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PROCEEDINGS B

Intensive human contact correlates with smaller brains: differential brain size reduction in cattle types

A. M. Balcarcel, K. Veitschegger, M. Clauss and M. R. Sánchez-Villagra

Article citation details
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Review timeline
Original submission: 16 December 2020
1st revised submission: 6 April 2021
2nd revised submission: 12 May 2021
Final acceptance: 17 May 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-3132.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This study is aimed at examining the brain-to-body size allometry in cattle with the goal of providing insight into ongoing discussions of domestication and its effect on brain size.

While the use of 'estimated endocranial volume' as a proxy for brain size (i.e., estimated using skeletal dimensions of the cranial vault), is a notable limitation (sure to disappoint comparative neuroanatomists), I believe the authors provide a good argument for its use in this case (especially given the direct comparisons made with the fossil data). I do however recommend a change in title to reflect the distinction between a comparison of endocranial volumes/capacities and brain size as this study more correctly looks at the former. Other than this I believe the authors provide a sound study design to compare between their groups.

Aside for this limitation, I believe this manuscript along with the accompanying dataset is an important contribution towards our understanding of domestication. I believe your readership will find this manuscript of broad scientific value and interest and that the focus on artiodactyls, is likely to rekindle interest in this fairly understudied group of animals, especially as it pertains to questions on domestication and brain evolution.

For me some of the major highlights of this work was the careful consideration given in grouping the domesticates by breed (i.e., bullfighting, beef or dairy) to help develop a good comparative dataset to compare with the wild type. This is an important and significant step in comparison to previous studies on domestication, as aptly highlighted in the introduction. I found the resulting statistical analyses and figures appropriate, correctly interpreted and in accordance with that used by others in the field. There are however a few important comments (see below) which I would like the authors to kindly address.

Comments:
While the authors note (in the Discussion section) based on previous studies on other mammals that reductions in brain size in domesticates have been shown to be driven by changes in limbic regions, I find this assertion in the current study rather speculative. It seems a large leap of faith to assume (without direct investigation) that changes in whole brain size in cattle are reflective of limbic changes, when no volumetric comparisons of the subcortical structures were undertaken in this study (and in fact no analysis of the brain or brain surface/endocast was performed). To me, this seems a rather convenient conclusion given the observation of increased brain sizes in bullfighting cattle. Why should the reader be convinced that this observation of increased brain sizes in the more aggressive cattle is indeed aligned with changes in the limbic system? Are there

other subcortical changes that might also explain these differences in brain size? For example, what about changes in underlying white matter volume effecting connectivity in these regions (e.g., Spocter et al 2018; Hecht et al 2019) or perhaps changes in cortical grey matter within non limbic structures such as the basal nuclei which could impact impulse control or movement. It seems to me that the potential search space is less narrow than just the limbic regions and with no direct study of the underlying brain anatomy the authors have to remain open to other changes in brain structure which might explain the differences described here. I wondered if the authors could kindly comment on why they feel that this assumption is justified.

Given the use of endocranial volume and not actual brain size in the current study, I wondered if the authors could kindly comment on the limitations of their current study to correctly assess brain size in cattle.

Although the argument in favor of the use of endocranial volume in this study is the seemingly close association between the brain and the skull, it is important to remember that numerous neural and non-neural structures are found within the endocranium. Aside for the brain, this includes the cerebrospinal fluid, meninges, subarachnoid cisterns, cerebral vessels, cranial venous sinuses and cranial nerves. I wondered if the authors could kindly comment on the likely possibility that these endocranial subcomponents also vary in size within their groups and that the differences in endocranial volume observed here is not reflective of brain size differences but rather differences in the relative contributions of these other subcomponents to total endocranial volume.

