigh's Test of Uniformity

148   performed on the angles of translocation indicate that they were not preferentially grouped in

149   any direction (p = 0.20). Translocation distances exceed the average movements observed in

150   cane toads in Hawai'i [36] and French Guiana [30,35] but are within the range of the maximum

151   movement distances observed, meaning we cannot be certain that all toads were moved to an

152   unfamiliar location. At the release site, the distance from the toad's nearest baseline point was

153   confirmed on a GPS-connected handheld device, the net cage was left at the release point for 5

154   minutes, and the toad was gently released from the cage.

155   Following release, toads were relocated approximately once per hour during nighttime,

156   beginning at 20:00 and continuing until tagged animals had stopped moving (06:40 at the

157   latest). On subsequent days, toads were relocated once in the early evening to determine

158   location before nocturnal movements began. If a toad was not active at night (e.g. not moving or

159   making small, multidirectional movements), the frequency of observations was reduced to every

160   2-3 hours while tracking moving toads.

161   Tracking ended once the animal reached a point within 200 meters of one of its recorded

162   baseline positions (homing, n = 33) or 72 hours had passed after translocation (non-homing, n =

163   27). Homing toads (captured within 200-meters of a baseline point) were euthanized and tissues

164   were collected for downstream analyses (described below) within 30 minutes of crossing the

165   200-meter threshold. Non-homing toads were sacrificed after 20:00 wherever they were at that

166   time. Two toads in the homing group were unintentionally caught before they crossed the 200-

167   meter threshold (B8 at 233-m and D5 at 218-m) and two more toads were caught and

168   euthanized approximately 300-m from home after encountering physical obstacles following

169   large homeward movements (C8 and F5) but were included in the homing condition when

170   considering behavior and neuronal activity. One toad moved outside of the study area and was

171   euthanized because it became impossible to track, and its behavior and brain data were not

172   included in analysis.

173   ## (e)   Sensory Ablations

174   A subset of translocated toads (n = 43; 22 females and 21 males) received

175   magnetoreception or olfactory ablations to test the roles of these sensory systems in homing.

176   Each modality had experimental (i.e. ablated) animals and control animals receiving sham

177   treatments. Toads were tracked for at least three days prior to the ablation procedure. After the

178   procedure, they were tracked for at least an additional 48 hours before translocation to evaluate

179   any effects of the ablation on space use. Olfactory ablations were performed through zinc

180   sulfate (ZnSO4) treatment, which temporarily damages the olfactory epithelium in amphibians

181   [37,38] and other vertebrates [39,40]. Magnetoreception ablations were carried out by attaching

182   a small Nickel-plated Neodymium magnet (N50, 10 mm x 6 mm x 2 mm, ~1 g; Super Magnet

183   Man, Pelham, Alabama, USA) with cyanoacrylate based adhesive (EM-150, Starbond,

184   Torrance, CA, USA) to the skin over the skulls of toads, similar to methodology performed in

185   other amphibians [21,41]. Detailed descriptions of ablation and sham procedures can be found

186   in SM1. Toads in ablation experiments were euthanized and tissues collected at the same time

187   points described above.

188   ## (f) Tissue collection

189   Translocated toads were euthanized at the time points described above. Additional

190   toads were euthanized without translocation after the completion of at least three days of

191   baseline tracking (baseline, n = 12). Toads were euthanized through MS-222 injection (0.7%

192   solution, 300 mg/kg body weight) followed by cervical transection. Brains were immediately

193   dissected, placed in 1 mL of 4% paraformaldehyde (PFA), and kept on ice until they could be

194   placed into a 4℃ refrigerator. Brains were fixed overnight in PFA, washed three times in 1x PBS

195   and then placed into 1x PBS with 0.02% sodium azide, or 1x PBS if transportation to the

196   laboratory site would happen in two days. Brains were kept in 1x PBS with sodium azide < 1

197   week. Two days prior to travel to the laboratory, the brains were transferred to 1 mL of 30%

198   sucrose dissolved in 1x PBS for cryoprotection. Once the brains sank in sucrose, they were

199   transported on ice to a laboratory at the University of Hawaiʻi at Manoa, where they were

200   embedded in Tissue-Tek OCT Compound (Electron Microscopy Sciences), frozen on dry ice,

201   and stored at -80℃. Seventy-three brains were collected (61 translocated and 12 baseline). One

202   homing toad lost its tag within 200-meters of its home area, so its brain was not collected.

203   ## (g)   Immunohistochemistry

204   Brains were sectioned on a cryostat in 20 μm slices and thaw-mounted onto slides in

205   four series. Slides dried at room temperature for ~48 hours before being transferred to -80℃ for

206   storage prior to staining. We used immunohistochemistry to detect Phospho-S6 Ribosomal

207   Protein (pS6) as a proxy for neural activity [42] as previously described by our laboratory [43,44]

208   (full protocol presented in SM2). Of the 73 collected brains, two were discarded due to poor

209   tissue quality and four were discarded for being collected at the wrong time point, leaving 67 for

210   analysis.

211   ## (h)   Microscopy and Cell Counting

212   Brains were imaged on a Leica DM6B microscope with brightfield capability at 20x

213   magnification. One hemisphere was selected to image for each brain. Nine sections along the

214   AP axis were chosen based on anatomical landmarks: three from the anterior of the

215   telencephalon (posterior to the olfactory bulb), three from the medial portion, and three from the

216   posterior of the telencephalon (anterior to the ventral hypothalamus) (Figure S2). ImageJ (NIH,

217   Bethesda, MD, USA) was used to count pS6-positive cells in each of six brain regions: medial

218   pallium (Mp), lateral pallium (Lp), dorsal pallium (Dp), medial septum (Ms), lateral septum (Ls),

219   and the striatum (Str) given their role in spatial behavior in toads [10–12] and other vertebrates

220   [45–47]. Delineation of brain regions was based on Northcutt and Kicliter (1980) and Sotelo et

221   al. (2016), which defined pallial regions by Westhoff and Roth (2002) and ventral regions by

222   Moreno and González (2004) [10,15,48,49]. ImageJ was used to calculate the surface area of

223   each region. Densities of pS6-positive neurons for each region were calculated by dividing the

224   number of pS6-positive cells by the area.

225   For toads in the olfaction ablation experiment, ImageJ was used to count active cells in a

226   300 μm by 300 μm box positioned at the lateral edge of the granule cell layer in the olfactory

227   bulb (Figure S3) to measure the effect of the olfactory ablation in comparison to the sham

228   ablation.

(i) Data analysis

(i) Toad movement data

231   GPS points were visualized in ArcGIS online and ArcGIS Pro 2 (ESRI). Spatial and

232   temporal attributes were calculated using the package 'adehabitatLT' [50] in R Studio (v

233   2024.12.0+467, Posit Software, PBC, Sunnyvale, CA) running R (v 4.4.2, R Foundation for

234   Statistical Computing, Vienna, Austria). Several descriptors of toad movement and homing

235   were calculated: cumulative movement, daily movement, movement range, straightness index,

236   homing duration/speed, active homing duration/speed, and similarity of toad's home range

237   before and after ablation treatment (details of descriptor calculations in SM3). Detailed

238   description of statistical treatment of movement data can be found in SM4.

(ii) Cell Count Data

240   Generalized linear mixed models were used ('glmmTMB' in R [51]) to test for

241   differences in brain activity and its relation with behavior, with homing condition, brain region,

242   and their interactions as the main predictors. Post hoc pairwise contrasts (between homing

243   condition groups and ablation treatments) were calculated with estimated marginal means using

244   the "emmeans" package in R [52]. Birds [53] and mammals [54] show variation in hippocampal

245   spatial activity along the anterior-posterior axis, so we also tested for differences in activity

246   along this axis in the Mp. To test if brain activity was related to either straightness or duration of

247   homing, linear mixed effects models were performed testing whether brain activity could be

248   predicted by straightness or duration. Detailed descriptions of statistical treatment of cell count

249   data can be found in SM5.

