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Neuron numbers link innovativeness with both absolute and relative brain size

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Short Title: Brains, neurons and cognition

Abstract

A longstanding issue in biology is whether the intelligence of animals can be predicted by absolute or relative brain size. However, progress has been hampered by an insufficient understanding of how neuron numbers shape internal brain organization and cognitive performance. Based on estimations of neuron numbers for 111 bird species, we show here that the number of neurons in the pallial telencephalon is positively associated with a major expression of intelligence: innovation propensity. The number of pallial neurons, in turn, is greater in brains that are larger in both absolute and relative terms, and positively co-varies with longer post-hatching development periods. Thus, our analyses show that neuron numbers link cognitive performance to both absolute and relative brain size through developmental adjustments. These findings help unify neuro-anatomical measures at multiple levels, reconciling contradictory views over the biological significance of brain expansion. The results also highlight the value of a life history perspective to advance our understanding of the evolutionary bases of the connections between brain and cognition.

Main

Encephalization—the evolutionary increase of the brain beyond that expected for a given body size[1]—has long been thought to be a major factor in the evolution of intelligence[2,3]. Comparisons across species have provided some support for this theory, showing that encephalization is associated with several facets of intelligence like innovativeness, learning and culture[4–8]. The theory has also stimulated an extended research program on the ecological and evolutionary implications of brain size and architecture[9–11]. Yet, the reasons why a disproportionately larger brain should provide cognitive advantages remain unclear[3,12,13].

The rationale of the encephalization theory, as originally envisioned by Jerison[14], is that the "extra tissue" that makes the brain larger than expected for a given body size (i.e., larger relative brain size) reflects extra neurons that are available for cognitive tasks. However, the notion that cognitive performance depends on neuron numbers and increases with encephalization is backed by insufficient evidence[3,15,16]. Moreover, given that neuron numbers increase with absolute brain size[3,17], should we not expect that cognitive differences across species will be better predicted by absolute rather than relative brain size? There is indeed evidence that absolute brain size sometimes predicts cognitive performance across species better than relative measures of the brain[18–20]. Such results are not surprising given that increases in relative brain size can be reached by both brain enlargement and body size reduction, and therefore may not always be associated with an increase in brain information-processing capacity[21,22]. The debate regarding the biological significance of absolute and relative brain sizes has been further complicated by the finding that not all brains are made in the same way; rather, brains may show different neuron densities and distributions among brain areas across species[12,15,23–25]. Thus, the intuitively appealing notion that larger brains translate into greater intelligence remains contentious[3,12,13].

To address this longstanding controversy, we provide theoretical and empirical grounds for the hypothesis that increased intelligence—operationally defined here as the ability to solve problems through mental or behavioural flexibility[3]— requires brains that are large in both absolute and relative terms (**Fig. 1**). This possibility has probably gone unrecognized because previous studies have used an 'either-or' approach and pitted absolute against relative brain size measures[18,20]. Yet if enhanced cognition requires more neurons in sensory, associative

and premotor areas of the telencephalon —the pallial areas in birds and the neocortex in mammals[3,24] — and these areas represent a large fraction of the telencephalon and the whole brain[26], then the accumulation of a disproportionately large number of pallial neurons should produce brains that are larger both in absolute terms and relative to body size[27,28]. Such co-variation between absolute and relative brain size should be more accentuated if the selective advantages of accumulating greater numbers of pallial neurons is higher for larger species than for small ones. This is to be expected because body size is a major correlate of longevity[29], and a long life increases the fitness value of gathering information and learning[30–33] while reducing the costs of delaying reproduction[34,35]. One mechanism that may allow a greater accumulation of pallial neurons is, according to some evo-devo models, an extension of development periods, particularly the later stages in altricial offspring that are born underdeveloped[36,37]. Thus, selection on cognition might link intelligence with larger absolute and relative brain size through developmental adjustments (**Fig. 1**).

Testing the above tenets is challenging owing to the difficulties of accurately estimating neuron numbers of different brain regions for many species[15]. The isotropic fractionator—a new method of assessing neuron numbers developed by Herculano-Houzel and collaborators[38]— now makes it possible. Our study is based on a substantially updated dataset[24,25,39] quantifying neuron numbers in the whole brain and three brain areas (the pallium, the cerebellum and the brainstem) for 111 species of 24 avian families, representing both basal and crown avian lineages (**Fig. 1e; Supplementary Fig. 1**) and encompassing a large fraction of the morphospace occupied by avian brains (**Supplementary Fig. 2**). To test associations with cognition, we focus on a major component of intelligence, innovativeness[40], by quantifying its product —innovation frequency[4–6,41]. Our innovation data were extracted from a database including >4400 published reports of bird species using novel foods or new feeding techniques in the wild[42]. On this basis, we first ask whether innovation propensity increases with the number of neurons in the pallium (and potentially also in the cerebellum, which is thought to co-evolve and function in tandem with the pallium[43,44]), but not with those in areas less directly involved in cognition like the brainstem. Next, we investigate whether the proliferation of neurons in the pallium makes the brain increase disproportionally with body size, linking innovativeness with both absolute and relative brain size. Finally, we test whether the accumulation of neurons in the pallium is associated with an extension of later stages of

development. We test these predictions by combining random forests, a type of machine-learning algorithm that allows us to accommodate complex non-linear interactions among predictors with minimal assumptions[45], with Bayesian mixed models that explicitly account for phylogenetic relatedness among species[46]. Because nocturnal species are difficult to observe, and hence are not present in the innovation data set, we exclude owls from all the analyses that follow; results with the entire dataset are shown in the Supplementary Information.

Results and discussion

Cognitive performance has long been thought to depend on the number of neurons in the brain[47,48], but this hypothesis is currently backed by surprisingly little empirical evidence[15]. A comparison of apes, corvids and pigeons in five cognitive domains concluded that neuron number is a poor predictor of absolute cognitive performance, but it may predict learning speed and the ability to plastically adjust rules to novel situations[47]. A broader comparative analysis across primates and birds revealed that performance in a cognitive task (the detour test) does tend to increase with the total number of cortical/pallial neurons[15], yet this study did not rule out the possibility that the association was driven by phylogenetic relatedness.

Using Bayesian phylogenetic mixed models, we found that the number of neurons in the entire brain is positively associated with behavioural innovation propensity (**Fig. 2a**), particularly technical innovations that are assumed to require more advanced cognition[5] **(Supplementary Table 1)**. The pattern holds when body mass is included as a covariate in the model (**Fig. 2b; Supplementary Table 2**), suggesting that innovation propensity is higher in birds with a disproportionately larger number of neurons than expected on the basis of body size. While we find that the brain of innovative species contains more neurons than the brain of less innovative species, there is no parallel increase in neuron density; rather, innovation propensity decreases with neuron density (**Fig. 2c**). Because avian neuron densities tend to decrease with brain size and the total number of neurons, these results support the notion that cognitive performance is primarily limited by the absolute and relative number of neurons rather than by neuron densities.