Unless overlooked, I was not able to find any age estimates for individuals (from the extant sample) in the current study. In the primate literature there is good evidence that the amount of neural tissue expressed as a percentage of endocranial volume is inversely correlated with age up to adulthood such that in humans the non -neural component of endocranial volume varies from 6 % at birth to 20 % into early adulthood (Blinkov & Glezer,1968; Tobias, 1994). I wondered if the authors could kindly add age estimates in to the data table or provide a blanket statement on the age range and discuss briefly if they think this might impact any of their conclusions.

It is well known that brain size and the folding of the cortical surface are tightly constrained and that larger brains tend to have more folded cortices (e.g., Grewal et al., 2020), I wondered if the authors could comment on the expected trend in gyrification predicted from their observation of larger endocranial volumes in the aggressive cattle varieties. Do we expect to see increased cortical folding in the brains of bullfighting cattle as opposed to beef and dairy cattle? What would such an increase in folding mean for the question of cortical complexity within the larger brained cattle varieties? I believe, adding this short comment to the text would help to bring the discussion back to discussion of brain structure and function and provide a hypothesis worth testing for others in the field.

Grewal, J. S., Gloe, T., Hegedus, J., Bitterman, K., Billings, B. K., Chengetanai, S., Bentil, S., Wang, V. X., Ng, J. C., Tang, C. Y., Geletta, S., Wicinski, B., Bertelson, M., Tendler, B. C., Mars, R. B., Aguirre, G. K., Rusbridge, C., Hof, P. R., Sherwood, C. C., Manger, P. R., … Spocter, M. A. (2020). Brain gyrification in wild and domestic canids: Has domestication changed the gyrification index in domestic dogs?. The Journal of comparative neurology, 528(18), 3209–3228.

Minor comments:

Please clarify in the Methods section if any slopes and intercept comparisons were undertaken to test if there exists any significant differences in regression lines between the groups.

I wondered if you the authors could kindly include the regression equations and R squared for the plots in the figure legends so it is readily accessible to the reader next to the image.

Review form: Reviewer 2

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please see attachment.

Decision letter (RSPB-2020-3132.R0)

25-Jan-2021

Dear Ms Balcarcel:

I am writing to inform you that your manuscript RSPB-2020-3132 entitled "Aggressive behaviour correlates with larger brains: differential brain size reduction in cattle types" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

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Sincerely,
Dr Robert Barton
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
Reviewers agree that the data you present provides a good opportunity to tackle a fundamental question on animal domestication. Several aspects will need to be addressed before the manuscript can be considered.
As reviewer 1 points out, there is a need for a more nuanced discussion on which neuroanatomical features may explain the observed changes in brain size. Reviewer 1 also suggests including a more in-depth discussion of the possible influence of non-neural structures on variation in endocranial size, and age estimates for the individuals.
Reviewer 2 raises concerns that differences within some of the three groups of cattle could significantly alter the results and should therefore be explored in more depth. For example, the effect of artificial selection for muzzle width may be a confounding factor when it is used as an indicator of body size. I also agree with reviewer 2 that a more thorough statistical analysis to ascertain the differences among the breeds is needed.
Lastly, the structure of the writing should also be improved. As reviewer 2 highlights, a central line of argument in the introduction is the result. Results should only be discussed in the discussion section. An introduction serves only to introduce the research, not to discuss its results.
Reviewer 2 has kindly provided a detailed road map on how to address these and other concerns.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
This study is aimed at examining the brain-to-body size allometry in cattle with the goal of providing insight into ongoing discussions of domestication and its effect on brain size.

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Minor comments:

Please clarify in the Methods section if any slopes and intercept comparisons were undertaken to test if there exists any significant differences in regression lines between the groups.
I wondered if you the authors could kindly include the regression equations and R squared for the plots in the figure legends so it is readily accessible to the reader next to the image.

Referee: 2
Comments to the Author(s)
Please see attachment.

Author's Response to Decision Letter for (RSPB-2020-3132.R0)

See Appendix A.

