BIOLOGY LETTERS

royalsocietypublishing.org/journal/rsbl

Research

Cite this article: Storks L, Garcia J, Perez-Martinez CA, Leal M. 2024 Habitat complexity influences neuron number in six species of Puerto Rican *Anolis*. *Biol. Lett.* **20**: 20230419.
https://doi.org/10.1098/rsbl.2023.0419

Received: 9 September 2023
Accepted: 15 January 2024

Subject Category:
Neurobiology

Subject Areas:
neuroscience, cognition, behaviour

Keywords:
habitat complexity, neuron number, neuroanatomy, *Anolis*, isotropic fractionator

Author for correspondence:
Levi Storks
e-mail: storksle@gmail.com

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.7043223.

THE ROYAL SOCIETY PUBLISHING

Habitat complexity influences neuron number in six species of Puerto Rican *Anolis*

Levi Storks[1,2], Jessica Garcia[1,3], Christian A. Perez-Martinez[1] and Manuel Leal[1]

[1]Division of Biological Sciences, University of Missouri, Columbia, MO, USA
[2]Department of Biology, University of Detroit Mercy, Detroit, MI, USA
[3]College of Veterinary Medicine, University of Minnesota, Minneapolis, MN, USA

LS, 0000-0003-3118-6234; ML, 0000-0002-7441-1991

Elucidating the selective forces shaping the diversity of vertebrate brains continues to be a major area of inquiry, particularly as it relates to cognition. Historically brain evolution was interpreted through the lens of relative brain size; however, recent evidence has challenged this approach. Investigating neuroanatomy at a finer scale, such as neuron number, can provide new insights into the forces shaping brain evolution in the context of information processing capacity. Ecological factors, such as the complexity of a species' habitat, place demands on cognition that could shape neuroanatomy. In this study, we investigate the relationship between neuron number and habitat complexity in three brain regions across six closely related anole species from Puerto Rico. After controlling for brain mass, we found that the number of neurons increased with habitat complexity across species in the telencephalon and 'rest of the brain,' but not in the cerebellum. Our results demonstrate that habitat complexity has shaped neuroanatomy in the Puerto Rican anole radiation and provide further evidence of the role of habitat complexity in vertebrate brain evolution.

1. Introduction

The impact of cognitive processes on the evolution of neuroanatomy continues to be a major area of inquiry. Historically, research primarily focused on relative brain size, which is a proxy for information processing capacity of the brain [1]. Larger values of relative brain size indicate larger brain size than expected for body size, which is typically associated with greater cognitive capacities [2]. However, the utility of relative brain size has been questioned as it is unclear how brain size correlates with the functional neuronal structure of the brain [3,4]. Information processing in the brain is highly complex and is a function of many factors (e.g. neuron number, diversity of cell types, synapse density) [5]. Recently, the number of neurons has emerged as an alternative proxy that, although still imperfect, better reflects information processing capacity than brain size [6–8]. Investigation into neuron numbers has already revealed insights into the evolution of vertebrate neuroanatomy (e.g. [9–11]).

An animal performs all behaviors required for survival and reproduction in the context of its habitat. The cognitive demands of effectively performing these behaviors, including motor coordination, integrating sensory information and spatial learning, are influenced by the habitat's structural heterogeneity [12,13]. Thus, an animal's fitness is impacted by how it meets the cognitive demands posed by the complexity of the habitat, which could ultimately affect selection on neuroanatomy. In fact, relationships between habitat complexity and neuroanatomy have been documented in diverse taxa [14–21]. For example, chipmunks found in habitats with denser vegetation cover have larger relative brain size than those in more open habitats [20].

West Indian *Anolis* have become a textbook example of adaptive radiation. On each island of the Greater Antilles, closely related species have each evolved

Table 1. Summarized data by species and brain region. Ecomorph classification is indicated under species name. Means are presented ± standard error. RHC = relevant habitat complexity; SVL = snout–vent length; *n* = sample size; *N* = neuron number.