Additional analyses revealed that the number of neurons in the pallium and, to a lesser extent, the cerebellum are better predictors of innovation propensity than neurons in the brainstem, a brain area less directly involved in cognition (**Fig. 2;**

Supplementary Tables 1 and 2). Although pallial areas are thought to co-evolve and function in tandem with the cerebellum[43,44], it remains to be determined whether the avian cerebellum subserves motor skills only (as its association with technical innovations but not resource innovations suggests; **Supplementary Tables 2**) or is also directly involved in cognitive functions like the mammalian cerebellum[43]. Nonetheless, our findings align with growing evidence that cognitive processes associated with intelligence are controlled by widely-distributed networks integrating several brain areas[49].

Corvids and parrots are regarded as the most innovative birds, a conclusion that is backed by ample experimental evidence[47,48,50,51]. These taxa also share both the highest inferred rates of brain-body size evolution among Neoaves and the steepest allometric slopes among all birds[52]. This contrasts with less innovative taxa like early-diverging birds (Palaeognathae, basal Neognathae), Anseriformes (waterfowl), and predatory core landbirds (hawks & eagles, falcons and owls), whose allometric exponents have diverged little from the ancestral avian grade and hence represent low-slope grades. To assess whether the proliferation of neurons in the pallium can explain deviations from the "ancestral" allometric scaling relationship, we estimated the allometric exponents of the neuron numbers for clades with the highest slope and low-slope grades (sensu [52]); we then compared these with the allometric exponents for the cerebellum and brainstem. We find that while the allometric exponents for the cerebellum and brainstem were similar between the two slope grade groups, clades that share a high slope tended to accumulate disproportionately more neurons in the pallium as they become larger (**Fig. 3**, **Supplementary Figs. 3-5**). Thus, as expected, the accumulation of pallial neurons makes the brain increase in both absolute and relative terms (**Fig. 4, Supplementary Figs. 6 and 7**).

While the number of neurons in the whole brain and the pallium increases in a similar way with both absolute and relative brain size (**Fig. 4**), the number of neurons in the cerebellum is more strongly related to absolute brain size alone; those in the brainstem do not follow any clear pattern. These conclusions are consistent regardless of the method used to estimate relative brain size (**Supplementary Fig. 7**); they also hold when we include owls (**Supplementary Figs. 6 and 7**), whose large forebrain results in part from expanding the visual Wulst for sensory rather than associative purposes. Although the evolutionary repatterning of the brain-body relationship cannot be circumscribed to selection on brain size alone, our results support the notion

194 that cognition can form a major driver of adaptive shifts to higher grades deviations
195 from the "ancestral" allometric scaling.
196 Presumably because brain cellular scaling rules can be clade-specific[15,23–25,53]
197 and because avian neuron densities decrease with brain mass[24,25], the relationship
198 between neuron numbers and brain size is complex. The relationship tends to be
199 roughly linear for relative brain size, especially when we exclude owls, but only for
200 the entire brain and the pallium (**Fig. 4b**). In contrast, neuron numbers tend to
201 asymptote at larger absolute brain sizes in all cases (**Fig. 4c**). This latter finding agrees
202 with the notion that animals that have large brains merely because they have very big
203 bodies are not necessarily the most intelligent, as it is the case for Ratites and large
204 Galliformes.
205 Several developmental mechanisms, including differences in early morphogen
206 patterning and expansion of stem cell pool, diversification of neural progenitors,
207 variation of cell-cycle rates and protracted neurogenesis are responsible for expansion
208 of the telencephalon in amniotes[37,54–56]. We asked whether these mechanisms were
209 reflected in the duration of embryonic and post-hatching development periods. We
210 found that longer development time leads to a greater accumulation of number of
211 neurons in the pallium of clades with high-slope grades than in those showing low-
212 slope grades (**Fig. 5; Supplementary Fig. 8**). This accumulation of neurons is
213 associated with an extension of the post-natal (fledging) development relative to the
214 embryonic period. Importantly, scaling of pallial neuron counts with development
215 strongly resembles that found for the total number of brain neurons, reinforcing the
216 notion that the number of pallial neurons largely accounts for the much larger number
217 of neurons in the brains of altricial species.
218 Growing evidence suggests that different mechanisms underlie telencephalon
219 growth and maturation in precocial and altricial birds, leading to relatively larger
220 relative brains in the latter. Precocial birds like ducks and grouse enlarge their
221 telencephalon early in development (before the onset of neurogenesis) presumably by
222 an increase in the number of telencephalic progenitors[57]. In contrast, expansion of the
223 telencephalon in altricial birds like songbirds and parrots is associated with protracted
224 neurogenesis and delayed neuronal maturation[58]. Our analyses are consistent with
225 these patterns (**Supplementary Fig. 9**), showing that longer development time leads
226 to greater accumulation of neurons in the pallium in altricial than in precocial species.
227 Indeed, all species from our data set belonging to the highest slope grade category

show high degree of altriciality (i.e. they are classified as super-altricial). Altogether, our results are consistent with the view that increases in absolute and relative brain size are made possible through the evolutionary transition to altriciality and changes in neurogenesis schedules related to a disproportionate lengthening of the later stages of development. While this pattern is predicted by some models of brain development, like the 'late is large' rule[37], additional research is needed to elucidate the exact mechanisms.

Our analyses unify neuro-anatomical measures at multiple levels. First, we provide firm support for the intuitively appealing notion that cognitive performance is limited by the number of neurons in the pallium and, to a lesser extent, the cerebellum. Thus, our results support the hypothesis[14] that intelligence reflects a disproportionate allocation of neurons to cognitive tasks, but also aligns with suggestions that cognitive performance ultimately depends on the total number of neurons and the way neurons connect different brain areas[3,15,23]. Second, we show that an increase in the number of neurons in the areas most closely involved in cognitive performance, the pallium, increases brain size in both absolute and relative terms. Although the number of neurons in the cerebellum scaled primarily with absolute brain size, the effect of total neuron numbers on relative brain size persisted because in birds larger brains contain increasing proportions of neurons in the pallium and decreasing proportions in the cerebellum and other brain regions[24]. Third, we provide an adaptive explanation for some of the patterns of brain-body co-variation in deep time detected by Ksepka et al.[52]: Clades that have a higher brain-body slope than others tend to be the ones that are most innovative. A higher brain-body slope means that as body size gets bigger, the brain increases disproportionately more in size than it does in non-innovative clades; this increase in both absolute and relative brain size is, according to our analyses, mostly due to an increase in pallial neurons. Finally, we provide a developmental rationale for the observed patterns, suggesting that the elongation of the fledging period in altricial species links neuron numbers with absolute and relative brain size. The failure to find a similar pattern in precocial species supports the notion that not all brains are made in a same way[25,53], highlighting the key role of life history in brain evolution (**Supplementary Fig. 10**).