RSPB-2021-0813.R0

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
See attached

Decision letter (RSPB-2021-0813.R0)

29-Apr-2021

Dear Ms Balcarcel:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article (https://royalsociety.org/journals/authors/author-guidelines/#data). Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link
http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Dr Robert Barton
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
I thank the authors for substantially improving the paper following the reviewer's suggestions. I agree with the reviewer that the paper is now excellent and well-referenced. However, I also agree with the reviewer that more work is needed on the narrative.
In my view, the reviewer highlights the key issues. For example, in the introduction it is of paramount importance that the reason as to why investigating cattle is important is described in clear terms. Simply because something has not been done before does not necessarily make it valuable to do. In this case, it is of course very valuable to do, but the reason why needs to be clearly stated.
Adding a central narrative to the discussion section is equally important to find resonance with the broad audience of Proc B.
The reviewer further highlights some minor methodological aspects that should also be resolved. For multiple testing, the reviewer suggests the possibility to do this manually (p-value/number of comparisons). This is a very good approach, though has been shown to be too conservative in some instances. An alternative approach would be to use the approach by Benjamin & Hochberg ('p.adjust' function with method 'BH' in the 'stats' R package).

Reviewer(s)' Comments to Author:
Referee: 2
Comments to the Author(s).
See attached

Author's Response to Decision Letter for (RSPB-2021-0813.R0)

See Appendix B.

Decision letter (RSPB-2021-0813.R1)

17-May-2021

Dear Ms Balcarcel

I am pleased to inform you that your manuscript entitled "Intensive human contact correlates with smaller brains: differential brain size reduction in cattle types" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

Data Accessibility section
Please remember to make any data sets live prior to publication, and update any links as needed when you receive a proof to check. It is good practice to also add data sets to your reference list.

Open Access
You are invited to opt for Open Access, making your freely available to all as soon as it is ready for publication under a CCBY licence. Our article processing charge for Open Access is £1700. Corresponding authors from member institutions (http://royalsocietypublishing.org/site/librarians/allmembers.xhtml) receive a 25% discount to these charges. For more information please visit http://royalsocietypublishing.org/open-access.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Dr Robert Barton
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Intensive human contact correlates with smaller brains: differential brain size reduction in cattle types

Balcarcel, A.M.[1], Veitschegger, K.[1], Clauss, M.[2], Sánchez-Villagra, M.R.[1]

List of revisions based on Referees

C= referee comment, A= answer/response

Associate Editor, Board Member: 1

C: '…more in-depth discussion of the possible influence of non-neural structures on variation in endocranial size…'

A: New analyses test for influences of phylogeny, geographical distribution, and a combination of specialization (use) and phylogeny (see Methods, Results, and Discussion).

C: '…the effect of artificial selection for muzzle width may be a confounding factor when it is used as an indicator of body size. I also agree with reviewer 2 that a more thorough statistical analysis to ascertain the differences among the breeds is needed…'

A: We address this extensively in this new version with a new analysis on the covariation of muzzle width and brain reduction (see Figure 2B, Methods, Results, and Supp. Data). Results confirm that muzzle reduction in is not influencing the differential brain reduction signal. In fact, breeds with smaller muzzles are showing the greatest reduction.

C: '…structure of the writing should also be improved…'

A: Writing has been restructured throughout.

Referee: 1 (most requests were for anatomical comparisons which were unfortunately not a part of our study)

C: It seems a large leap of faith to assume (without direct investigation) that changes in whole brain size in cattle are reflective of limbic changes, when no volumetric comparisons of the subcortical structures were undertaken…'

A: Indeed, we agree that having access to dissected brains of various breeds, and a large number of them, would allow a valuable analysis of what areas of the brain are changing and to what degree. This is beyond our capabilities at present, as we have access only to morphometric data of the skulls of both fossil and extant cattle. We mention the limbic system in the discussion as a possible explanation, given the

number of artiodactyls that have expressed such changes. However, we do not claim any direct correlation with it.