species	RHC	mean SVL (cm)	mean mass $_{Body}$ (g)	brain region	*n*	mean $N \times 10^6$	mean mass$_{Region}$ (mg)
A. cristatellus	2.6196	5.5 ± 0.088	4.6 ± 0.24	telencephalon	14	1.88 ± 0.0885	1.77 ± 0.0681
trunk–ground				ROB	14	2.26 ± 0.0907	2.81 ± 0.0744
				cerebellum	13	0.840 ± 0.0547	0.19 ± 0.010
A. evermanni	−5.1598	5.6 ± 0.20	4.4 ± 0.41	telencephalon	10	1.73 ± 0.0800	1.56 ± 0.103
trunk–crown				ROB	10	2.62 ± 0.128	2.64 ± 0.168
				cerebellum	8	0.619 ± 0.0622	0.17 ± 0.012
A. gundlachi	8.1306	6 ± 0.075	4.9 ± 0.24	telencephalon	10	1.59 ± 0.144	1.85 ± 0.0776
trunk–ground				ROB	10	2.06 ± 0.0784	3.08 ± 0.0835
				cerebellum	10	0.650 ± 0.0741	0.24 ± 0.012
A. krugi	3.2436	4.8 ± 0.097	2.5 ± 0.19	telencephalon	10	1.37 ± 0.101	1.33 ± 0.0603
grass–bush				ROB	9	1.90 ± 0.132	2.10 ± 0.0664
				cerebellum	10	0.532 ± 0.0787	0.15 ± 0.0075
A. pulchellus	−0.3601	4.4 ± 0.12	1.7 ± 0.14	telencephalon	10	1.24 ± 0.0512	0.914 ± 0.0489
grass–bush				ROB	10	1.79 ± 0.0635	1.59 ± 0.0713
				cerebellum	10	0.522 ± 0.0452	0.12 ± 0.0076
A. stratulus	−8.3974	4.3 ± 0.11	1.6 ± 0.11	telencephalon	10	1.61 ± 0.110	1.11 ± 0.0248
trunk–crown				ROB	10	2.22 ± 0.0943	1.80 ± 0.0629
				cerebellum	10	0.705 ± 0.0578	0.14 ± 0.0097

an array of adaptive morphological traits to effectively exploit distinct structural habitats (i.e. ecomorphs) [22–25]. This clade enables us to compare neuroanatomy among closely related species that differ in one trait of interest (i.e. habitat complexity) yet share similar social structure, diet and sensory systems [22]. Using this approach, two previous studies evaluated whether patterns in habitat specialization extended to neuroanatomy in the same six species used in this study [17,26]. Results showed that species differed in habitat complexity, but these differences were not related to the relative volume of six brain regions [17]. Variation in the volume of each brain region was primarily driven by total brain volume [26]. Two non-exclusive hypotheses could explain these findings: neuroanatomy is not as evolutionarily labile as other traits in *Anolis*, or relevant variation in neuroanatomy is not captured at the level of brain volume. Evidence to support or reject these hypotheses is lacking in most taxa.

In this study we compare habitat complexity with the number of neurons in three brain regions across six species of Puerto Rican anoles to evaluate whether structural habitat and neuroanatomical differences are correlated. We predict that neuron number in each brain region increases with habitat complexity across species.

2. Methods

(a) Study species

We collected adult males of six species: *Anolis evermanni* (*n* = 10), *Anolis stratulus* (*n* = 10), *Anolis cristatellus* (*n* = 14), *Anolis gundlachi* (*n* = 10), *Anolis krugi* (*n* = 10) *and Anolis pulchellus* (*n* = 10). Lizards were collected from sites with minimally disturbed habitat and within the active breeding season for each species [27] (see electronic supplementary material, for more details). Individuals were sexed upon capture via visual identification, transported to the University of Missouri, and housed briefly prior to euthanasia (August 2019; May 2021). Lizards were held in the lab for a maximum of 7 days before euthanasia. Our protocols followed all guidelines of the University of Missouri Animal Care and Use Committee.

(b) Neuronal analysis

We quantified neuron numbers following the isotropic fractionation methods outlined in Storks *et al*. [28]. See electronic supplementary material, for detailed methods. We measured snout–vent length (SVL; ± 0.1 cm) and weighed (± 0.1 g) each lizard prior to euthanasia. Lizards were perfused intracardially after which whole heads and then dissected brains were post-fixed in 4% paraformaldehyde. We dissected the brain into three regions: the telencephalon, cerebellum, and the 'rest of the brain' (ROB; representing diencephalon, mesencephalon, pons and myelencephalon). Each region represents a distinct functional division of the brain and could be reliably dissected based on gross neuroanatomy. The telencephalon is involved in sensory integration and higher cognition [29], cerebellum in motor coordination and learning [30], and ROB in sensory input and motor output [31]. Each region was weighed and homogenized separately to create an isotropic suspension of cell nuclei at a set volume. The number of total cells and neurons was quantified by counting cell nuclei within samples of the suspension that were differentially labelled using immunofluorescence. Cell nuclei were labelled with DAPI (4′,6-diamidino-2-phenylindole) and neuronal nuclei were labelled with a Cy3-conjugated antibody for NeuN (ABN78C3, Millipore Sigma). Estimates for each brain region (table 1) differ in sample size due to tissues lost during processing.