The reason why the dual role of absolute and relative brain size in cognition has been under-appreciated in the past probably reflects the common practice of removing the allometric effects of body size in comparative analyses of brain size. As

suggested by Jerison[2], this is probably legitimate when comparing brains of species with striking differences in body size, like an ostrich and a hummingbird. Yet by treating body size as a statistical nuisance, we appear to be missing important information. A larger body is often associated with greater longevity[59] and can reduce juvenile mortality risk[35], which should increase the value of learning and reduce the costs of a long development time[30,31,60]. Alternatively, the same environmental pressures that favour a slow pace of life could generate correlated selection on both cognition and body size[31]. Whether a large body facilitates selection for cognition or co-varies with cognition due to either correlated selection or shared developmental processes, the consequence for functional architecture of the brain is to link neuron numbers and cognitive performance to both absolute and relative brain size.

Methods

Neuron numbers estimation. Our study is based on an updated database quantifying neuron numbers in the whole brain and three brain areas – the pallium (comprising the hyperpallium, mesopallium, nidopallium, acropallium and hippocampus), the cerebellum and the brainstem (comprising the medula oblongata and midbrain tegmentum) for bird species. Information for 65 avian species were extracted from the literature[24,25]. Numbers of brain, pallial and cerebellar neurons for an additional 81 individuals representing 46 species and number of brainstem neurons for an additional 172 individuals representing 83 avian species were newly estimated using the isotropic fractionator, following experimental procedures described in Olkowicz *et al.*[24]. Briefly, animals were killed by an overdose of halothane, weighed and immediately perfused transcardially with warmed phosphate-buffered saline containing 0.1% heparin followed by cold phosphate-buffered 4% paraformaldehyde solution. The brains were immediately removed, weighted, postfixed for an additional 7-21 days and then dissected into the examined brain divisions. The cerebral hemispheres (including the olfactory bulbs) were detached from the diencephalon by a straight cut separating the subpallium from the thalamus. The cerebellum was cut off at the surface of the brainstem. The tectum (optic lobe) was bilaterally excised from the surface of the brainstem. The excised parts included most of the tectal gray, optic tectum and torus semicircularis. The remaining structures were dissected into diencephalon (rostral part) and brainstem (caudal part comprising the medula oblongata and midbrain tegmentum) along the plane connecting the posterior commissure dorsally and hypothalamus-mesencephalon boundary ventrally. The latter is visible macroscopically as a groove between the convex ventral part of the midbrain and the hypothalamus, caudally to the infundibulum and mammillary bodies. In one individual per species, one hemisphere was

dissected into the pallium and the subpallium. These hemispheres were embedded in agarose and sectioned on a vibratome at 300–500 μm (depending on size of a hemisphere) in the coronal plane. Under oblique transmitted light at the stereomicroscope and with the use of a microsurgical knife (Stab Knife Straight, 5.5 mm, ref 7516, Surgical Specialities Corporation, Reading, PA, USA) we manually dissected the pallium from subpallium on each section by cutting along the pallial-subpallial lamina, as defined by Reiner et al.[62].

The dissected structures were dried with a paper towel, weighed to the nearest 0.1 milligram, incubated in 30% sucrose solution until they sank, then transferred into antifreeze (30% glycerol, 30% ethylene glycol, 40% phosphate buffer) and frozen for further processing. The examined brain parts were homogenized in 40 mM sodium citrate with 1% Triton X-100 using Tenbroeck tissue grinders (Wheaton, Millville, NY, USA) to obtain a suspension of free cell nuclei. The fluorescent DNA marker 4′,6-diamidino-2-phenylindole (DAPI) was added (0.5 mg / l) to stain the nuclei. Afterwards the homogenate was adjusted to defined volume and the mixture was kept homogenous by agitation. The total number of cells was estimated by counting at least five aliquots of 10 μl using a Neubauer improved counting chamber (BDH, Dagenham, Essex, UK) with an Olympus BX51 microscope equipped with epifluorescence and appropriate filter settings; additional aliquots were counted if needed to reach the coefficient of variation among counts ≤0.10. The proportion of neurons was determined by immunocytochemical detection of the neuronal nuclear marker NeuN[63]. This neuron-specific protein was detected by an anti-NeuN mouse monoclonal antibody (clone A60, Sigma-Aldrich; dilution 1:800), which was characterized by Western blotting with chick brain samples and shown to react with a protein of the same molecular weight as in mammals, indicating that it does not cross-react with other proteins in birds[64]. The binding sites of the primary antibody were revealed by Alexa Fluor 546-conjugated goat anti-mouse IgG antibody (Life Technologies, Carlsbad, CA, USA; dilution 1:400). An electronic hematologic counter (Alchem Grupa, Torun, Poland) was used to count the proportion of double-labelled nuclei in the Neubauer chamber. At least 500 nuclei were examined for each sample. The final data set included information on neuron numbers for 240 specimens belonging to 111 species. For *Caloenas nicobarica* and *Eudromia Formosa,* information on pallial neurons was missing and had to be imputed to avoid comparing results with different sample sizes. We estimated these missing data by combining phylogenetic imputation with multivariate data (brain and body size, and number of neurons in the entire brain, the pallium, the cerebellum and the brainstem), as implemented in the R-package *phytools*[65]. We note that results hold whether or not these two species were included in the analyses.

Innovation data. Our innovation data were taken from Lefebvre[42], compiled by systematically searching for reports of new behaviours in the short notes of 204 ornithology journals published between 1960 and 2020. The criterion to accept an innovation was that the report described the behaviour with key words such as 'novel', 'not noted before' and/or 'unusual'. Each innovation was classified as a resource innovation —if it involved a novel food item—, or a technical innovation —if the searching and handling techniques were themselves novel regardless of whether the food type was novel or not[5]. Nocturnal clades were excluded due to the difficulty of being observed. The frequency with which a species was observed innovating in the wild was used to characterize the propensity of the species to innovate. Innovation propensity depends not only on innovative ability, however, but also on the probability that new behaviours are observed and reported. Thus, a species may have a low number of innovations not because it cannot innovate but because it is rare or secretive, and hence difficult to observe and study. We tackled this issue by considering research effort in the analyses[5,11,31], using data on number of papers published per species[66]. The probability of reporting an innovation may also increase with geographic range, urbanisation and island living, and it can decrease with migratory behaviour[5,11,31]. Therefore, we also included these variables as covariates in the models (see below). Data were drawn from previously assembled datasets. Geographic range (number of 1 degree x 1 degree grid cells overlapping breeding/resident range), mobility (resident, nomadic, migrant and altitudinal migrant) and insularity (proportion of breeding/resident range intersecting with islands of landmass below 2,000 sq km) were extracted from[67], while the occurrence in urban environments was taken from[11].