C: '…Aside for the brain, this includes the cerebrospinal fluid, meninges, subarachnoid cisterns, cerebral vessels, cranial venous sinuses and cranial nerves…'

A: Once again, a great point. However, we are presenting a proxy for endocranial volume—itself a proxy for brain size. The error stemming from meningeal tissues is minimal considering the double proxy. However, this is the best approximation possible given the partial preservation of fossil aurochs skull sampled. Any bias is also distributed to the domestic sample, since the same method is used across both samples, so we consider the comparison to be a sound one.

C:'… no volumetric comparisons of the subcortical structures were undertaken in this study…'…' Are there other subcortical changes that might also explain these differences in brain size? For example, what about changes in underlying white matter volume effecting connectivity in these regions…'

A: Unfortunately, this is not possible with our data set. However, even an endocranial cast would not be able to capture changes in the inner brain where the limbic system is found. Anatomical dissection work is beyond the scope of our study. But this would be a great future study, although complicated in terms of sampling.

C: '… the amount of neural tissue expressed as a percentage of endocranial volume is inversely correlated with age up to adulthood such that in humans the non -neural component of endocranial volume varies from 6 % at birth to 20 % into early adulthood…'

A: We state in Methods that our sample is composed of adults only. We make no ontogenetic inferences. Also, anatomical dissection work is beyond the scope of our study.

C: Please clarify in the Methods section if any slopes and intercept comparisons were undertaken to test if there are any significant differences in regression lines between the groups…''

A: Executed in Methods, and Supp. Data files. R code is also provided.

Referee: 2 (most changes reflect suggestions by Referee 2)

C: A more thorough investigation into the differences in ECV/MZW within breeds.

A: Done. This was a great addition to our study. It has made the investigation more thorough and has reinforced our original study. Thank you. Results: lines170-180, and Figure 2B. All analyses in Supp Data_D7.

C: A pairwise test to understand if dairy, beef and fighting differ in intercept too

A: ANCOVA tests provided in Table 2 in Supp. Data_D6. Tests were performed for all analyses and comparisons.

C: An investigation of how variable breeds with more than one data point are; given the small sample size of the fighting bulls, this seems like an important way of strengthening the argument.

A: Done. We investigated brain size variation, and muzzle width variation across breed clusters and specialized/non-specialized breeds. Within these small clusters we explore the positions of specific member breeds, some which only had one to three datapoints, but were nonetheless informative. Supplementary Data files D9-D10.

C:'Include more information on age of the breed, or broad breeding history, and whether some breeds are related to each other (e.g. it could be that the relatively largest- ECV breeds are all Schwarzvieh or all showed some particular trait). True phylogenetic analysis is of course not possible but each cattle breed has … historical information, breeding history, and traits that contribute to breeding stan dards should be findable.'

A: A full section in Methods, Results, and Discussion is devoted to this: "*Brain reduction between phylogenetic clusters".* This has greatly improved our study and we thank all Referees for guiding us toward further exploration.

C: Consider adding at least a figure with dual- purpose/multipurpose datapoints included.

A: Supplementary Data D8. Regression with non-specialized cattle, Heck and Swissfighting breeds (Herens).

C: '…introduction requires a re- structure. As it stands, the result is given away and discussed in the paragraph from p. 3, l.56. I would focus more on explicitly explaining why aggressive behaviour might result in larger brain sizes (currently done on p. 3, l. 58), and make this the chief hypothesis.
A: Introduction and Methods restructured. Hypotheses are described more clearly and earlier in the manuscript.

C: 'Some of the explanations as to why aggressiveness might correlate with brain size

discussion, but it would be good to include them in the introduction already.'

A: Done. Introduction was restructure to reflect these suggestions.

C:'Working towards better resolution of the fascinatingly diverse traits in the various breeds might be a go od way of getting more value out of the dataset…Adding some properties to the data, either by adding gro uping factors or by colouring in different groups, might help explain a bit more of the variation in the data set and give it greater depth.'

A: Grouping factors added, new analyses added. See Methods, Results.