(c) Habitat complexity

Measures of habitat complexity were extracted for each species from Powell & Leal [17]. Relevant habitat complexity (RHC) is a composite variable resulting from detailed characterization of the structural habitat traversed during 20 min focal observations of undisturbed lizards (see [17] for further description). This produces a normalized index in which more negative values indicate a higher level of structural habitat complexity.

(d) Statistical analyses

All statistical analyses were performed in R Statistical Software version 4.3.1 [32] and RStudio version 2023.06.0 [33]. Data for SVL, body mass, brain region mass, and neuron number were log-10 transformed. Three samples were excluded as outliers because they were (1) beyond 1.5 times the interquartile range from the quartiles (i.e. 1.5 IQR rule) and (2) exclusion could be justified by anomalies noted during processing (e.g. poor distribution of nuclei, weak staining).

We evaluated the relationship between neuron number, brain region mass, and habitat complexity across species with generalized linear models fit using Markov chain Monte Carlo (MCMC) methods using the R package *MCMCglmm* [34]. We used brain region mass in our analyses rather than body size because brain region mass and SVL were highly correlated ($r \sim$ 0.65–0.92 in our data). All models yielded quantitatively similar results when SVL or body mass was substituted for brain region mass (see electronic supplementary material). Furthermore, studies utilizing a similar approach use brain mass rather than body size as a scaling factor for neuron number, as body size obscures scaling relationships that are apparent when using brain size [8,35,36]. Number of neurons was modelled as a response variable within each brain region across species with predictors brain region mass and RHC. Each model was run with 155 000 iterations at a thinning rate of 100 following 500 burn-in iterations. We used default weakly informative diffuse normal priors centred at zero for fixed effects. Uninformative inverse-Wishart priors were specified for residual variance ($V = 1$, nu = 0.01). Model output was evaluated by inspecting credible intervals for posterior sample means and MCMC probability (p) values. All models showed minimal autocorrelation ($r < 0.1$), adequate model convergence, and normality of residuals.

Accounting for evolutionary history is challenging in analyses focused on a limited number of species (e.g. [37,38]). Our species sample size (spp. $n = 6$) limited our ability to construct robust models accounting for phylogenetic correlation. However, for transparency we provide output from models that are corrected for phylogenetic non-independence (PCM models) as electronic supplementary material. The variance explained by the phylogenetic random effect in our PCM models is near zero and we obtained highly variable estimates of phylogenetic signal. Further, our PCM models did not find a relationship between neuron number and brain region mass, which is not in accordance with other studies using this methodology [7,9,35,36]. Our study design focuses on closely related species and as such minimizes phylogenetic differences. For these reasons, we focus on models that do not account for phylogenetic non-independence (non-PCM models) in the main text of the article.

3. Results

In table 1, we present RHC, mean SVL, and mean body mass for each species and sample size (n), mean neuron number (N), and mean region mass for each brain region within species.

We present the posterior sample mean (β) and the 95% credible interval (CI) for each parameter in models for each brain region. Significant fixed effects are evaluated based on MCMC probability values (p). Interaction effects were not significant in all models and removed. In the model predicting neuron number in the telencephalon (figure 1*a*; $N_{TEL} \sim Mass_{TEL} + RHC$), both telencephalon mass ($\beta = 0.51$; CI = 0.32, 0.73; $p < 0.001$) and RHC ($\beta = -0.0065$; CI = −0.011, −0.0018; $p = 0.01$) significantly predicted neuron number across species. In the ROB model (figure 1*b*; $N_{ROB} \sim Mass_{ROB} + RHC$), again both ROB mass ($\beta = 0.38$; CI = 0.21, 0.55; $p < 0.001$) and RHC ($\beta = -0.0082$; CI = −0.0119, −0.0045; $p < 0.001$) significantly predicted neuron number across species. However, in the cerebellum model (figure 1*c*; $N_{CER} \sim Mass_{CER} + RHC$), cerebellum mass ($\beta = 0.43$; CI = 0.028, 0.81; $p = 0.03$) significantly predicted neuron number while RHC ($\beta = -0.0064$; CI = −0.015, 0.0029; $p = 0.17$) did not. Added-variable plots in the electronic supplementary material show the effect of each predictor conditioned on the other predictors in the model (electronic supplementary material, figure S5).