Life history data. We extracted published information on the duration of incubation (embryonic stage) and fledging periods (post-natal growth) from previously compiled datasets[11,31], updated with information from the online edition of the Handbook of birds of the world (https://birdsoftheworld.org). Information was available for 108 species for incubation duration and 102 species for fledging duration. Post-natal growth fraction was estimated as [fledging/(incubation + fledging)]$^{0.5}$, following[68]. To assign species to different developmental modes, we used the classification recently proposed by Bothelo[69], which divides species in super-precocial, precocial, semi-precocial, semi-altricial, altricial and super-altricial. However, for the questions addressed in the study, and because some categories were insufficiently represented in our data set, we pooled together super-precocial, precocial and semi-precocial species in a general precocial category and semi-altricial, altricial and super-altricial species in an altricial category.

Modelling neurons, brains and innovations. We used Bayesian Generalised Linear Mixed models (BGLMM) based on Markov Chain Monte Carlo (MCMC) approximations to model variation in neuron numbers, brain size and innovation propensity, as implemented in the R-packages *MCMCglmm*[46] and *BRMS*[70]. To ensure that neuron numbers and brain measures were species characters, we first used intra-specific data to assess within-species consistency by means of Gaussian BGLMM, including sex as fixed effect and species as random effect (**Supplementary Table 3**). Consistency was estimated as the Intraclass Correlation Coefficient (ICC), calculated by dividing the variation among species by the total variation (i.e. variation among species plus variation within species, the latter including natural variation and measurement error). The consistency attributed to shared ancestors was estimated in a similar model, but incorporating a variance-covariance matrix of phylogenetic distances as a random effect. This allowed us estimating varying intercepts among species adjusted by phylogenetic dependency. Phylogenetic heritability was estimated as the fraction of total variation accounted for the phylogenetic distance between species (**Supplementary Table 3**).

To test whether neuron numbers affect innovation frequency, we then averaged the values for each species and used phylogenetic BGLMMs with the response variable (innovation frequency) fitted as Gaussian. In these models, biogeographic realm[71] was included as a random effect together with the phylogenetic variance-covariance matrix to allow the integration of global information originating from different regions[31]. To control for potential confounding effects, research effort, geographic range, tolerance to urbanisation, insularity and mobility were included as fixed effects.

Species-level phylogenetic BGLMMs were also used to model neuron numbers as a function of body mass, development duration (incubation and fledging) and incubation fraction. We generally used BRMS with Gaussian responses, switching to Weibull distributions when divergent transitions affected model convergence. To assess whether the relationship varied between low-slope and highest-slope grades, sensu Ksepka et al.[52], we included in the models an interaction with a variable coding for these two groups. Differences between precocial and altricial species was investigated in a similar way.

The phylogenetic hypothesis was a summary trees based on 10,000 trees from one of the backbones of the complete phylogeny of birds[72] available at www.birdtree.org. We note that using the alternative phylogenetic backbone yielded similar results.

For all models, the number of MCMC iterations and the burn-in interval were chosen so as to ensure satisfactory convergence. The priors settings are described in the Supplementary R code. The parameters reported for fixed and random effects are the posterior mode and the 95% lower and upper credibility intervals (CI). We considered the fixed effects

statistically significant when 95% CIs did not include the zero. Conditional effects plots and 95% credible intervals were used to visualize the relationship between predictors and response variables.

Describing neuron numbers as a function of relative and absolute brain size. We used regression-based Random Forests (RF), a type of machine-learning algorithm, to describe neuron numbers as a function of both absolute and relative brain size[73]. When modelling quantitative response variables, RF uses linear regressions to recursively partition the data by means of decision trees. Instead of selecting a best tree, however, the method does so by taking a random sample of the training data and a random selection of variables at each step[45]. For each tree in the RF, the fitted value of each terminal node is the mean of the response variable values, which is averaged over all trees to estimate the fitted values of the RF. The data not used to train the model, the out-of-bag (OOB) sample, provides a way to stabilise the error without having to sacrifice training data to use for validation. In this way, RF allows to efficiently model non-linear relationships and deal with complex interactions between predictors while avoiding over-fitting, producing stable patterns that are more difficult to change with new data and that are less sensitive to outliers.

We modelled neuron numbers (response variable) as a function of relative and absolute brain size (predictors) with the R-package *randomForest*[74]. Following the protocol suggested by Brieuc et al.[45], we ran 500 trees twice and compared the stability of the results (correlation >0.97 in all cases). Deviations between the fitted and observed values were used to compute a "pseudo" R^2. Bi-variate partial dependence (i.e., marginal effects) plots for the last tree in the forest, once the model had converged, were used to visualize the co-variation of neuron numbers with absolute and relative brain size while univariate plots were used to visualize the influence of each predictor separately.

To be included together with absolute brain size in the RF, we estimated relative brain size by means of the *normalized scaled brain index*[61] (NSBI). This approach uses the equation of allometric growth to adjust the brain size of species to that which they would have if all had the same body size, making the values directly comparable:

$$NSBI = Y_i \left[\frac{X_0}{X_i}\right]^b$$

where b is the allometric exponent, Y_i and X_i are, respectively, brain size and body size for the individual i and X_0 is the ancestral body size used to scale all species to the same size. We estimated the allometric exponent b based on a log-log phylogenetic Gaussian BGLMMs of absolute brain size against body mass. To this purpose, we used a previously assembled dataset of brain mass (g) and volume (ml)[75], updated with information on brain mass from

the specimens used to estimate neuron numbers (see above) and with new endocast measures of specimens from museums in Europe and North America (n = 114 specimens). Volumes were obtained by means of the endocast method and converted to mass by multiplying by the density of brain tissue (i.e. 1.036g ml^{-1})[75]. We only used specimens of known body mass, yielding information for 10,523 specimens belonging to 1,977 species. To scale all species to the same size, we used the body mass of the presumed ancestor of current birds (2400 g), as suggested by Torres et al.[76]. In our dataset, for example, the greater adjutant (*Leptoptilos dubius*) has the largest brain (~34g) of the 1976 species from our dataset but this mainly reflects the fact that it is a large bird (~7400g). Using the above normalization technique to scale the brain, the NSBI of the greater adjutant (*Leptoptilos dubius*) —estimated around 6.29— brings the species down to the 300th position of the ranking.

The NSBI is equivalent to the residual approach used in previous studies and hence presumes that species share the same brain:body allometric equation. However, there is evidence that the allometric exponent can exhibit some differences across lineages[52]. Consequently, we used a second NSBI ($NSBI_{grades}$) based on an allometric exponent *b* estimated excluding clades that have been found to exhibit substantial grade shifts in brain:body allometries (i.e. *Anseriformes* and *Neoaves*)[52]. This exponent represents the scaling relationship of birds before some lineages experienced grade shifts (**Fig. 1**), and it is remarkably close to that estimated with the entire dataset (**Supplementary Fig. 11**). Thus, in the main text we present the results based on the $NSBI_{grades}$, which better fit to the proposed theoretical framework (**Fig. 1**).