250   (j) Permits and Ethical Statement

251   All work was done in adherence to Protected Wildlife Permit WL21-16 issued by the

252   State of Hawaiʻi Department of Land and Natural Resources, Division of Forestry and Wildlife.

253   All procedures were approved by the Institutional Animal Care and Use Committee of Stanford

254   University (Protocol #33530).

255   Results

256   Toads generally exhibit site fidelity

257   Before performing translocations, we observed baseline movements and found a mean

258   movement range of 129.45 m (s.d. 140.22 m), mean cumulative movement of 547.97 m (s.d.

259   758.00), and mean daily movement of 114.23 m (s.d. 158.98). Movement range varied between

260   sites (Kruskal-Wallis, $\chi^2_{(3)}$ = 14.21, p = 0.0026), with toads at the Waimea Valley forest site

261   exhibiting smaller ranges than toads at golf courses (Figure 1). There was no sex-based

262   difference in movement range (Wilcoxon, W = 750, p = 0.77). All three movement ranges more

263   than three standard deviations from the mean were male (example in Figure S4). Size did not

264   have an effect on movement range ($t_{(68.60)}$ = -0.814, p = 0.42; Figure S5).
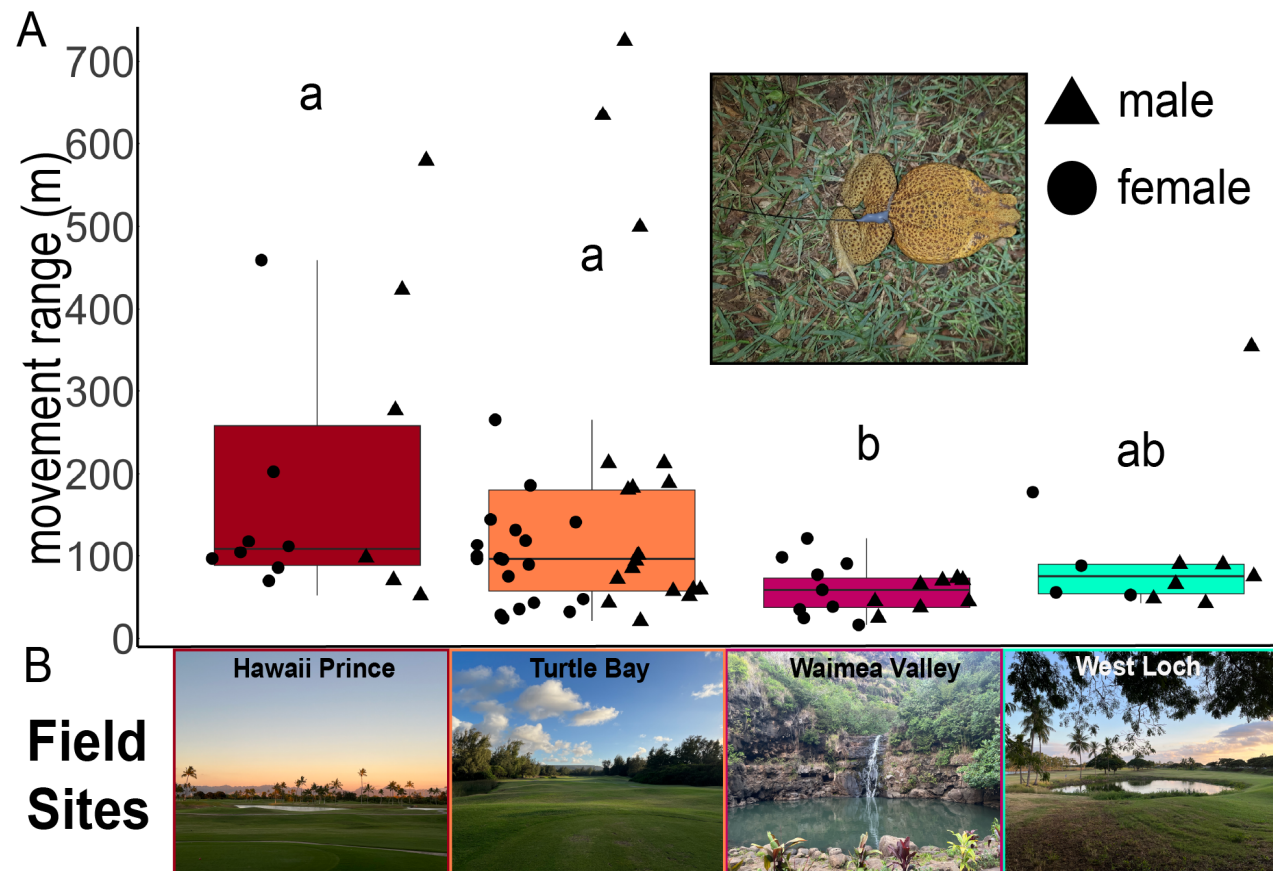
Figure 1. Movement range varies across locations. (A) Observed movement range of cane toads at each tracking site. Letters represent significant differences between sites (Kruskal-Wallis, $\chi^2_{(3)}$ = 14.21, p = 0.0026) from post-hoc Dunn's Test (with Benjamini-Hochberg adjustment). Shapes represent sex (▲ = male, ●= female). Inset shows toad with radio tag. **(B)** Photographs depicting typical landscapes for each site.
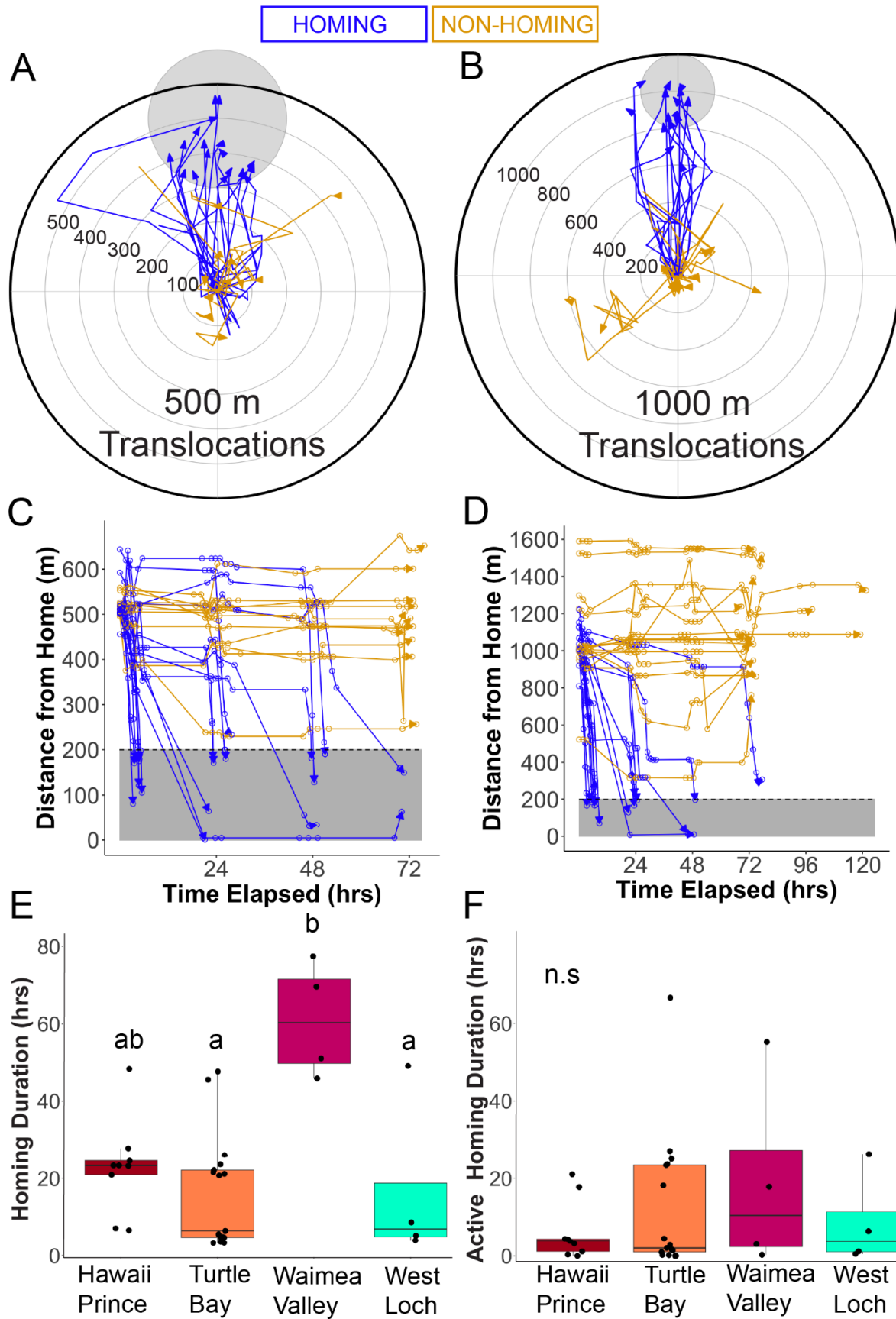
Toads exhibited long-distance homing