4. Discussion

Selection favours the evolution of adaptive traits that enable species to effectively exploit their preferred habitat. After controlling for brain mass, species occupying habitats of greater complexity (i.e. more negative RHC values) had more neurons in the telencephalon and ROB, but not in the cerebellum (figure 1). Although brain mass had a larger effect on neuron number than habitat complexity, our results show that both forces shape neuroanatomy across the radiation of Puerto Rican *Anolis*. These results suggest a link between habitat complexity and neuroanatomy that was not observed when examining brain size across the same six species [17]. A large-scale study across 171 squamate species found a similar result, observing no differences in relative whole brain size between species inhabiting four ecological guilds (i.e. habitats) [39]. These results suggest that brain volume may be too coarse a metric to detect neuroanatomical differences related to habitat preferences in squamates, at least in some cases. For example, the discrepancy between our results and those of Powell & Leal [17] could be explained by neurons varying in number, size and structure (e.g. more numerous, smaller neurons), which could result in variation in neuron number while brain volume remains constant [6,7,40]. Differences in neuron number are expected to be more closely linked to functional differences related to behaviour and cognition compared to relative brain size.

Our interpretations are based on models that do not account for phylogenetic non-independence (see Methods). Ancestral state reconstruction shows that neuron number is interspersed across species (electronic supplementary material, figures S5–7), suggesting that neuron number is evolutionarily labile. We present phylogenetically corrected models (PCM) in the electronic supplementary material. However, the PCM models yielded disparate results from our non-PCM models and failed to support established patterns of neuronal scaling with respect to brain mass, likely related to the small number of species examined. We encourage future studies with larger samples of species to consider phylogeny in analyses exploring the link between habitat complexity and neuroanatomy in *Anolis*.

Our results suggest that species found in habitats of higher complexity have a greater number of neurons at a given brain mass, leading to higher neuron densities in the telencephalon