References

1. Shultz, S. & Dunbar, R. Encephalization is not a universal macroevolutionary phenomenon in mammals but is associated with sociality. Proceedings of the National Academy of Sciences of the United States of America 107, 21582–21586 (2010).
2. Jerison, H. J. Animal intelligence as encephalization. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 308, 21–35 (1985).
3. Roth, G. & Dicke, U. Evolution of the brain and intelligence. Trends in Cognitive Sciences 9, 250–257 (2005).
4. Lefebvre, L., Whitle, P., Lascaris, E. & Finkelstein, A. Feeding innovations and forebrain size in birds. Animal Behaviour 53, 549–560 (1997).
5. Overington, S. E., Morand-Ferron, J., Boogert, N. J. & Lefebvre, L. Technical innovations drive the relationship between innovativeness and residual brain size in birds. Animal Behaviour 78, 1001–1010 (2009).
6. Reader, S. M., Hager, Y. & Laland, K. N. The evolution of primate general and cultural

intelligence. Philosophical Transactions of the Royal Society B: Biological Sciences 366, 1017–1027 (2011).

7. Benson-Amram, S., Dantzer, B., Stricker, G., Swanson, E. M. & Holekamp, K. E. Brain size predicts problem-solving ability in mammalian carnivores. Proceedings of the National Academy of Sciences 113, 2532–2537 (2016).

8. Reader, S. M. & Laland, K. N. Social intelligence, innovation, and enhanced brain size in primates. Proceedings of the National Academy of Sciences 99, 4436–4441 (2002).

9. Fristoe, T. S., Iwaniuk, A. N. & Botero, C. A. Big brains stabilize populations and facilitate colonization of variable habitats in birds. Nature Ecology & Evolution 1, 1706–1715 (2017).

10. van Woerden, J. T., van Schaik, C. P. & Isler, K. Effects of seasonality on brain size evolution: evidence from Strepsirrhine primates. The American Naturalist 176, 758–767 (2010).

11. Ducatez, S., Sol, D., Sayol, F. & Lefebvre, L. Behavioural plasticity is associated with reduced extinction risk in birds. Nature Ecology & Evolution 4, 788–793 (2020).

12. Herculano-Houzel, S. Brains matter, bodies maybe not: The case for examining neuron numbers irrespective of body size. Annals of the New York Academy of Sciences 1225, 191–199 (2011).

13. Logan, C. J. et al. Beyond brain size: Uncovering the neural correlates of behavioral and cognitive specialization. Comparative Cognition & Behavior Reviews 13, 55–89 (2018).

14. Jerison, H. Evolution of the Brain and Intelligence. (Academic Press, 1973).

15. Herculano-Houzel, S. Numbers of neurons as biological correlates of cognitive capability. Current Opinion in Behavioral Sciences 16, 1–7 (2017).

16. Van Schaik, C. P., Triki, Z., Bshary, R. & Heldstab, S. A. A Farewell to the Encephalization Quotient: A New Brain Size Measure for Comparative Primate Cognition. Brain, Behavior and Evolution 96, 1–12 (2021).

17. Striedter, G. F. Principles of brain evolution. (Sinauer Associates, Inc. Sunderland, Mass, 2005).

18. MacLean, E. L. et al. The evolution of self-control. Proceedings of the National Academy of Sciences 111, E2140–E2148 (2014).

19. Matějů, J. et al. Absolute, not relative brain size correlates with sociality in ground squirrels. Proceedings of the Royal Society B: Biological Sciences 283, 20152725 (2016).

20. Deaner, R. O., Isler, K., Burkart, J. & Van Schaik, C. Overall brain size, and not encephalization quotient, best predicts cognitive ability across non-human primates. Brain, Behavior and Evolution 70, 115–124 (2007).

21. Smaers, J. B., Dechmann, D. K. N., Goswami, A., Soligo, C. & Safi, K. Comparative analyses of evolutionary rates reveal different pathways to encephalization in bats, carnivorans, and primates. Proceedings of the National Academy of Sciences 109, 18006–18011 (2012).

22. Smaers, J. B. et al. The evolution of mammalian brain size. Science Advances 7, eabe2101 (2021).

23. Němec, P. & Osten, P. The evolution of brain structure captured in stereotyped cell count and cell type distributions. Curr. Opin. Neurobiol 60, 176–183 (2020).

24. Olkowicz, S. et al. Birds have primate-like numbers of neurons in the forebrain. Proceedings of the National Academy of Sciences USA 113, 7255–7260 (2016).

25. Kverková, K. et al. The evolution of brain neuron numbers in amniotes. Proceedings of the National Academy of Sciences of the United States of America 119, e2121624119 (2022).

26. Iwaniuk, A. N. & Hurd, P. L. The evolution of cerebrotypes in birds. Brain, Behavior and Evolution 65, 215–230 (2005).

27. Timmermans, S., Lefebvre, L., Boire, D. & Basu, P. Relative size of the hyperstriatum ventrale is the best predictor of feeding innovation rate in birds. Brain, Behavior and Evolution 56, 196–203 (2000).

28. Sayol, F., Lefebvre, L. & Sol, D. Relative brain size and its relation with the associative pallium in birds. Brain, Behavior and Evolution 87, 69–77 (2016).

29. Healy, K. et al. Ecology and mode-of-life explain lifespan variation in birds and mammals. Proceedings of the Royal Society B: Biological Sciences 281, 20140298–20140298 (2014).

30. Deaner, R. O., Barton, R. A. & van Schaik, C. P. Primate brains and life histories: Renewing the connection. in Primate life histories and socioecology 233–265 (University of Chicago Press, 2003).

31. Sol, D., Sayol, F., Ducatez, S. & Lefebvre, L. The life-history basis of behavioural innovations. Philosophical Transactions of the Royal Society B 371, 20150187 (2016).

32. Dukas, R. Evolutionary biology of animal cognition. Annual Review of Ecology, Evolution, and Systematics 35, 347–374 (2004).

33. Ricklefs, R. E. The cognitive face of life histories. Wilson Bull. 116, 119–133 (2004).

34. Martin, T. E., Oteyza, J. C., Boyce, A. J., Lloyd, P. & Ton, R. Adult mortality probability and nest predation rates explain parental effort in warming eggs with consequences for embryonic development time. American Naturalist 186, 223–236 (2015).

35. Unzeta, M., Martin, T. E. & Sol, D. Daily Nest Predation Rates Decrease with Body Size in Passerine Birds. The American Naturalist 196, 743–754 (2020).

36. Charvet, C. J. & Striedter, G. F. Developmental modes and developmental mechanisms can channel brain evolution. Frontiers in Neuroanatomy 5, 1–5 (2011).

37. Finlay, B. L. & Darlington, R. B. Linked regularities in the development and evolution of mammalian brains. Science 268, 1578–1584 (1995).

38. Herculano-Houzel, S. Isotropic fractionator: A simple, rapid method for the quantification of total cell and neuron numbers in the brain. Journal of Neuroscience 25, 2518–2521 (2005).

39. Massen, J. J. M. et al. Brain size and neuron numbers drive differences in yawn duration across mammals and birds. Communications Biology 4, 1–10 (2021).