Next, we tested whether toads could navigate back to home areas following displacements of 500- or 1000-m. Out of 62 translocated toads, 34 exhibited homing behavior (Figure 2A-D). Homing success did not vary with sex ($\chi^2_{(1)}$= 0.16, p = 0.69), but did vary with field site ($\chi^2_{(3)}$= 10.33, p = 0.016). Specifically, toads at Hawaii Prince homed with greater success than toads in Turtle Bay (Fisher exact test, p = 0.04) and Waimea Valley (Fisher exact test, p = 0.03). Toads returned home in 3.27 to 77.48 hours (25.50 +/- 22.23) with a mean straightness of 0.81 (+/- 0.16). Considering all toads (including those in ablation experiments), 19 out of 31 toads returned home from 500-meters and 15 out of 30 toads returned from 1000-

280　meters. There was no effect of translocation distance on homing success (Pearson's Chi-

281　squared, $\chi^2_{(1)}$ = 0.40, p = 0.53). Distance had no effect on homing duration (Wilcoxon, W =

282　143.5, p = 0.99) (Figure S6A) and toads translocated 1000-meters had faster homing speeds

283　(Wilcoxon, W = 72, p = 0.015; Figure S6B). A linear mixed-effects model accounting for the

284　unequal distribution of toad size across field sites showed no significant effect of size on homing

285　duration ($t_{(30.52)}$ = -0.51, p = 0.61; Figure S7).

286　　　　Homing speed and duration varied significantly between sites, with toads at Waimea

287　Valley homing slower than toads at Turtle Bay and West Loch (Speed: Kruskal-Wallis, $\chi^2_{(3)}$=

288　10.52, p = 0.015; Duration: Kruskal-Wallis, $\chi^2_{(3)}$= 12.38, p = 0.0062; Figure 2E). Toads often did

289　not immediately move homeward, so we measured an "active homing speed" using only

290　movement and time after the first 100-meters of homeward movement. Active homing speed

291　and duration did not vary between sites (Speed: Kruskal-Wallis, $\chi^2_{(3)}$ = 4.93 p = 0.18; Duration:

292　Kruskal-Wallis, $\chi^2_{(3)}$= 0.44, p = 0.93), suggesting that the main differences in homing time are

293　due to stationary periods prior to homeward movements (Figure 2F). On average, the time to

294　move at least 100-meters homeward was 64% of the total homing duration.

295　　　　Twenty-seven of the 62 translocated toads did not exhibit homing behavior. Cumulative

296　movement of non-homing toads averaged 547.07 m (+/- 488.5) and the mean minimum

297　distance from home reached was 719.3 m (+/- 359.5). Mean movement angles of homing toads

298　showed a significant orientation towards the home direction (Rayleigh p = 0.024) and non-

299　homing toads did not (Rayleigh p = 0.83). However, there was only a marginal difference in

300　distribution of angles of movement between homing and non-homing toads (MANOVA, Pillai =

301　0.092, $F_{(2,58)}$ = 2.95, p = 0.06; Figure S8).

302
303
304
305
306
307
308

309

310 **Figure 2. Toads exhibit long-distance homing**. Normalized trajectories of toads translocated
311 **(A)** 500-m and **(B)** 1000-m. Plot centers represent translocation release points and the gray
312 circles at the top represent the 200-m buffer threshold around baseline points. 19/31 toads
313 returned from 500-m and 15/30 from 1000-m. Distance to home over time for toads translocated
314 **(C)** 500-m and **(D)** 1000-m. Gray rectangle at the bottom of the plot represents the 200-m buffer
315 threshold around baseline points **(E)** Duration of homing differs between the experimental sites
316 (Kruskal-Wallis, $\chi^2_{(3)}$= 12.38, p = 0.0062) Letters represent significant differences from post-hoc
317 Dunn's Test (with Benjamini-Hochberg adjustment). **(F)** Active homing time shows no difference
318 between locations (Kruskal-Wallis, $\chi^2_{(3)}$= 0.44, p = 0.93).

Olfaction and magnetoreception ablations affect toad movements

320   We next asked whether ablation of olfaction led to a disruption in spatial behavior and

321 navigation, as has been demonstrated in other amphibians [21,22]. There was no significant

322 difference in movement range pre- and post-ablation for sham (Paired-Wilcoxon, V = 38, p =

323 0.70) or ablated animals (Paired Wilcoxon V = 36, p = 0.83). However, when comparing the

324 space occupied pre- and post-ablation, ablated toads showed greater divergence from their pre-

325 ablation area than sham animals (Wilcoxon, W = 340, p = 0.021), suggesting that loss of

326 olfaction might disrupt routine movements and space use. There were no significant differences

327 of homing success ($\chi^2_{(1)}$ = 0.73, p = 0.39) or duration (Wilcoxon, W = 12, p = 0.43) between

328 sham and ablated groups (ablated success = 45.5%, sham success = 63.6%)(Figure 3A). After

329 translocation, ablated toads moved more than sham toads when considering homing and non-

330 homing toads together (Wilcoxon, W = 28, p = 0.034) (Figure 3B). Though the sample is too

331 small for statistical comparison, ablated toads that homed showed more cumulative movement

332 (831.90 m +/- 288.07) than sham toads that homed (629.79 m +/- 334.19). Olfactory ablated

333 toads had significantly less pS6-positive cells in their olfactory bulbs than sham toads,

334 confirming reduced olfactory function from chemical ablation (Wilcoxon, W = 93.5, p =

335 0.0074)(Figure S9).
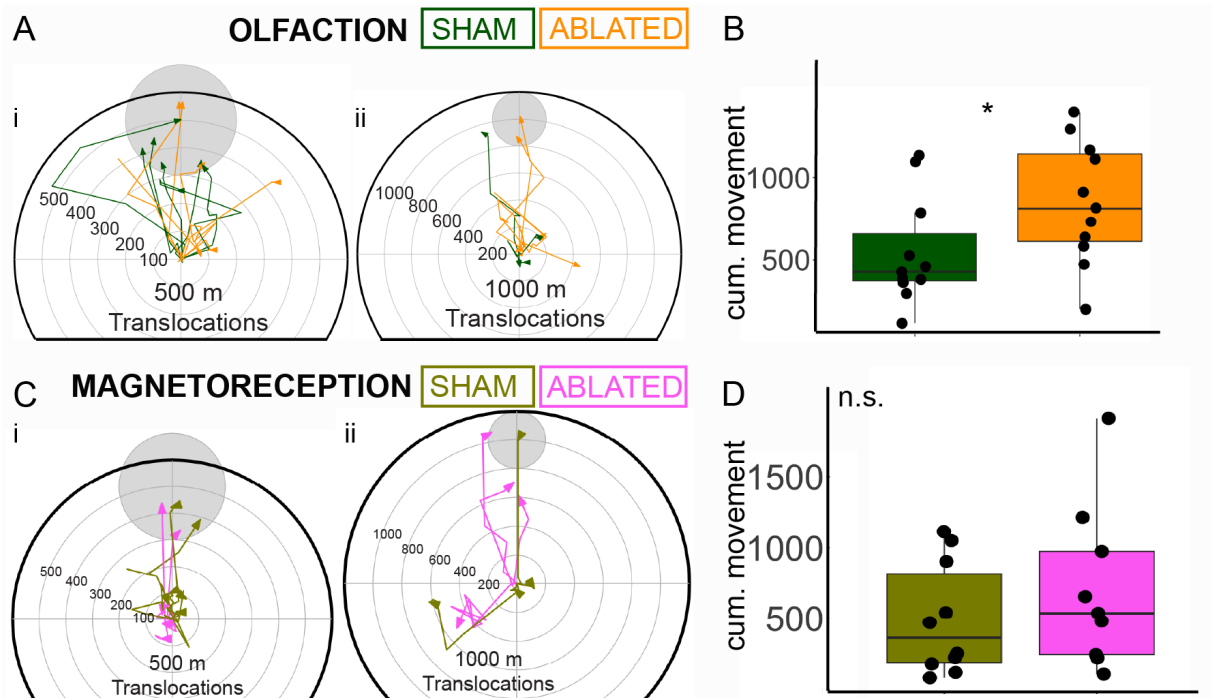
Figure 3. Sensory ablations did not prevent homing. (A) Trajectories of toads in the olfactory ablation experiment translocated (i) 500-meters and (ii) 1000-meters. **(B)** Olfaction ablated toads exhibited higher cumulative movement post- translocation than sham toads (Wilcoxon, W = 28, p = 0.034). **(C)** Trajectories of toads in the magnetoreception ablation experiment translocated (i) 500-meters and (ii) 1000-meters. **(D)** Magnetoreception ablated toads exhibited no difference in cumulative movement post- translocation than sham toads (W = 35, p = 0.447).