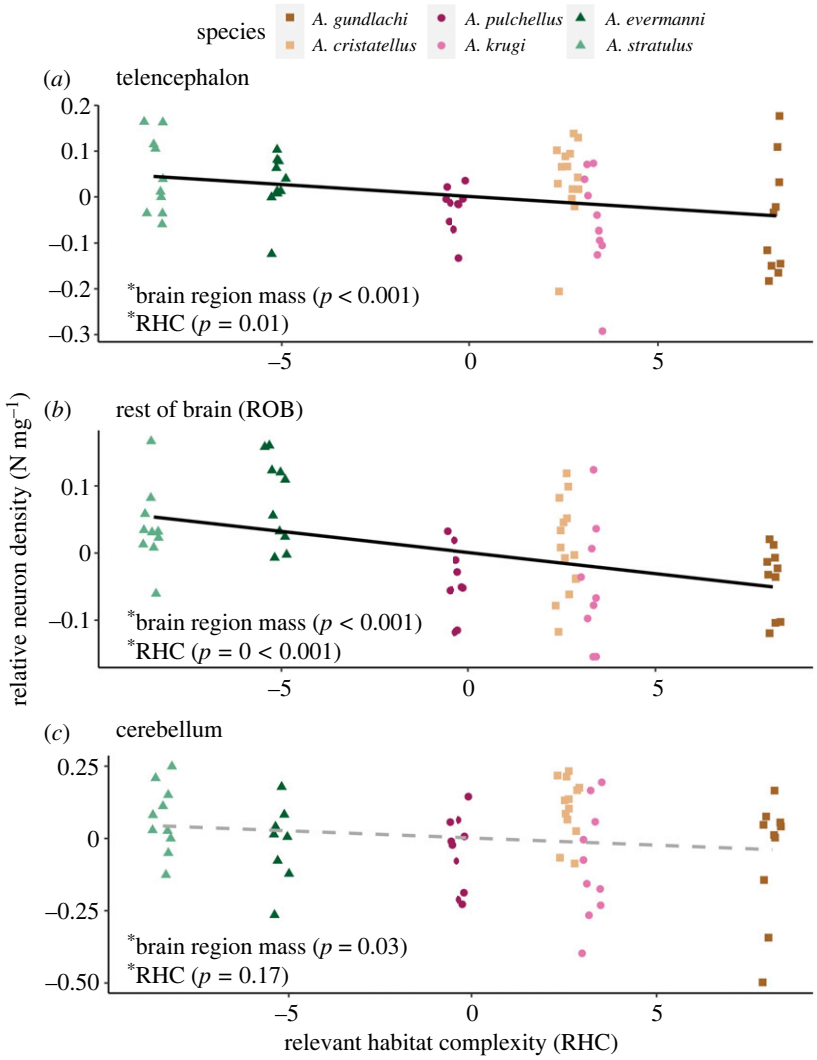

Figure 1. Relationship between relative neuron density and relevant habitat complexity. Each point represents an estimate for a single individual, with colour representing species and shape distinguishing different ecomorphological classes. Relevant neuron density is the residual for each individual when the number of neurons is regressed on brain region mass, yielding a normalized measure of neuron density in neurons per milligram. Habitat complexity decreases with increasing values of RHC. Significant relationships are plotted with a solid line.

and ROB (i.e. more neurons per milligram of brain mass). In Puerto Rican anoles, species living in the canopy experience the most complex habitats while species living in the understorey, such as on tree trunks, grass and shrubs, experience the least complex habitats. Cognitive demands are likely higher in more complex habitats, as an animal must coordinate movement along a larger number of potential paths. Furthermore, for territorial species like anoles, the degree of habitat complexity also impacts maintenance of territorial boundaries and recognition of neighboring conspecifics [22]. Increases in neuron density could support the greater cognitive demands of these behaviors in more complex habitats. Experimental studies have shown anoles can solve multiple cognitive tasks [41,42], suggesting an unexpected level of behavioural flexibility. Although neuron density is linked to cognition [5], it is still unclear how neuron density relates to cognition in anoles [28] and in vertebrates more generally.

The association we observed between habitat complexity and neuroanatomy in *Anolis* parallels findings in other vertebrate taxa. Studies in Aegean wall lizards [43], three-spine sticklebacks [44] and zebrafish [45] found that individuals from more complex habitats performed better in spatial learning tasks. Other studies report similar findings at the level of neuroanatomy [14–16,18,20,21,46,47] (but see [19,48]) and cognition [49].

Few empirical studies have explored the effect of neuron density on behaviour and cognition. Theory suggests that higher neuron packing density enables signals to transmit more efficiently in the brain [5,6,50,51]. In primates, neuron density correlates with connectivity [52]. Cognitive abilities observed in primates [7,35,53] and core land birds [7,54] could be related to their high neuron densities. Three species of corvids were found to have higher neuron densities relative to other birds in the associative pallium, a brain region that supports complex cognition in these species [11]. Cortical neuron density is linked to quantitative discrimination [55] (see also [56]). Diurnal primates, which rely heavily on vision, have high neuron densities in visual areas of the cerebral cortex [53]. Nevertheless, the functional implications of neuron density on behaviour are largely unknown and require further investigation.

Historically, the evolution of neuroanatomy and its relationship to species ecology has been investigated primarily at the level of brain size. Here we found that the number of neurons in the telencephalon and ROB increased with habitat complexity across six closely related species of Puerto Rican anoles. These results suggest that habitat complexity has potentially selected for interspecific differences in neuroanatomy across the Puerto Rican anole radiation. Further studies

are needed to test if this pattern is present across other anole radiations. Furthermore, our results demonstrate that novel patterns in brain evolution can be revealed by using alternative measures of neuroanatomy [6,8].

Ethics. This research adhered to the guidelines of the Institutional Animal Care and Use Committee at University of Missouri, protocol (no. 8244). We followed the Recommendations for the Care of Amphibians and Reptiles [57] in the treatment of all animals used in this study. The Departamento de Recursos Naturales y Ambientales of Puerto Rico issued permits (DRNA 2022-IC-045) for the collection of animals used in this study.

Data accessibility. The datasets generated and analysed during the current study are available as electronic supplementary material [58].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. L.S.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing—original draft, writing—review and editing; J.G.: data curation, investigation, writing—review and editing; C.A.P.-M.: data curation, investigation, methodology, writing—review and editing; M.L.: conceptualization, funding acquisition, methodology, resources, supervision, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare that we have no competing interests.