40. Ramsey, G., Bastian, M. L. & Schaik, C. Van. Animal innovation defined and operationalized. Behavioral and Brain Sciences 30, 393–437 (2007).

41. Reader, S. M. & Laland, K. N. Social intelligence, innovation, and enhanced brain size in primates. Proceedings of the National Academy of Science, USA 99, 4436–4441 (2002).

42. Lefebvre, L. A global database of feeding innovations in birds. The Wilson Journal of

Ornithology 132, 803–809 (2021).

43. Barton, R. A. Embodied cognitive evolution and the cerebellum. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 2097–2107 (2012).

44. Gutiérrez-Ibáñez, C., Iwaniuk, A. N. & Wylie, D. R. Parrots have evolved a primate-like telencephalic-midbrain-cerebellar circuit. Scientific Reports 8, 9960 (2018).

45. Brieuc, M. S. O. O., Waters, C. D., Drinan, D. P. & Naish, K. A. A practical introduction to random forest for genetic association studies in ecology and evolution. Molecular Ecology Resources 18, 755–766 (2018).

46. Hadfield, J. D. & Nakagawa, S. General quantitative genetic methods for comparative biology: Phylogenies, taxonomies and multi-trait models for continuous and categorical characters. Journal of Evolutionary Biology 23, 494–508 (2010).

47. Güntürkün, O., Ströckens, F., Scarf, D. & Colombo, M. Apes, feathered apes, and pigeons: differences and similarities. Current Opinion in Behavioral Sciences 16, 35–40 (2017).

48. Ströckens, F. et al. High associative neuron numbers could drive cognitive performance in corvid species. Journal of Comparative Neurology (2022) doi:10.1002/cne.25298.

49. Shanahan, M., Bingman, V. P., Shimizu, T., Wild, M. & Güntürkün, O. Large-scale network organisation in the avian forebrain: A connectivity matrix and theoretical analysis. Frontiers in Computational Neuroscience 7, 1–17 (2013).

50. Emery, N. J. Cognitive ornithology: the evolution of avian intelligence. Philosophical Transactions of the Royal Society B: Biological Sciences 361, 23–43 (2006).

51. Lambert, M. L., Jacobs, I., Osvath, M. & von Bayern, A. M. P. Birds of a feather? Parrot and corvid cognition compared. Behaviour vol. 156 505–594 (2019).

52. Ksepka, D. T. et al. Tempo and pattern of avian brain size evolution. Current Biology 30, 2026-2036.e3 (2020).

53. Herculano-Houzel, S., Manger, P. R. & Kaas, J. H. Brain scaling in mammalian evolution as a consequence of concerted and mosaic changes in numbers of neurons and average neuronal cell size. Frontiers in Neuroanatomy 8, 1–28 (2014).

54. Smaers, J. B., Mongle, C. S., Safi, K. & Dechmann, D. K. N. Allometry, evolution and development of neocortex size in mammals. Progress in Brain Research 250, 83–107 (2019).

55. Cárdenas, A. & Borrell, V. Molecular and cellular evolution of corticogenesis in amniotes. Cell Mol Life Sci 77, 435–1460 (2020).

56. García-Moreno, F. & Molnár, Z. Variations of telencephalic development that paved the way for neocortical evolution. Progress in Neurobiology 194, 101865 (2020).

57. Charvet, C. J. & Striedter, G. F. Developmental basis for telencephalon expansion in waterfowl: Enlargement prior to neurogenesis. Proceedings of the Royal Society B: Biological Sciences 276, 3421–3427 (2009).

58. Striedter, G. F. & Charvet, C. J. Developmental origins of species differences in telencephalon and tectum size: Morphometric comparisons between a parakeet (Melopsittacus undulatus) and a quail (Colinus virgianus). Journal of Comparative Neurology 507, 1663–1675 (2008).

59. Sibly, R. M. & Brown, J. H. Effects of body size and lifestyle on evolution of mammal life histories. Proceedings of the National Academy of Sciences 104, 17707–17712

(2007).

60. Uomini, N., Fairlie, J., Gray, R. D. & Griesser, M. Extended parenting and the evolution of cognition. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 375, 20190495 (2020).

61. Lleonart, J., Salat, J. & Torres, G. J. Removing allometric effects of body size in morphological analysis. Journal of Theoretical Biology 205, 85–93 (2000).

62. Reiner, A. et al. Revised Nomenclature for Avian Telencephalon and Some Related Brainstem Nuclei. Journal of Comparative Neurology 473, 377–414 (2004).

63. Mullen, R. J., Buck, C. R. & Smith, A. M. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211 (1992).

64. Mezey, S. et al. Postnatal changes in the distribution and density of neuronal nuclei and doublecortin antigens in domestic chicks (Gallus domesticus). Journal of Comparative Neurology 520, 100–116 (2012).

65. Revell, L. J. phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution 3, 217–223 (2012).

66. Ducatez, S. & Lefebvre, L. Patterns of Research Effort in Birds. PLoS ONE 9, e89955 (2014).

67. Sheard, C. et al. Ecological drivers of global gradients in avian dispersal inferred from wing morphology. Nature Communications 11, (2020).

68. Cooney, C. R. et al. Ecology and allometry predict the evolution of avian developmental durations. Nature Communications 11, 2383 (2020).

69. Botelho, J. F. & Faunes, M. The evolution of developmental modes in the new avian phylogenetic tree. Evolution & Development 17, 221–223 (2015).

70. Bürkner, P.-C. Brms: An R Package for Bayesian Multilevel Models using Stan. Journal of Statistical Software 80, 1–28 (2017).

71. Pigot, A. L. et al. Macroevolutionary convergence connects morphological form to ecological function in birds. Nature Ecology & Evolution 4, 230–239 (2020).

72. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. Nature 491, 444–448 (2012).

73. Berk, R. A. Statistical learning from a regression perspective. (Springer International Publishing Switzerland, 2017).

74. Liaw, A. & Wiener, M. Classification and regression by randomForest. R News 2, 18–22 (2002).

75. Sayol, F., Downing, P. A., Iwaniuk, A. N., Maspons, J. & Sol, D. Predictable evolution towards larger brains in birds colonizing oceanic islands. Nature Communications 9, 2820 (2018).

76. Torres, C. R., Norell, M. A. & Clarke, J. A. Bird neurocranial and body mass evolution across the end-Cretaceous mass extinction: The avian brain shape left other dinosaurs behind. Science Advances 7, 1–10 (2021).