Magnetoreception is a sensory modality linked to homing in several amphibians [29,55], so we tested the hypothesis that cane toads also use magnetoreception for homing. Movement range was significantly lower in both sham (Paired Wilcoxon, V = 56, p = 0.042) and ablated (Paired Wilcoxon, V = 62, p = 0.0068) animals. There was no significant difference when comparing similarity in positions pre- and post-ablation (Wilcoxon, W = 236, p = 0.90). There were no significant differences of homing success ($\chi^2_{(1)}$ = 1.27, p = 0.26) or duration (Wilcoxon, W = 10, p = 0.57) between sham and ablated groups (ablated success = 55.6%, sham success = 30.0%) (Figure 3C). There was no difference in cumulative movement post-translocation between sham and ablated animals (Wilcoxon, W = 55, p = 0.44)(Figure 3D).

Pallial and septal brain regions show increased neural activity in homing toads

Given the variability in homing performance, we next asked whether neural activity in brain regions is correlated with homing success by quantifying the number of pS6-positive cells in six brain regions (Figure 4). We first asked whether toads, regardless of sensory treatment, showed differences in brain activity due to homing behavior. We found a significant effect of homing behavior on neural activity in a brain region specific manner (brain region x homing condition: $\chi^2_{(10)}$ = 112.80, p < 2.2e-16) (Table S1). We found that homing animals had more pS6-positive neurons than both non-homing and baseline toads in the medial pallium (Mp), medial septum (Ms), and the lateral septum (Ls) (Table 1). In the lateral pallium (Lp), we also found significant homing-non-homing differences and marginal homing-baseline differences. The dorsal pallium (Dp) and striatum (Str) were not different in any comparison. When we split the Mp into three sections along the A-P axis, we found significant differences between homing toads and the other two conditions for every section, indicating no variation from front to back (Table S2). There were no differences in brain activity across brain regions due to path straightness (Tables S3 and S4). However, the Mp (p = 0.0076) and Ls (p = 0.00076) showed significant differences in activity related to duration of homing compared to other brain regions (Table S5) and duration showed a significant effect of homing on neuronal activity in the Ms ($t_{(27)}$ =-2.55, p = 0.017) and Ls ($t_{(27)}$ = -2.93, p = 0.0069) (Table S6).

385 **Table 1. Pairwise contrasts by Homing Condition in all brains**

Brain Region	Contrast	estimate	std. error	z-ratio	p-value
Dp	Baseline-Homing	-0.4071	0.202	-2.013	0.1091
Dp	Baseline-Nonhoming	-0.1087	0.204	-0.532	0.8558
Dp	Homing-Nonhoming	0.2984	0.153	1.957	0.1231
Lp	Baseline-Homing	-0.457	0.198	-2.303	0.0554
Lp	Baseline-Nonhoming	-0.0521	0.201	-0.26	0.9635
Lp	Homing-Nonhoming	0.405	0.15	2.703	0.0188
Mp	Baseline-Homing	-0.7123	0.198	-3.595	0.0009
Mp	Baseline-Nonhoming	-0.096	0.2	-0.48	0.881
Mp	Homing-Nonhoming	0.6163	0.15	4.12	0.0001
Ms	Baseline-Homing	-0.7186	0.203	-3.533	0.0012
Ms	Baseline-Nonhoming	-0.058	0.206	-0.282	0.9572
Ms	Homing-Nonhoming	0.6606	0.153	4.312	<.0001
Ls	Baseline-Homing	-0.716	0.202	-3.544	0.0011
Ls	Baseline-Nonhoming	-0.0598	0.204	-0.293	0.9537
Ls	Homing-Nonhoming	0.6561	0.152	4.303	0.0001
Str	Baseline-Homing	-0.191	0.207	-0.923	0.6259
Str	Baseline-Nonhoming	-0.0897	0.209	-0.428	0.9038
Str	Homing-Nonhoming	0.1013	0.156	0.651	0.7917

386 *Shading represents significant relationships, light shading represents marginal significance
387
388   To better account for the effect of sensory ablations on brain activity, we also modeled

389   brain activity in all translocated toads with sensory ablation as a predictor (Table S7). This

390   model predicts significant effects of both homing condition ($\chi^2_{(5)}$ = 119.09, p < 2.2e-16) and

391   ablation ($\chi^2_{(20)}$ = 127.59, p < 2.2e-16) on neural activity in brain region specific manners.

392   However, the magnitude of the effect was insufficient to detect significant pairwise differences in

393   region-specific activity between ablated and sham groups in both olfaction and

394   magnetoreception experiments (Figures S10; Table S8). The model also indicated significant

395   differences between homing and non-homing toads in the Mp ($z = 4.138$, $p < 0.0001$), Ms ($z =$

396   2.909, $p = 0.0036$), and Ls ($z = 3.916$, $p = 0.0001$), but not the Lp ($z = 1.876$, $p = 0.0607$).

