

Review of the manuscript entitled: “Digitized endocasts and brains: measurements and analyses of the evolution of 172 fossil and extant amniote specimens”

Thank you for giving a new opportunity to review this manuscript and thank you to the authors for incorporating the majority of my suggestions. Because it was first reviewed in 2021, I hope you can understand that I have new comments related to articles that have been published in the last 4 years and that are relevant to different aspects of the paper. There is quite a bit of areas in text that need to be better justified or clarified. My comments below are meant to be constructive feedback.

I understand the authors want to compare the graphs here with the data that were generated using the same methods by Jerison. However, this is highly problematic because you are not incorporating newly published data including all of the virtual endocasts of fossils ever published (from what I understand the sample is) and not keeping up with current hypotheses about brain evolution. For example, the current paper does not have many Paleocene mammals and therefore fails to see the decrease in relative brain size found by Bertrand et al. (2022).

So, I think it is important to better frame this paper with this in mind. It is crucial to better justify throughout the manuscript, abstract and conclusion, what aspects of the papers are providing 1) truly novel results, 2) what aspects are supporting previous results, 3) acknowledge that virtual endocasts are not included and therefore some of the conclusions on the quantitative analyses here might be outdated and 4) the fact that many of the natural endocasts cannot be included in future quantitative analyses because of incompleteness.

Something that should be put more forward in this paper is the fact that natural endocasts can be great for morphological descriptions and identifying important features such as the exposure of the midbrain including the colliculi as well as sulci pattern on gyrencephalic brains.

I am sorry, but I will not be able to look in the supplementary data, figures and table legend in depth because of time constraints.

Detailed comments: The line numbers correspond to the PDF version of the manuscript

The abstract needs to be updated to include more recent work. An increase in neocortical surface area that occurred independently in mammals throughout the Paleocene to Eocene has been published more recently (Bertrand et al., 2022, see below). It would be more accurate to write “as previously reported, on average, neocorticalization of mammals increased over time...”

Bertrand, O. C. *et al.* Brawn before brains in placental mammals after the end-Cretaceous extinction. *Science* **376**, 80-85 (2022).

Lines 58-60: “Encephalization, the evolutionary increase in brain complexity or relative size reflecting environmental adaptations, is ... independent of their phylogenetic details”

This sentence needs clarification. As it stands, I would not agree with that especially in mammals. There are clear significant differences between the slope and intercept of various mammalian clades today. Please see Smaer et al. (2021). Bruger et al. (2019) proposed different equations for various mammalian clades.

Smaers, J. B. *et al.* The evolution of mammalian brain size. *Sci. Adv.* **7**, eabe2101, doi:10.1126/sciadv.abe2101 (2021).

Burger, J. R., George, M. A., Jr., Leadbetter, C. & Shaikh, F. The allometry of brain size in mammals. *J. Mammal.* **100**, 276-283, doi:https://doi.org/10.1093/jmammal/gyz043 (2019).

Lines 62-63: Barton and Harvey (2000) are proponent of the mosaic model of evolution and the concerted so I am not sure why this publication is cited here. The work of Finlay would be more appropriate here (Finlay and Darlington, 1995 for example but there are others).

For example: Finlay, B. & Darlington, R. Linked regularities in the development and evolution of mammalian brains. *Science* **268**, 1578-1584, doi:10.1126/science.7777856 (1995).

Lines 73-75: I think this is an important goal and I agree with the authors, but it would be good to mention previous work that has starting doing so using virtual endocasts of fossils from the Paleocene and Eocene (see Bertrand et al., 2022).

Lines 78-79 and Lines 84-86: Please cite and discuss the results from Bertrand et al. (2022).

Line 76: I would add that there are limitations in using natural endocasts from fossils because sometimes regions are not well preserved and broken. It would be good to justify how the authors dealt with this issue specifically in the statistical analyses. It is not clear if all of the specimens here were used in the quantitative analyses or only the ones that are well preserved.

Lines 137-138: Again, a lot of specimens are incomplete. Please specify which ones were measured and which ones were too damaged.

Lines 148-149: Do you mean that it is not visible in cetaceans? Please clarify this sentence.