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Figures

Fig. 1. Framework linking cognition, neuron numbers and brain size. a, Enhanced cognition is assumed to require more neurons in the pallial telencephalon, and perhaps also in the cerebellum. Thus, an increase in pallial neurons relative to the ancestors is expected in lineages that have been selected for higher intelligence. **b,** Because pallium comprises a large fraction of the mass of the brain, a disproportionate accumulation of pallial neurons should enlarge the brain relative to body size. **c,** If the benefits of enhanced cognition increase with body size, selection for cognition should further increase brain size in large species. As a result, species that excel at cognitive performance should have brains that are large in both absolute and relative terms. **d,** A mechanism that may allow accumulation of more neurons in the pallium is to extend the period of development, particularly in the later stages. According to some evo-devo theories, extending the later stages of development increases neurogenesis in the areas of the brain where progenitor cell multiplication stops later, i.e. the pallial areas of the telencephalon. Thus, if a longer development period facilitates neurogenesis in pallial regions, it may be targeted by selection for increased intelligence. **e**, Phylogenetic relationships among the species analysed for neuron numbers to address hypotheses b-c (for an enlarged tree with species names see Supplementary Fig. 1). Silhouette illustrations are from PhyloPic (http://phylopic.org), contributed by Anthony Caravaggi and Ferran Sayol under public domain licence.

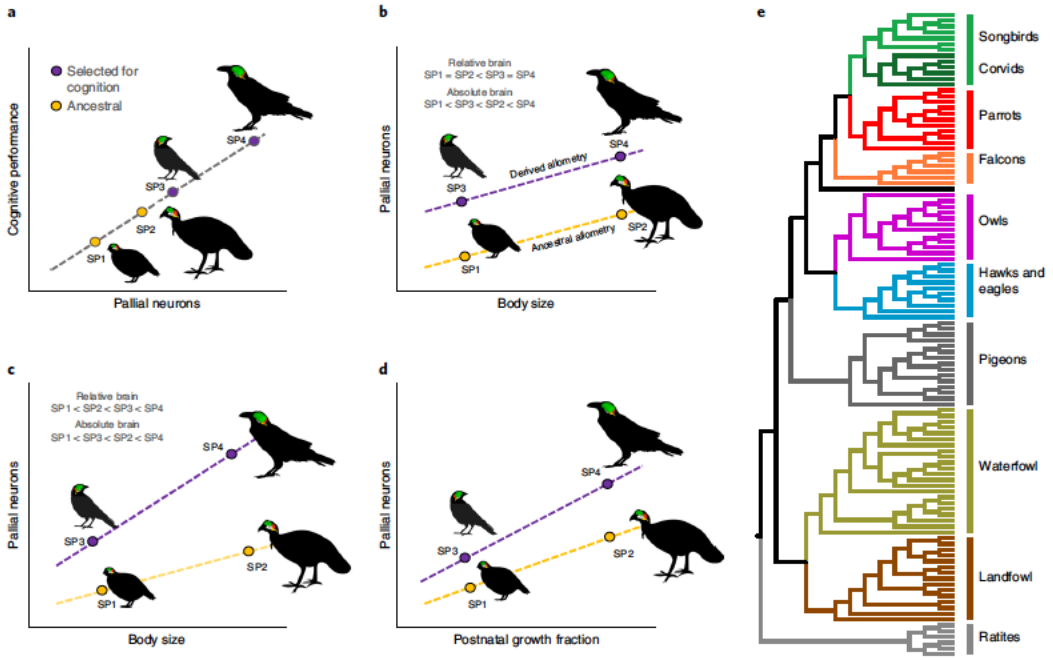

Fig. 2. Neurons and innovation propensity. Relationship between neuron numbers and innovation propensity for the entire brain and the pallium, cerebellum and brainstem, as predicted by models. **a,** Absolute neuron numbers. **b,** Neuron numbers adjusted by body size by including body mass (previously subtracting brain mass) as co-variate in the model. **c**, Density of neurons (cells per mg). All models account for the effect of phylogeny, biogeographic realm and confounding variables (see Supplementary Tables 1 and 2 for details). Lines and credibility intervals are derived from Bayesian phylogenetic mixed models. Sample size is 99 species, as nocturnal specialists (i.e. owls) are excluded from the innovation database.

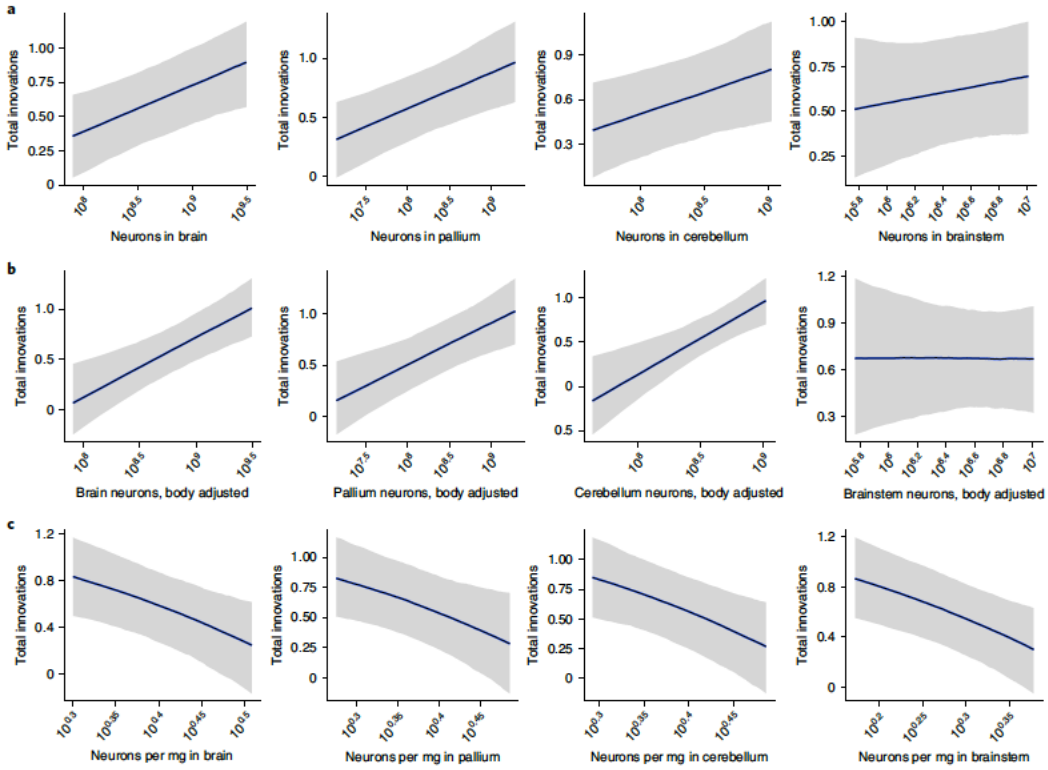

Fig. 3. Neuron numbers and brain mass as a function of body size. a-b, Distribution of neuron numbers among pallium, cerebellum and brainstem for clades belonging to low-slope (a) and the highest slope (b) grades. The assignation of species to each slope grade group is based on Ksepka et al.[52]. **c,** Variation in neuron numbers in the entire brain as a function of body size. **d-e,** Variation in brain mass as a function of body size for the sample of species used in analyses of neurons (d) and for the entire brain-body data set (e). In c-d, clades with low-slope grades are shown in blue while clades with the highest slope grades are shown in red. In all plots, owls have been excluded. For plots based on the entire sample of species, see Supplementary Fig. 3.