Funding. Funding was provided by the National Science Foundation (grant no. IOS-1051793) to M.L. and Sigma Xi Grant-in-Aid of Research to L.S.

Acknowledgements. We thank University of Missouri Molecular Cytology Core for supporting our microscopy needs; the members of the Chipojo lab; and anonymous reviewers that provided comments on this manuscript.

References

1. Jerison HJ. 1973 *Evolution of the brain and intelligence*. New York, NY: Academic Press.
2. Striedter GF. 2005 *Principles of brain evolution*. Sunderland, MA: Sinauer Associates.
3. Healy SD, Rowe C. 2007 A critique of comparative studies of brain size. *Proc. R. Soc. B* **274**, 453–464. (doi:10.1098/rspb.2006.3748)
4. Logan CJ *et al.* 2018 Beyond brain size: uncovering the neural correlates of behavioral and cognitive specialization. *Comp. Cogn. Behav. Rev.* **13**, 55–89. (doi:10.3819/ccbr.2018.130008)
5. Dicke U, Roth G. 2016 Neuronal factors determining high intelligence. *Phil. Trans. R. Soc. B* **371**, 20150180. (doi:10.1098/rstb.2015.0180)
6. Nemec P, Osten P. 2020 The evolution of brain structure captured in stereotyped cell count and cell type distributions. *Curr. Opin. Neurobiol.* **60**, 176–183. (doi:10.1016/j.conb.2019.12.005)
7. Kverková K *et al.* 2022 The evolution of brain neuron numbers in amniotes. *Proc. Natl Acad. Sci. USA* **119**, e2121624119. (doi:10.1073/pnas.2121624119)
8. Herculano-Houzel S. 2011 Brains matter, bodies maybe not: the case for examining neuron numbers irrespective of body size. *Ann. NY Acad. Sci.* **1225**, 191–199. (doi:10.1111/j.1749-6632.2011.05976.x)
9. Marhounova L, Kotrschal A, Kverkova K, Kolm N, Nemec P. 2019 Artificial selection on brain size leads to matching changes in overall number of neurons. *Evolution* **73**, 2003–2012. (doi:10.1111/evo.13805)
10. Sol D *et al.* 2022 Neuron numbers link innovativeness with both absolute and relative brain size in birds. *Nat. Ecol. Evol.* **6**, 1381–1389. (doi:10.1038/s41559-022-01815-x)
11. Ströckens F, Neves K, Kirchem S, Schwab C, Herculano-Houzel S, Güntürkün O. 2022 High associative neuron numbers could drive cognitive performance in corvid species. *J. Comp. Neurol.* **530**, 1588–1605. (doi:10.1002/cne.25298)
12. Godfrey-Smith P. 2002 Environmental complexity and the evolution of cognition. In *The evolution of intelligence* (eds RJ Sternberg, JC Kaufman), pp. 223–249. Mahwah, NJ: Lawrence Erlbaum Associates Publishers.
13. Shumway C. 2008 Habitat complexity, brain, and behavior. *Brain. Behav. Evol.* **72**, 123–134. (doi:10.1159/000151472)
14. Bauchot R, Bauchot M, Platel R, Ridet JM. 1977 Brains of Hawaiian tropical fishes; brain size and evolution. *Copeia* **1977**, 42–46. (doi:10.2307/1443502)
15. Abbott ML, Walsh CJ, Storey AE, Stenhouse IJ, Harley CW. 1999 Hippocampal volume is related to complexity of nesting habitat in Leach's storm-petrel, a nocturnal procellariiform seabird. *Brain. Behav. Evol.* **53**, 271–276. (doi:10.1159/000006598)
16. Safi K, Dechmann DKN. 2005 Adaptation of brain regions to habitat complexity: a comparative analysis in bats (Chiroptera). *Proc. Biol. Sci.* **272**, 179–186. (doi:10.1098/rspb.2004.2924)