397
398
399
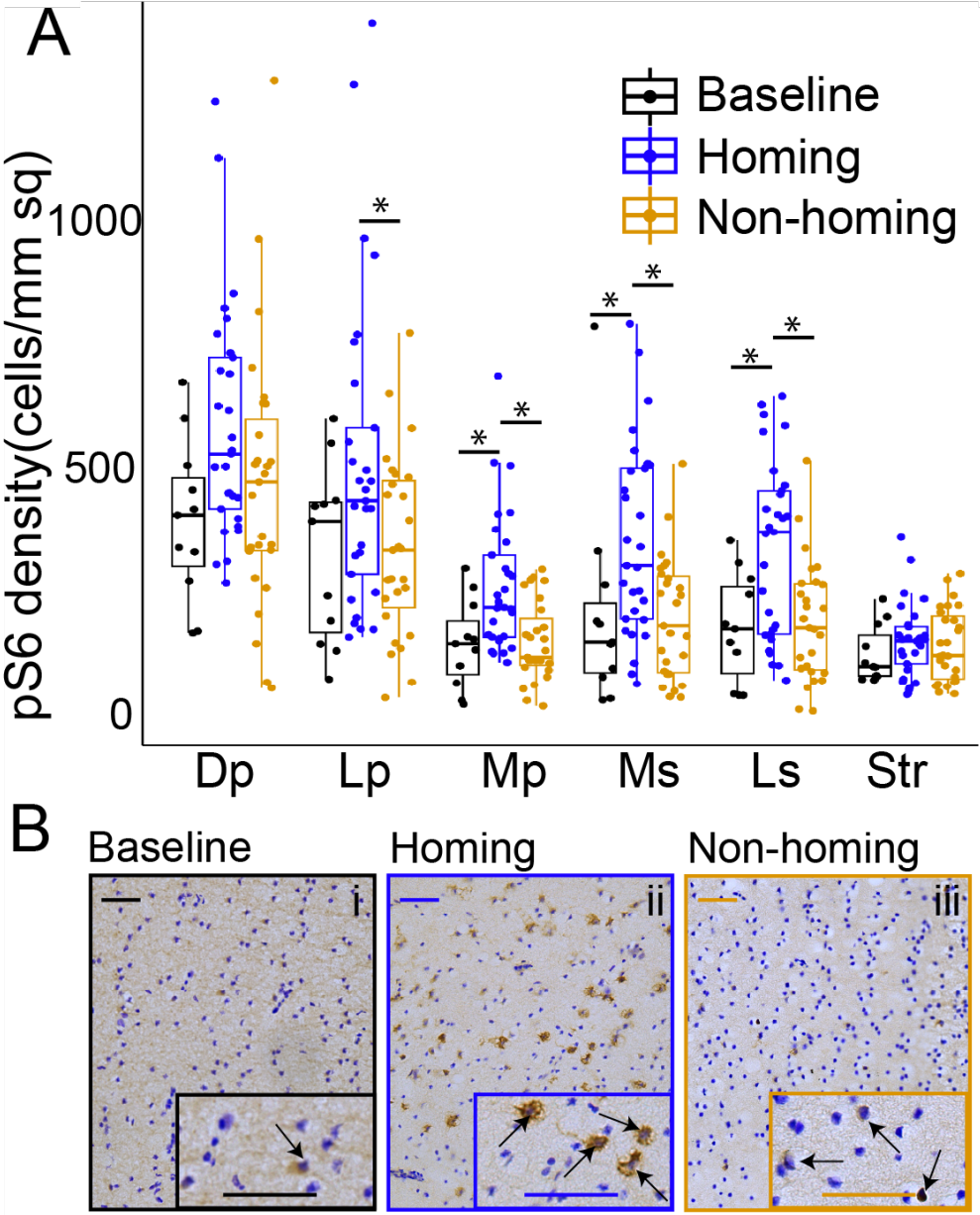
400 **Figure 4. Pallial regions and the septum are more active due to homing behavior.**
401 **(A)** pS6-positive cells per mm^2 averaged per individual for each brain region across homing
402 conditions. Brain activity within brain regions varied significantly due to homing conditions ($\chi^2_{(10)}$
403 = 112.800, p < 2.2e-16). Stars represent significant pairwise differences within brain regions
404 based on a mixed model with cell count as a response factor. Abbreviations: Dp, dorsal pallium;
405 Lp, lateral pallium; Mp, medial pallium; Ms, medial septum; Ls, lateral septum; Str, striatum. **(B)**
406 Representative examples of stained Mp brain tissue from each homing group. Scale bars
407 represent 50 μm. In inset, arrows indicate pS6-positive cells.

Discussion

409   In the current study, we examined the neural correlates and sensory basis of cane toad

410 homing. Our results demonstrate that more than half of toads displayed a map-like navigation

411 ability from distances exceeding regular movements. We found that areas associated with

412 navigation in mammals and birds [3,6,45,56] and which have previously been implicated in

413 amphibian lab studies [10–13], such as medial pallium and the septum, show increased activity

414 in homing toads. Contrary to our predictions, we found that olfactory or magnetic ablation did

415 not disrupt homing, suggesting this behavior is multimodal, as has been demonstrated in other

416 amphibians [21,57]. These results contribute to filling in gaps in the understanding of amphibian

417 spatial behaviors and neural architecture of vertebrate spatial cognition.

(a) Homing in toads

419   We found that most toads could navigate home from up to 1 kilometer. Amphibian

420 homing abilities have previously been linked to the spatial challenges of parental care and

421 seasonal migrations [23,57]. However, cane toad homing suggests that refined navigation may

422 not be reserved for species with specialized reproductive behaviors but is instead more widely

423 present across amphibians. Cane toads outside the Australian invasive front typically moved

424 less than 200-meters, but rare movements of 500-meters to 2-kilometers have been observed

425 [30,35,36]. Thus, our translocation distances moved toads beyond the area of regular

426 movements, but were still within the range that toads could occasionally explore. In addition,

427 toads were found distributed throughout each field site, rather than clustered at specific sites.

428   Therefore, for most translocated toads, suitable habitat was available closer to the release site

429   than home, which suggests that toads were navigating to specific locations and not simply

430   finding the nearest suitable habitat.

431   Many toads did not home, suggesting strong variability in ability or motivation to move

432   back home. Some variation may be attributable to environmental features, as the more wooded

433   sites (Waimea Valley and Turtle Bay) had lower levels of success than Hawaii Prince.

434   Vegetation may create landscape resistance and occlude sensory information or provide

435   additional shelter that reduces motivation to return. This is in line with the results of a homing

436   experiment with *R. marina* in primary rainforest [30], where homing was generally slower than

437   that observed in this experiment. Turtle Bay and Waimea Valley also experienced more

438   precipitation than West Loch and Hawaii Prince. More available water could reduce pressure to

439   return to previously occupied areas, resulting in lower rates of homing (in Australia, availability

440   of water has been shown to influence daily movements of cane toads [58]). However, individual

441   variation in ability, experience, and motivation leading to variable homing success might also

442   play in an important role. Inter-individual variability unaccounted for by environmental variation

443   has been often observed in amphibian navigation studies [23,59] and is likely caused by

444   individual differences that should be further explored.

445   # (b) Use of sensory systems in homing

446   Olfaction and magnetoreception have been implicated as important senses in navigation

447   studies in many amphibians [19–22,27,29,57] and other vertebrates [60–64]. Ablation

448   experiments strongly suggested that *B. bufo* relies on these senses to orient towards breeding

449   ponds [21]*,* while *B. japonicus* seemed to rely solely on olfaction [22]. In a variety of salamander

450   species, both magnetoreception and olfaction are used to orient and return to breeding streams

451   [19,20,27,28]. However, in our study, these ablations did not disrupt *R. marina* homing. If either

452   is used in cane toad navigation, they likely work in combination with other senses or can

453   compensate for each other.

454   Olfaction is used in multiple ways in navigation. Birds use odors distributed by wind to

455   determine goal locations [60]. Mammals sample odor information through sniffing to gain

456   information about their environment [61]. For amphibians, cues can come directly from aquatic

457   navigation goals, as seems to be the case in other toad species [21,22] and poison frogs [26].

458   Some salamanders may smell pheromones left by themselves or conspecifics [19]. Although we

459   did not observe a reduced success in homing with olfactory ablations, ablated toads showed

460   less similarity in space occupied post-ablation and moved more post-translocation than sham

461   toads. These results suggest that olfactory cues may help toads move in more direct paths and

462   are important for recognizing home areas. This is somewhat different from what has been

463   observed in migratory bufonids: when olfaction was ablated, *B. bufo* could not orient correctly

464   but still moved in a direct path [21] and *B. japonicus* was completely disoriented [22].

465   Differences in the specific utility of olfaction during navigation may arise from the observed

466   variations in life history.

467   Magnetic orientation has been demonstrated in several amphibians [27,65,66], including

468   other bufonids [21,67], but we found no evidence that magnetic field disruption impacts long-

469   distance homing in cane toads. Other vertebrates, such as pigeons, sea turtles, and cetaceans

470   exhibit long range navigation using magnetic cues [62–64]. Much of the functional

471   understanding of magnetoreception comes from salamanders [27,29,65], which can use

472   magnetoreception as both compass and map senses (similarly to sea turtles and birds), even

473   though their movements are on much smaller scales [55,68]. The effect of magnetoreception

474   ablation on homing in other toad species is variable: *B. bufo* showed less accuracy in orientation

475   following translocation [21], while *B. japonicus* showed no response to ablation [22]. We cannot

476   discount magnetoreception in *R. marina* on the basis of the current experiment, as the sham

477   group performed poorly with only 30% of toads returning home. Although we used methodology

478   previously deployed in other amphibians [21,41], both ablated and sham groups moved less

479   following ablation. Treatment may have had an unintended consequence on behavior, possibly

480   through physically covering the pineal gland, which contains photoreceptors [69] that may play a

481   role in light dependent magnetic compass orientation [28]. Future experiments are necessary to

482   further explore the possible role of the pineal gland in navigation or determine other factors

483   affecting locomotion and navigation.