Lines 177-178: Just something to clarify, did the authors used the EQ equation from Jerison (1973)? If that's the case, it is important to acknowledge that this equation was done on a very limited sample compared to the equations that have been produced more recently in Burger et al. (2019; all mammals) and Lopez-Torres et al., (2023; Eurachontoglires). Why not sure a more updated equation like the one from Burger et al (2019) for all mammals?

Burger, J. R., George, M. A., Jr., Leadbetter, C. & Shaikh, F. The allometry of brain size in mammals. *J. Mammal.* **100**, 276-283, doi:https://doi.org/10.1093/jmammal/gyz043 (2019).

López-Torres, S. *et al.* The allometry of brain size in Euarchontoglires: clade-specific patterns and their impact on encephalization quotients. *J. Mammal.* **105**, 1430-1445, doi:10.1093/jmammal/gyae084 (2024).

Lines 187-188: I am not sure that I understand what that means. Could you please clarify?

Lines 188-189: Could you provide an example for point 3? I am not sure how this can be considered more recent to measure brain and brain region sizes in 2D.

Lines 194-196: I am not sure I see a need to do that when we can scan and segment complete virtual endocasts. Those different natural endocasts are damaged and therefore are problematic to include in quantitative analyses.

Line 198: I am not sure that it is fair to say that this work would represent a foundation for future studies when so much has been done since 1973 in the field of paleoneurology regarding ideas and discussions. If the authors want to keep this part, please make a clearer case for it in the paper. Please also consider reading the recently published book by Dozo *et al.* (2023). There is a wealth of information about paleoneurology and should be cited in the introduction.

Dozo MT, Paulina-Carabajal A, Macrini TE, Walsh S (2023) *Paleoneurology of amniotes: New directions in the study of fossil endocasts*, Cham: Springer.

Line 206: Please also cite Bertrand *et al.* (2022), we looked at relative brain size and the size of brain regions through time in mammals.

Bertrand, O. C. *et al.* Brawn before brains in placental mammals after the end-Cretaceous extinction. *Science* **376**, 80-85 (2022).

Lines 298-299: Relative brain size has been shown now to not be a good way to estimate “intelligence”. Even the term “intelligence” should be avoided. I would say that it is quite an outdated idea and an oversimplification of behaviour. See van Schaik *et al.* (2021) and add a few lines about this aspect in the manuscript.

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 Behav. Evol.* **96**, 1-12, doi:10.1159/000517013 (2021).

Lines 305-307: Could you please rephrase this sentence? The meaning is not clear.

Lines 307-309: Do you mean changes in cortical organization? I would modify the sentence so it is clearer.

Lines 309-311: There have been papers showing a link between ecology and brain region size using endocasts and the fossil record. Admittedly not many but some. I am not trying to just cite my papers, there is just not so many that have been doing this yet. We found that there is a

correlation between arboreality and the increase in the size of the neocortex and petrosal lobules when squirrels transition to an arboreal lifestyle using extant and extinct taxa. We also looked at the influence of diet in the *Trogosus* paper and auditory capabilities in the *Incamys* paper. This is just to provide some background on the topic.

Bertrand, O. C., Püschel, H. P., Schwab, J. A., Silcox, M. T. & Brusatte, S. L. The impact of locomotion on the brain evolution of squirrels and close relatives. *Commun. Biol.* **4**, 1-15, doi:<https://doi.org/10.1038/s42003-021-01887-8> (2021).

Bertrand, O. C. *et al.* The virtual brain endocast of *Trogosus* (Mammalia, Tillodontia) and its relevance in understanding the extinction of archaic placental mammals. *J. Anat.* **244**, 1-21, doi:<https://doi.org/10.1111/joa.13951> (2024).

Bertrand, O. C. *et al.* The virtual brain endocast of *Incamys bolivianus*: Insight from the neurosensory system into the adaptive radiation of south American rodents. *Pap. Palaeontol.* **10**, e1562, doi:<https://doi.org/10.1002/spp2.1562> (2024).

Lines 312-313: If I recall, there is potentially an issue in the way the brain was preserved for the koala in that original publication and it may have shrunken post-mortem before they measured the brain. There are more recent images published in Taylor et al. (2006) that show that the brain is against the endocranial cavity (Fig. 1).