17. Powell BJ, Leal M. 2014 Brain organization and habitat complexity in *Anolis* lizards. *Brain Behav. Evol.* **84**, 8–18. (doi:10.1159/000362197)
18. Sobrero R, Fernandez-Aburto P, Ly-Prieto A, Delgado SE, Mpodozis J, Ebensperger LA. 2016 Effects of habitat and social complexity on brain size, brain asymmetry and dentate gyrus morphology in two octodontid rodents. *Brain. Behav. Evol.* **87**, 51–64. (doi:10.1159/000444741)
19. Ahmed NI, Thompson C, Bolnick DI, Stuart YE. 2017 Brain morphology of the threespine stickleback (*Gasterosteus aculeatus*) varies inconsistently with respect to habitat complexity: a test of the Clever Foraging Hypothesis. *Ecol. Evol.* **7**, 3372–3380. (doi:10.1002/ece3.2918)
20. Budeau DA, Verts BJ. 1986 Relative brain size and structural complexity of habitats of chipmunks. *J. Mammal.* **67**, 579–581. (doi:10.2307/1381291)
21. Calisi RM, Chintamen S, Ennin E, Kriegsfeld L, Rosenblum EB. 2017 Neuroanatomical changes related to a changing environment in lesser earless lizards. *J. Herpetol.* **51**, 258–262. (doi:10.1670/16-056)
22. Losos JB. 2009 *Lizards in an evolutionary tree: ecology and adaptive radiation of anoles*. Berkeley, CA: University of California Press.
23. Gunderson AR, Mahler DL, Leal M. 2018 Thermal niche evolution across replicated *Anolis* lizard adaptive radiations. *Proc. R. Soc. B* **285**, 20172241. (doi:10.1098/rspb.2017.2241)
24. Johnson MA, Revell LJ, Losos JB. 2010 Behavioral convergence and adaptive radiation: effects of habitat use on territorial behavior in *Anolis* lizards. *Evolution* **64**, 1151–1159. (doi:10.1111/j.1558-5646.2009.00881.x)
25. Williams EE. 1972 The origin of faunas. Evolution of lizard congeners in a complex island fauna: a trial analysis. In *Evolutionary biology* (eds T Dobzhansky, MK Hecht, WC Steere), pp. 47–89. New York, NY: Springer.
26. Powell BJ, Leal M. 2012 Brain evolution across the Puerto Rican anole radiation. *Brain Behav. Evol.* **80**, 170–180. (doi:10.1159/000341161)
27. Gorman GC, Licht P. 1974 Seasonality in ovarian cycles among tropical *Anolis* lizards. *Ecology* **55**, 360–369. (doi:10.2307/1935223)
28. Storks L, Powell BJ, Leal M. 2020 Peeking inside the lizard brain: neuron numbers in *Anolis* and its implications for cognitive performance and vertebrate brain evolution. *Int. Comp. Biol.* **63**, 223–237. (doi:10.1093/icb/icaa129)
29. Briscoe SD, Ragsdale CW. 2019 Evolution of the chordate telencephalon. *Curr. Biol.* **29**, R647–R662. (doi:10.1016/j.cub.2019.05.026)
30. Sultan F, Glickstein M. 2007 The cerebellum: comparative and animal studies. *Cerebellum* **6**, 168. (doi:10.1080/14734220701332486)
31. Wyneken J. 2007 Reptilian neurology: anatomy and function. *Vet. Clin. North Am. Exot. Anim. Pract.* **10**, 837–853. (doi:10.1016/j.cvex.2007.05.004)
32. R Development Core Team. 2008 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org/.
33. RStudio Team. 2015 *RStudio: integrated development environment for R*. Boston, MA: RStudio, Inc.
34. Hadfield JD. 2010 MCMC methods for multi-response generalized linear mixed models: the