484   Other senses may play a role in homing behavior. For example, both male and female

485   cane toads exhibit phonotaxis to male calls [12,70]. However, we observed toads distributed

486   over several ponds and inconsistent calling, making the use of auditory cues unlikely. Vision

487   may also play a role and is used by toads and poison frogs in laboratory arenas, both in the

488   context of identifying feature cues and environmental geometry [10,71,72]. It is unlikely to be the

489   primary sense involved in homing, as the visual information toads receive (ground-level, at

490   night) is very limited in relation to the scale of the translocation distance. While environmental

491   geometry could play some role in cane toad navigation, geometry in amphibians and rodents is

492   considered on much smaller scales in enclosed arenas [10,72,73] and does not directly

493   translate to large scale movements in complex environments. In addition, migratory amphibians

494   with visual ablations are able to return home [20,21]. However, vision may be used to sense

495   celestial cues, which provide compass information to some amphibians [57,74]. Further study is

496   necessary to assess the functionality of sensory modalities during navigation.

497   (c) Conserved role of pallial brain regions in spatial tasks

498   Our study contributes to the accumulating evidence that medial pallium is functionally

499   homologous to the mammalian hippocampus. The hippocampus performs many navigation-

500   related functions, such as encoding place [1,45] and consolidating spatial memory [2]. In

501   pigeons, hippocampal lesions impair homing [5] fish and turtles require homologous regions to

502   solve a spatial task [9]. Extensive neuronal activation and lesion studies in the toad *Rhinella*

503   *arenarum* suggest that the Mp is used in spatial tasks involving environmental geometry [10],

504   feature cues [11], and conspecific mating calls [12]. The Mp is also shown to have increased

505   activity in tadpole-transporting poison frogs, a parental care task that requires spatial memory

506   [44]. Our work corroborates this accumulating evidence that the Mp is involved in the regulation

507   of spatial behaviors in amphibians.

508   In our experiment, the lateral pallium showed increased activity in homing animals, while

509   the dorsal pallium did not. There is ongoing uncertainty regarding specific functions and

510   homologies of pallial divisions in amphibians [15,75]. Some evidence points to the Dp being

511   homologous to the mammalian entorhinal cortex and subiculum [76], though it has also been

512   considered similar to the general cortex [75,77]. Meanwhile, the Lp is proposed to be

513   homologous to the piriform or olfactory cortex [14,75,77]. However, we saw no evidence of

514   activity changes in the Lp associated with olfactory ablations (Figure S10, Table S8). Sotelo et

515   al. 2024 [13] presents evidence from the toad *R. arenarum* [10] and the newt *Triturus alpestris*

516   [78] suggesting the Dp and Lp are necessary for learning associated with visual stimuli and thus

517   support the Mp in navigation. The potential homologies these regions represent to the

518   mammalian brain and their interconnectedness with the Mp [14] further support the idea that

519   they may be involved in spatial functions. We found that lateral, but not dorsal, pallium was

520   associated with long-distance homing, suggesting distinct and non-visual functions of these

521   regions in relation to navigation.

522   Our results showed increased activity in the septum in homing animals and increasing

523   activity with faster homing. Both medial and lateral portions of the septum have been implicated

524   in spatial tasks in rodents, with the Ms integrating circuitry important to path integration [56] and

525   the Ls exhibiting reward-related place fields [79]. The septum has also been implicated in

526   navigation in amphibians: in *R. arenarum,* the Ms, but not Ls, showed heightened activity when

527   animals located goal locations in relation to mating calls [12], environmental geometry, and

528   feature cues [13]. Our results differ in this regard, as we find that both the Ms and Ls show

529   increased activity in homing toads compared to non-homing and non-translocated toads. These

530   data corroborate findings in *R. arenarum* but also suggest that the Ls may govern navigation

531   that requires large movements and not necessarily smaller scale spatial learning.

532   One important caveat of our experiment is the relationship of the behavior and the timing

533   of the endpoint. Homing animals were often stationary before moving back in a straight line,

534   suggesting that the animals gain their bearings during this time. The snapshot of brain activity

535   captured by our pS6 immunohistochemistry does not account for this period, but for brain

536   activity happening during active homing. In other animals, the hippocampal formation is

537   responsible for specific homing processes. For example, in homing pigeons, it governs use of

538   landmarks when approaching home, but not initial homeward orientation [6]. In future toad

539   experiments, it would be useful to compare active-homing brain activity to that of the pre-homing

540   period to identify a neural signature of orientation.

Conclusion

542   Our work combines intensive field-based telemetry with laboratory analysis of brain

543   activity to study the neural and sensory basis of navigation. We demonstrate that cane toads (a)

544   are capable of long-distance navigation to specific sites, (b) exhibit conserved function of the

545   pallial and septal regions in relation to spatial behavior, and (c) likely have a multimodal basis of

546   navigation. In the future, lesion experiments are needed to identify the specific function of

547   pallium brain regions in navigation. In addition, further lab-based studies incorporating

548   electrophysiology may characterize how neurons encode spatial information in amphibians. The

549   current work provides a foundation upon which to begin further examinations of the neural and

550   sensory basis of amphibian navigation through the integration of field and laboratory data.

Acknowledgements

Data Availability

All data and code have been uploaded as supplemental information.

Author contributions

Conceptualization: D.A.S., A.P., L.A.O; Methodology: D.A.S.; Formal analysis: D.A.S.;

Investigation: D.A.S., D.P.M., C.G.; Resources: L.A.O.; Data curation: D.A.S., D.P.M., C.G;

Writing – original draft: D.A.S.; Writing – review and editing: D.A.S., L.A.O., A.P., D.P.M., C.G.;

Visualization: D.A.S.; Supervision: L.A.O.; Project administration: L.A.O.; Funding acquisition: D.A.S., L.A.O.

Funding

577 # Competing interests

578 The authors declare no competing or financial interests.

579 # Citations

580 1. O'Keefe J, Dostrovsky J. 1971 The hippocampus as a spatial map. Preliminary evidence
581   from unit activity in the freely-moving rat. *Brain Res.* **34**, 171–175. (doi:10.1016/0006-
582   8993(71)90358-1)
583 2. Florian C, Roullet P. 2004 Hippocampal CA3-region is crucial for acquisition and memory
584   consolidation in Morris water maze task in mice. *Behav. Brain Res.* **154**, 365–374.
585   (doi:10.1016/j.bbr.2004.03.003)
586 3. Yartsev MM, Ulanovsky N. 2013 Representation of Three-Dimensional Space in the
587   Hippocampus of Flying Bats. *Science* **340**, 367–372. (doi:10.1126/science.1235338)
588 4. Forli A, Yartsev MM. 2023 Hippocampal representation during collective spatial behaviour in
589   bats. *Nature* **621**, 796–803. (doi:10.1038/s41586-023-06478-7)
590 5. Bingman VP, Mench JA. 1990 Homing behavior of hippocampus and parahippocampus
591   lesioned pigeons following short-distance releases. *Behav. Brain Res.* **40**, 227–238.
592   (doi:10.1016/0166-4328(90)90079-T)
593 6. Gagliardo A, Ioalé P, Bingman VP. 1999 Homing in Pigeons: The Role of the Hippocampal
594   Formation in the Representation of Landmarks Used for Navigation. *J. Neurosci.* **19**, 311–
595   315. (doi:10.1523/JNEUROSCI.19-01-00311.1999)
596 7. Cohen L, Vinepinsky E, Donchin O, Segev R. 2023 Boundary vector cells in the goldfish
597   central telencephalon encode spatial information. *PLOS Biol.* **21**, e3001747.
598   (doi:10.1371/journal.pbio.3001747)
599 8. Yang C, Mammen L, Kim B, Li M, Robson DN, Li JM. 2024 A population code for spatial
600   representation in the zebrafish telencephalon. *Nature* **634**, 397–406. (doi:10.1038/s41586-
601   024-07867-2)
602 9. Rodríguez F, López JC, Vargas JP, Gómez Y, Broglio C, Salas C. 2002 Conservation of
603   spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J. Neurosci.*
604   *Off. J. Soc. Neurosci.* **22**, 2894–2903. (doi:20026211)
605 10. Sotelo MI, Daneri MF, Bingman VP, Muzio RN. 2016 Telencephalic Neuronal Activation
606   Associated with Spatial Memory in the Terrestrial Toad Rhinella arenarum: Participation of
607   the Medial Pallium during Navigation by Geometry. *Brain. Behav. Evol.* **88**, 149–160.
608   (doi:10.1159/000447441)
609 11. Sotelo MI, Bingman VP, Muzio RN. 2022 The medial pallium and the spatial encoding of
610   geometric and visual cues in the terrestrial toad, Rhinella arenarum. *Neurosci. Lett.* **786**,
611   136801. (doi:10.1016/j.neulet.2022.136801)
612 12. Sotelo MI, Bingman VP, Muzio RN. 2019 The Mating Call of the Terrestrial Toad, Rhinella
613   arenarum, as a Cue for Spatial Orientation and Its Associated Brain Activity. *Brain. Behav.*
614   *Evol.* **94**, 7–17. (doi:10.1159/000504122)
615 13. Sotelo MI, Daneri MF, Bingman VP, Muzio RN. 2024 Amphibian spatial cognition, medial
616   pallium and other supporting telencephalic structures. *Neurosci. Biobehav. Rev.* , 105739.
617   (doi:10.1016/j.neubiorev.2024.105739)
618 14. Roth G, Laberge F, Mühlenbrock-Lenter S, Grunwald W. 2007 Organization of the pallium in
619   the fire-bellied toad Bombina orientalis. I: Morphology and axonal projection pattern of
620   neurons revealed by intracellular biocytin labeling. *J. Comp. Neurol.* **501**, 443–464.
621   (doi:10.1002/cne.21255)