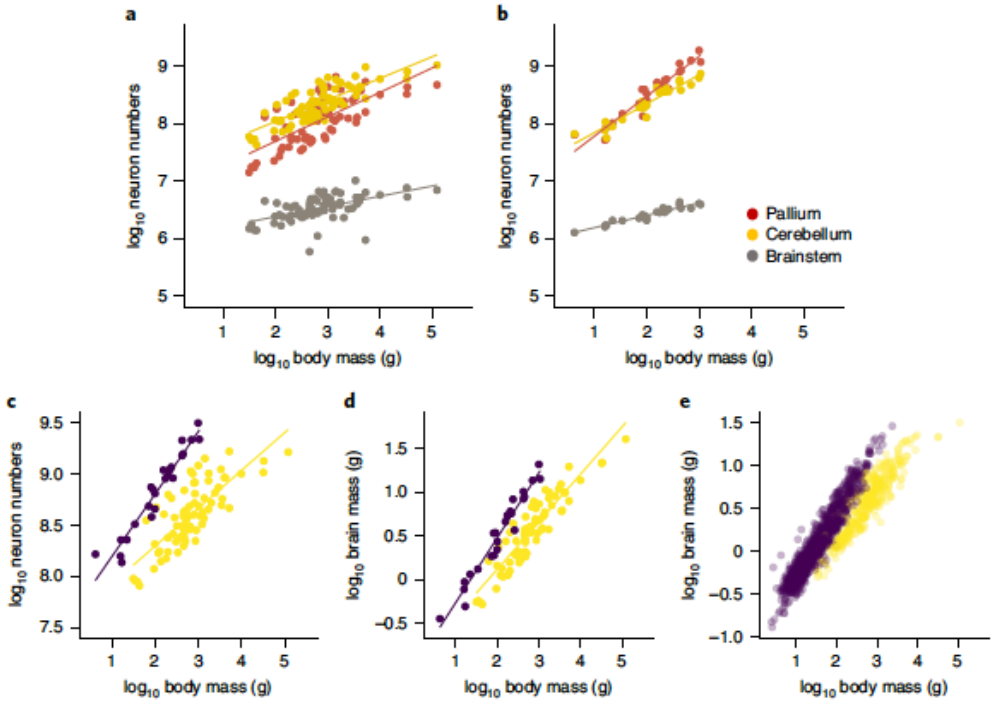

Fig. 4. Neuron numbers as a function of absolute and relative brain size. a, Bivariate dependence plots representing neuron numbers in the entire brain and main brain regions as a function of absolute and relative brain size, based on the predictions from random forests. Colours describe neuron numbers, with low numbers represented by dark-blue colours and higher numbers by yellow-green colours. Relative brain size was estimated by means of the *normalized scaled brain index*[61], with the allometric exponent estimated excluding clades that have been found to exhibit substantial grade shifts in brain:body allometries ($NSBI_{grades}$, see Methods).. **b**, Univariate representations (partial dependence plots) for relative brain size to further interpret the bivariate dependence plots. The plots show the dependence between neuron numbers and relative brain size, marginalizing over the values of absolute brain size. **c**, Univariate representation of the bivariate dependence plot for absolute brain size. In all analyses, owls have been excluded. For analyses with the entire sample of species, see Supplementary Figs. 6 and 7.

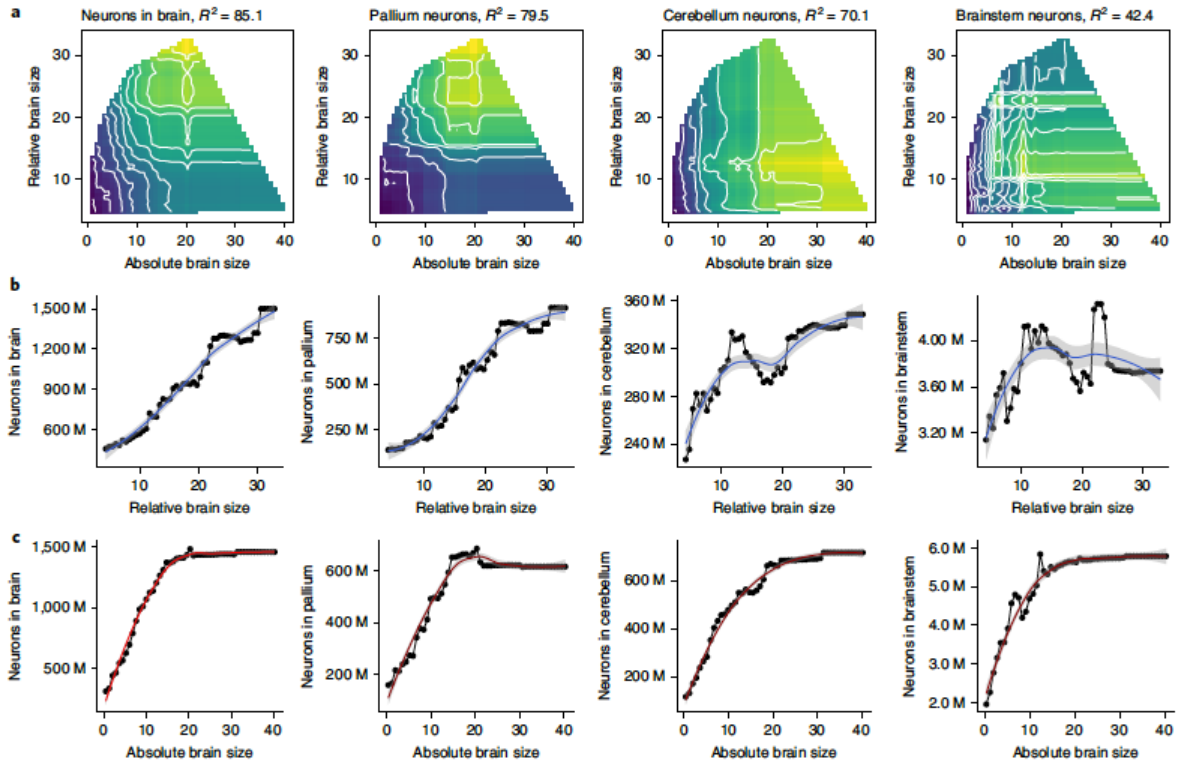

Fig. 5. Neurons and development in species belonging to low- and the highest slope grades. Neuron numbers as a function of the duration of development (embryonic stage plus post-natal growth)(a) and the fraction of total development time represented by the post-natal growth (b), for low-slope grades (blue bar) and the highest slope grades (red bar). Lines and credibility intervals are derived from Bayesian phylogenetic mixed models. In all analyses, owls have been excluded (for analyses with the entire sample size, see Supplementary Fig. 8).