Taylor, J., Brown, G., De Miguel, C., Henneberg, M. & Rühli, F. J. MR imaging of brain morphology, vascularisation and encephalization in the koala. *Australian Mammalogy* **28**, 243-247 (2006).

Lines 313-315: Please add a reference for this sentence.

Lines 327-329: I would rephrase this sentence. How variation in brain regions demonstrates why brain as a whole evolved? Maybe flesh out this idea a bit more to clarify what you mean.

Lines 332-333: A reference is needed here for this statement. I was able to compile quite a bit of olfactory bulb data throughout the years on fossil endocasts (see for example Bertrand et al., 2022).

I think it would be fairer to say that it is an issue for natural endocasts but not for virtual endocasts. The circular fissure is a narrow point and it can break easily, therefore the olfactory bulbs are not always preserved in natural endocasts.

Lines 341-342: Enlarged colliculi do not automatically mean that an animal has echolocation. It just means that it potentially uses more the midbrain to survive than the visual and auditory cortices. Additionally, please specify if the inferior or the superior colliculi are enlarged. We published on this in the *Incamys* paper (Bertrand et al., 2024) and found that the inferior colliculi were possibly enlarged in that Oligocene rodent. In squirrels, the superior colliculi are enlarged instead. Also, it is important to keep in mind that it is difficult to design experiment

that would show if indeed koalas use their vision or audition a lot. We do not have the same “Umwelt” as koalas, so we might not see how they would use these functions yet.

Lines 352-363: These results should be compared with Bertrand et al. (2022). We measured the neocortical size of *Arctocyon* on a virtual endocast and found 10.3% (Table S1) but this is in relation to the whole endocast (including the olfactory bulbs). We also found that some carnivoramorphs also had a relatively big neocortex. Feel free to check Table S1 for making comparisons with the current work. We also found an increase in the relative size of the neocortex through time (Fig. 3C, F, I of the same paper) with some interesting differences between stem taxa and crown clades of the Eocene. We also look at the middle Eocene specifically in the *Trogosus* paper (Bertrand et al., 2024).

Lines 402-403: This point makes me think of one important finding from Bertrand et al. (2022) is that we found a decrease in relative brain size in the Paleocene in comparison to the Mesozoic. I think it would good to discuss this point and how this affects the conclusion of the current study.

Lines 421-423: I would encourage you to check Caspar et al. (2024) for the part about which rule dinosaurs follow. This paper can be discussed in relation to the results here.

Caspar, K. R. *et al.* How smart was ? Testing claims of exceptional cognition in dinosaurs and the application of neuron count estimates in palaeontological research. *Anat. Rec.* **307**, 3685-3716, doi:<https://doi.org/10.1002/ar.25459> (2024).

Lines 454-457: For this particular point, you could check the very insightful paper from Boch et al. (2024). They look at sulci pattern and foraging behaviour in this group. This paper could be discussed in relation to the statement in the present paper.

Boch M, Karadachka K, Loh KK, et al. (2024) Comparative neuroimaging of the carnivoran brain: Neocortical sulcal anatomy.). eLife Sciences Publications, Ltd.

Lines 458-459: There are other regions that can be measured: the olfactory bulbs and the petrosal lobules with great accuracy. More broadly, there is quite a bit in the literature showing the impact of ecology on diverse brain regions. Check De Casien et al. (2019) for example. They look at diverse brain regions and the link to ecology and behaviour.

DeCasien AR, Higham JP (2019) Primate mosaic brain evolution reflects selection on sensory and cognitive specialization. *Nat. Ecol. Evol.*, **3**, 1483-1493.

Lines 467-468: I am not sure that I understand this sentence, why could researchers not study neocorticalization and brain surface areas before digitization?

Lines 481-486: Please include a few sentences on how this compares to the results from Bertrand et al. (2022). This is true that we did not include extant species, but we had 137 fossil taxa, which is a few more than the current study if the 172 endocasts include extant species.

Lines 489-490: I would add that it is important to make sure that future researcher keep in mind that many of these natural endocasts are incomplete (missing part of the olfactory bulbs) and a lot of them cannot be incorporated with data of virtual endocasts.

Yours sincerely,

Ornella Bertrand