MCMCglmm R package. *J. Stat. Softw.* **33**, 1–22. (doi:10.18637/jss.v033.i02)

35. Herculano-Houzel S, Manger PR, Kaas JH. 2014 Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. *Front. Neuroanat.* **8**, 77. (doi:10.3389/fnana.2014.00077)
36. Kverkova K, Polonyiova A, Kubicka L, Nemec P. 2020 Individual and age-related variation of cellular brain composition in a squamate reptile. *Biol. Lett.* **16**, 20200280. (doi:10.1098/rsbl.2020.0280)
37. Münkemüller T, Lavergne S, Bzeznik B, Dray S, Jombart T, Schiffers K, Thuiller W. 2012 How to measure and test phylogenetic signal. *Methods Ecol. Evol.* **3**, 743–756. (doi:10.1111/j.2041-210X.2012.00196.x)
38. Boettiger C, Coop G, Ralph P. 2012 Measuring the power of comparative methods: is your phylogeny informative? *Evolution* **66**, 2240–2251. (doi:10.1111/j.1558-5646.2011.01574.x)
39. De Meester G, Huyghe K, Van Damme R. 2019 Brain size, ecology and sociality: a reptilian perspective. *Biol. J. Linn. Soc.* **126**, 381–391. (doi:10.1093/biolinnean/bly206)
40. Smaers JB *et al.* 2021 The evolution of mammalian brain size. *Sci. Adv.* **7**, eabe2101. (doi:10.1126/sciadv.abe2101)
41. Leal M, Powell BJ. 2012 Behavioural flexibility and problem-solving in a tropical lizard. *Biol. Lett.* **8**, 28–30. (doi:10.1098/rsbl.2011.0480)
42. Storks L, Leal M. 2020 Thinking outside the box: problem-solving in free-living lizards. *Behav. Ecol. Sociobiol.* **74**, 75. (doi:10.1007/s00265-020-02852-x)
43. De Meester G, Pafilis P, Vasilakis G, Van Damme R. 2022 Exploration and spatial cognition show long-term repeatability but no heritability in the Aegean wall lizard. *Anim. Behav.* **190**, 167–185. (doi:10.1016/j.anbehav.2022.06.007)
44. Odling-Smee LC, Boughman JW, Braithwaite VA. 2008 Sympatric species of threespine stickleback differ in their performance in a spatial learning task. *Behav. Ecol. Sociobiol.* **62**, 1935–1945. (doi:10.1007/s00265-008-0625-1)
45. Roy TJ, Bhat A. 2016 Learning and memory in juvenile zebrafish: what makes the difference—population or rearing environment? *Ethology* **122**, 308–318. (doi:10.1111/eth.12470)
46. Shumway CA. 2010 The evolution of complex brains and behaviors in African cichlid fishes. *Curr. Zool.* **56**, 144–156. (doi:10.1093/czoolo/56.1.144)
47. Chung W-S, Kurniawan ND, Marshall NJ. 2022 Comparative brain structure and visual processing in octopus from different habitats. *Curr. Biol.* **32**, 97–110. (doi:10.1016/j.cub.2021.10.070)
48. Axelrod CJ, Laberge F, Robinson BW. 2021 Interspecific and intraspecific comparisons reveal the importance of evolutionary context in sunfish brain form divergence. *J. Evol. Biol.* **34**, 639–652. (doi:10.1111/jeb.13763)
49. White GE, Brown C. 2015 Cue choice and spatial learning ability are affected by habitat complexity in intertidal gobies. *Behav. Ecol.* **26**, 178–184. (doi:10.1093/beheco/aru178)
50. Changizi MA. 2001 Principles underlying mammalian neocortical scaling. *Biol. Cybern.* **84**, 207–215. (doi:10.1007/s004220000205)
51. Chittka L, Niven J. 2009 Are bigger brains better? *Curr. Biol.* **19**, R995–R1008. (doi:10.1016/j.cub.2009.08.023)
52. Beul SF, Hilgetag CC. 2019 Neuron density fundamentally relates to architecture and connectivity of the primate cerebral cortex. *Neuroimage* **189**, 777–792. (doi:10.1016/j.neuroimage.2019.01.010)
53. Collins CE, Airey DC, Young NA, Leitch DB, Kaas JH. 2010 Neuron densities vary across and within cortical areas in primates. *Proc. Natl Acad. Sci. USA* **107**, 15927. (doi:10.1073/pnas.1010356107)
54. Olkowicz S, Kocourek M, Lučan RK, Porteš M, Fitch WT, Herculano-Houzel S, Němec P. 2016 Birds have primate-like numbers of neurons in the forebrain. *Proc. Natl Acad. Sci. USA* **113**, 7255–7260. (doi:10.1073/pnas.1517131113)
55. Bryer MAH *et al.* 2022 The evolution of quantitative sensitivity. *Phil. Trans. R. Soc. B* **377**, 20200529. (doi:10.1098/rstb.2020.0529)
56. Fang WQ, Yuste R. 2017 Overproduction of neurons is correlated with enhanced cortical ensembles and increased perceptual discrimination. *Cell Rep.* **21**, 381–392. (doi:10.1016/j.celrep.2017.09.040)
57. Pough FH. 1991 Recommendations for the care of amphibians and reptiles in academic institutions. *ILAR J.* **33**, S1–S21. (doi:10.1093/ilar.33.4.S1)
58. Storks L, Garcia J, Perez-Martinez CA, Leal M. 2024 Habitat complexity influences neuron number in six species of Puerto Rican *Anolis*. Figshare. (doi:10.6084/m9.figshare.c.7043223)