15. Westhoff G, Roth G. 2002 Morphology and projection pattern of medial and dorsal pallial neurons in the frog Discoglossus pictus and the salamander Plethodon jordani. *J. Comp. Neurol.* **445**, 97–121. (doi:10.1002/cne.10136)

16. Muzio RN, Bingman VP. 2022 Brain and Spatial Cognition in Amphibians: Stem Adaptations in the Evolution of Tetrapod Cognition. In *Evolution of Learning and Memory Mechanisms*, pp. 105–124. Cambridge University Press.

17. Bingman VP, Muzio RN. 2017 Reflections on the Structural-Functional Evolution of the Hippocampus: What Is the Big Deal about a Dentate Gyrus? *Brain. Behav. Evol.* **90**, 53–61. (doi:10.1159/000475592)

18. Twitty V, Grant D, Anderson O. 1967 Home Range in Relation to Homing in the Newt Taricha rivularis (Amphibia: Caudata). *Copeia* **1967**, 649–653. (doi:10.2307/1442244)

19. Jaeger RG, Fortune D, Hill G, Palen A, Risher G. 1993 Salamander Homing Behavior and Territorial Pheromones: Alternative Hypotheses. *J. Herpetol.* **27**, 236–239. (doi:10.2307/1564945)

20. Madison DM. 1969 Homing behaviour of the red-cheeked salamander, Plethodon jordani. *Anim. Behav.* **17**, 25–39. (doi:10.1016/0003-3472(69)90109-2)

21. Sinsch U. 1987 Orientation behaviour of toads (Bufo bufo) displaced from the breeding site. *J. Comp. Physiol. A* **161**, 715–727. (doi:10.1007/BF00605013)

22. Ishii S, Kubokawa K, Kikuchi M, Nishio H. 1995 Orientation of the Toad, Bufo japonicus, toward the Breeding Pond. *Zoolog. Sci.* **12**, 475–484. (doi:10.2108/zsj.12.475)

23. Pašukonis A *et al.* 2022 Contrasting parental roles shape sex differences in poison frog space use but not navigational performance. *eLife* **11**, e80483. (doi:10.7554/eLife.80483)

24. Beck KB, Loretto M-C, Ringler M, Hödl W, Pašukonis A. 2017 Relying on known or exploring for new? Movement patterns and reproductive resource use in a tadpole-transporting frog. *PeerJ* **5**, e3745. (doi:10.7717/peerj.3745)

25. Stynoski JL. 2009 Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog, *Oophaga pumilio*. *Anim. Behav.* **78**, 1351–1356. (doi:10.1016/j.anbehav.2009.09.002)

26. Serrano-Rojas SJ, Pašukonis A. 2021 Tadpole-transporting frogs use stagnant water odor to find pools in the rainforest. *J. Exp. Biol.* **224**, jeb243122. (doi:10.1242/jeb.243122)

27. Phillips JB, Diego-Rasilla FJ. 2022 The amphibian magnetic sense(s). *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **208**, 723–742. (doi:10.1007/s00359-022-01584-9)

28. Phillips JB, Borland SC. 1992 Behavioural evidence for use of a light-dependent magnetoreception mechanism by a vertebrate. *Nature* **359**, 142–144. (doi:10.1038/359142a0)

29. Phillips JB. 1986 Two Magnetoreception Pathways in a Migratory Salamander. *Science* **233**, 765–767. (doi:10.1126/science.3738508)

30. Shaykevich DA, Pašukonis A, O'Connell LA. 2022 Long distance homing in the cane toad (Rhinella marina) in its native range. *J. Exp. Biol.* **225**, jeb243048. (doi:10.1242/jeb.243048)

31. Urban MC, Phillips BL, Skelly DK, Shine R. 2007 The cane toad's (Chaunus [Bufo] marinus) increasing ability to invade Australia is revealed by a dynamically updated range model. *Proc. R. Soc. B Biol. Sci.* **274**, 1413–1419. (doi:10.1098/rspb.2007.0114)

32. Urban MC, Phillips BL, Skelly DK, Shine R. 2008 A Toad More Traveled: The Heterogeneous Invasion Dynamics of Cane Toads in Australia. *Am. Nat.* **171**, E134–E148. (doi:10.1086/527494)

33. Shine R *et al.* 2021 Increased rates of dispersal of free-ranging cane toads (Rhinella marina) during their global invasion. *Sci. Rep.* **11**, 23574. (doi:10.1038/s41598-021-02828-5)

34. Zug GR, Zug PB. 1979 The marine toad, Bufo marinus : a natural history resumé of native populations.

35. DeVore JL, Shine R, Ducatez S. 2021 Spatial ecology of cane toads (Rhinella marina) in their native range: a radiotelemetric study from French Guiana. *Sci. Rep.* **11**, 11817. (doi:10.1038/s41598-021-91262-8)

36. Ward-Fear G, Greenlees MJ, Shine R. 2016 Toads on Lava: Spatial Ecology and Habitat Use of Invasive Cane Toads (Rhinella marina) in Hawai'i. *PLOS ONE* **11**, e0151700. (doi:10.1371/journal.pone.0151700)

37. Yovanovich CAM, Jungblut LD, Heer T, Pozzi AG, Paz DA. 2009 Amphibian larvae and zinc sulphate: a suitable model to study the role of brain-derived neurotrophic factor (BDNF) in the neuronal turnover of the olfactory epithelium. *Cell Tissue Res.* **336**, 1–9. (doi:10.1007/s00441-009-0752-7)

38. Smith CG. 1951 Regeneration of sensory olfactory epithelium and nerves in adult frogs. *Anat. Rec.* **109**, 661–671. (doi:10.1002/ar.1091090403)

39. Hentig JT, Byrd-Jacobs CA. 2016 Exposure to Zinc Sulfate Results in Differential Effects on Olfactory Sensory Neuron Subtypes in Adult Zebrafish. *Int. J. Mol. Sci.* **17**, 1445. (doi:10.3390/ijms17091445)

40. Keller M, Douhard Q, Baum MJ, Bakker J. 2006 Destruction of the Main Olfactory Epithelium Reduces Female Sexual Behavior and Olfactory Investigation in Female Mice. *Chem. Senses* **31**, 315–323. (doi:10.1093/chemse/bjj035)

41. Freake MJ, Borland SC, Phillips JB. 2002 Use of a Magnetic Compass for Y-Axis Orientation in Larval Bullfrogs, Rana catesbeiana. *Copeia* **2002**, 466–471. (doi:10.1643/0045-8511(2002)002[0466:UOAMCF]2.0.CO;2)

42. Knight ZA, Tan K, Birsoy K, Schmidt S, Garrison JL, Wysocki RW, Emiliano A, Ekstrand MI, Friedman JM. 2012 Molecular profiling of activated neurons by phosphorylated ribosome capture. *Cell* **151**, 1126–1137. (doi:10.1016/j.cell.2012.10.039)

43. Fischer EK, Alvarez H, Lagerstrom KM, McKinney JE, Petrillo R, Ellis G, O'Connell LA. 2020 Neural correlates of winning and losing fights in poison frog tadpoles. *Physiol. Behav.* **223**, 112973. (doi:10.1016/j.physbeh.2020.112973)

44. Fischer EK, Roland AB, Moskowitz NA, Tapia EE, Summers K, Coloma LA, O'Connell LA. 2019 The neural basis of tadpole transport in poison frogs. *Proc Biol Sci* **286**, 20191084.

45. Humphreys GW, Duncan J, Treisman A, O'Keefe J, Burgess N, Donnett JG, Jeffery KJ, Maguire EA. 1998 Place cells, navigational accuracy, and the human hippocampus. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **353**, 1333–1340. (doi:10.1098/rstb.1998.0287)

46. Chersi F, Burgess N. 2015 The Cognitive Architecture of Spatial Navigation: Hippocampal and Striatal Contributions. *Neuron* **88**, 64–77. (doi:10.1016/j.neuron.2015.09.021)

47. Vorhees CV, Williams MT. 2014 Assessing spatial learning and memory in rodents. *ILAR J.* **55**, 310–332. (doi:10.1093/ilar/ilu013)

48. Moreno N, González A. 2004 Localization and connectivity of the lateral amygdala in anuran amphibians. *J. Comp. Neurol.* **479**, 130–148. (doi:10.1002/cne.20298)

49. Northcutt RG, Kicliter E. 1980 Organization of the Amphibian Telencephalon. In *Comparative Neurology of the Telencephalon* (ed SOE Ebbesson), pp. 203–255. Boston, MA: Springer US. (doi:10.1007/978-1-4613-2988-6_8)

50. Calenge C. 2006 The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecol. Model.* **197**, 516–519. (doi:10.1016/j.ecolmodel.2006.03.017)

51. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Machler M, Bolker BM. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400. (doi:10.3929/ethz-b-000240890)

52. Lenth R. 2024 Package 'emmeans'. *CRAN*

53. Payne HL, Lynch GF, Aronov D. 2021 Neural representations of space in the hippocampus of a food-caching bird. *Science* **373**, 343–348. (doi:10.1126/science.abg2009)

54. Nadel L, Hoscheidt S, Ryan LR. 2013 Spatial Cognition and the Hippocampus: The Anterior–Posterior Axis. *J. Cogn. Neurosci.* **25**, 22–28. (doi:10.1162/jocn_a_00313)

55. Diego-Rasilla FJ, Phillips JB. 2021 Evidence for the use of a high-resolution magnetic map by a short-distance migrant, the Alpine newt (Ichthyosaura alpestris). *J. Exp. Biol.* **224**, jeb238345. (doi:10.1242/jeb.238345)

56. Tsanov M. 2017 Speed and Oscillations: Medial Septum Integration of Attention and Navigation. *Front. Syst. Neurosci.* **11**. (doi:10.3389/fnsys.2017.00067)

57. Sinsch U. 2006 Orientation and navigation in Amphibia. *Mar. Freshw. Behav. Physiol.* **39**, 65–71. (doi:10.1080/10236240600562794)

58. Tingley R, Shine R. 2011 Desiccation Risk Drives the Spatial Ecology of an Invasive Anuran (Rhinella marina) in the Australian Semi-Desert. *PLOS ONE* **6**, e25979. (doi:10.1371/journal.pone.0025979)

59. Pašukonis A, Loretto M-C, Hödl W. 2018 Map-like navigation from distances exceeding routine movements in the three-striped poison frog (Ameerega trivittata). *J. Exp. Biol.* **221**. (doi:10.1242/jeb.169714)

60. Gagliardo A. 2013 Forty years of olfactory navigation in birds. *J. Exp. Biol.* **216**, 2165–2171. (doi:10.1242/jeb.070250)

61. Marin AC, Schaefer AT, Ackels T. 2021 Spatial information from the odour environment in mammalian olfaction. *Cell Tissue Res.* **383**, 473–483. (doi:10.1007/s00441-020-03395-3)

62. Lohmann K, Lohmann C. 1996 Orientation and open-sea navigation in sea turtles. *J. Exp. Biol.* **199**, 73–81.

63. Mouritsen H, Ritz T. 2005 Magnetoreception and its use in bird navigation. *Curr. Opin. Neurobiol.* **15**, 406–414. (doi:10.1016/j.conb.2005.06.003)

64. Bauer GB, Fuller M, Perry A, Dunn JR, Zoeger J. 1985 Magnetoreception and Biomineralization of Magnetite in Cetaceans. In *Magnetite Biomineralization and Magnetoreception in Organisms: A New Biomagnetism* (eds JL Kirschvink, DS Jones, BJ MacFadden), pp. 489–507. Boston, MA: Springer US. (doi:10.1007/978-1-4613-0313-8_24)

65. Phillips JB. 1977 Use of the earth's magnetic field by orienting cave salamanders (Eurycea lucifuga). *J. Comp. Physiol.* **121**, 273–288. (doi:10.1007/BF00609616)

66. Deutschlander ME, Phillips JB, Borland SC. 2000 Magnetic Compass Orientation in the Eastern Red-Spotted Newt, Notophthalmus viridescens: Rapid Acquisition of the Shoreward Axis. *Copeia* **2000**, 413–419. (doi:10.1643/0045-8511(2000)000[0413:MCOITE]2.0.CO;2)

67. Landler L, Gollmann G. 2011 Magnetic orientation of the Common Toad: establishing an arena approach for adult anurans. *Front. Zool.* **8**, 6. (doi:10.1186/1742-9994-8-6)

68. Phillips JB, Freake MJ, Fischer JH, Borland CS. 2002 Behavioral titration of a magnetic map coordinate. *J. Comp. Physiol. A* **188**, 157–160. (doi:10.1007/s00359-002-0286-x)

69. Adler K. 1976 Exraocular Photoreception in Amphibians. In *Extraretinal Photoreception* (ed M Menaker), pp. 275–298. Pergamon. (doi:10.1016/B978-0-08-020965-4.50008-0)

70. Yasumiba K, Alford RA, Schwarzkopf L. 2015 Why do male and female cane toads, *Rhinella marina*, respond differently to advertisement calls? *Anim. Behav.* **109**, 141–147. (doi:10.1016/j.anbehav.2015.08.015)

71. Liu Y, Day LB, Summers K, Burmeister SS. 2019 A cognitive map in a poison frog. *J. Exp. Biol.* **222**, jeb197467. (doi:10.1242/jeb.197467)

72. Sorrell CA, Burmeister SS. 2023 Orientation by environmental geometry and feature cues in the green and black poison frog (Dendrobates auratus). *Anim. Cogn.* **26**, 2023–2030. (doi:10.1007/s10071-023-01820-y)

73. Cheng K. 1986 A purely geometric module in the rat's spatial representation. *Cognition* **23**, 149–178. (doi:10.1016/0010-0277(86)90041-7)

74. Ferguson DE. 1971 The Sensory Basis of Orientation in Amphibians. *Ann. N. Y. Acad. Sci.* **188**, 30–36. (doi:10.1111/j.1749-6632.1971.tb13087.x)

75. Bruce LL, Neary TJ. 1995 The limbic system of tetrapods: a comparative analysis of cortical

775   and amygdalar populations. *Brain. Behav. Evol.* **46**, 224–234. (doi:10.1159/000113276)

776   76. Woych J *et al.* 2022 Cell-type profiling in salamanders identifies innovations in vertebrate
777   forebrain evolution. *Science* (doi:10.1126/science.abp9186)

778   77. González A, López JM, Morona R, Moreno N. 2020 The Organization of the Central
779   Nervous System of Amphibians. In *Evolutionary Neuroscience (Second Edition)* (ed JH
780   Kaas), pp. 125–157. London: Academic Press. (doi:10.1016/B978-0-12-820584-6.00007-6)

781   78. Wenz E, Himstedt W. 1990 Telencephalic structures are involved in learning and memory in
782   the newt Triturus alpestris. *Naturwissenschaften* **77**, 239–240. (doi:10.1007/BF01138493)

783   79. Wirtshafter HS, Wilson MA. 2020 Differences in reward biased spatial representations in the
784   lateral septum and hippocampus. *eLife* **9**, e55252. (doi:10.7554/eLife.55252)