Peer Review Overview

Manuscript Title: “THE FUNCTIONAL CHARACTERIZATION OF CALLOSAL CONNECTIONS”

Received	06-Jun-2021
1st Decision	05-Aug-2021
1st Revision Submitted	26-Sep-2021
2nd Decision	01-Nov-2021
2nd Revision Submitted	05-Nov-2021
Accepted	11-Nov-2021

1st Decision Letter

Dear Prof. Caminiti,

Thank you for submitting your manuscript to Progress in Neurobiology.

We have completed our evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following major revision. We invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Oct 04, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/proneu/, and navigate to the "Submissions Needing Revision" folder.

Progress in Neurobiology values your contribution and we look forward to receiving your revised manuscript.

Kind regards,
Sabine Kastner, MD, PhD
Editor-in-Chief
Progress in Neurobiology

Editor and Reviewer comments:

Reviewer #1: This is a well written review that thoroughly discusses the breadth and depth of the field to date. I congratulate the authors on an excellent manuscript that expertly guides the reader through the published literature in a well-organized manner. I greatly appreciated how each section built on the prior and smoothly transitioned to the following section. I suggested very few small revisions in Sections 7, 8, and 9, as well as some minor edits to a few of the figures. Otherwise I think this work is appropriate for publication and will add value to the current literature.

Section 2 does an excellent job summarize the extant knowledge of axonal morphology and how this knowledge shapes our understanding of cerebral networks. The nature of axon tracing studies necessitates a synthesis of data across species, relying heavily on macaque and other non-human models given the invasive methodology. The authors do a good job relating these data to the limited tracer studies in human anatomy as well as relating to diffusion weighted MRI tractography studies.

Tractography studies are readily performed in human subject given its non-invasive modality but is less specific and the correlation to cellular anatomy is imperfect.

Section 3 explores the various ways that lesion and lesion like studies have investigation the role of the corpus callosum and function and cognition. Interestingly, as many questions were raised as they were answered from many of these studies. This finding points to the incredible complexity of the corpus callosum, the range of functions its connections serve, and the variance across areas, systems, and species.

Section 4 introduces several studies using various functional stimulation paradigms. This sections nicely samples the various modalities for stimulating the nervous system including direct and indirect electrical stimulation in humans and non-humans. Electrical stimulation has an excellent correlation with medical interventions, as hinted in the introductory paragraph referencing Penfield's functional mapping in human patients. The association of TMS mapping to the axonal diameter studies introduced in prior sections is an excellent example of how basic science in animal models supports non-invasive studies in humans and allows for validation of human non-invasive findings. This section continues with fascinating results from optogenetic studies representing cutting edge biomedical engineering solutions to the imperfection of electrical stimulation mapping.

Section 5 continues to explore cross species data that characterizes the relationship of structural connections through cortical projecting neurons and various physiologic functions. Great interest has been devoted to this topic with respect to hand sensory and motor. Also reviewed are visual pathways, which are also uniquely interesting in the relative weight of structural connections to visual cortex.

Section 6 begins by extending the information presented in the prior section regarding callosal projections in the visual system and expands to post-synaptic transcallosal transmission of information between the hemispheres with a novel experimental paradigm involving sectioning of the optic chiasm. This section continues to expand the concept of bilateral information transfer beyond direct callosal projecting neuros in the somatosensory and gustatory areas.

Section 7 continues to expand on the relationship between structure and function with functional imaging studies. It begins with a description of the observation that structural connectivity is not perfectly aligned to functional or effective connectivity as measured by fMRI, EEG, and other modalities. These concepts build well on the prior sections that setup an understanding of post-synaptic and poly-synaptic information transmission. The advantage of fMRI is it allows for non-invasive studies to be performed in behaving human subjects. This is then illustrated by the corpus callosotomy studies performed in human subjects being treated for medically refractory epilepsy.

The authors here make a mistake in their reading of Roland, et al, 2017. They describe this as a review of results obtained from several groups. That manuscript is not a review of data from other groups, it is novel data of 22 unique subjects at a single institution who had a complete or partial callosotomy for medically refractory epilepsy. The unique advantage of studying complete and partial callosotomies is these results showed effectively a dose response where the complete callosotomies affected the entire brain, yet the partial callosotomy affected primarily the portion implicated by the structure connectivity of the anterior two-thirds of the corpus callosum. This is what one would expect if the interhemispheric FC was primarily mediated by structural connectivity through the corpus callosum. Furthermore, that data diverged slightly from the results published by O'Reilly, et al, in 2013 in 3 monkeys where 2 had anterior commisurotomies and 1 did not. O'Reilly showed a large impact of the anterior commissure, which when spared was associated with significant interhemispheric functional connectivity remaining. In the Roland, et al, 2017 data, none of the human subjects had the anterior commissure sectioned yet the overwhelming majority of the interhemispheric functional connectivity was lost after complete callosotomy. All regions showed a decrease in interhemispheric functional connectivity, most to near zero. The motor and vision areas decreased, although less so than more associative areas. These are the relatively focal areas where interhemispheric, and more specifically homotopic, functional connectivity was hypothesized to have either additional polysynaptic (post-synaptic?) input non from callosal projecting neurons or possibly through subcortical structures (e.g. thalamocortical circuits). Figure 4A shows this well, where the pre/post central and primary visual areas show a small residual homotopic functional connectivity while surrounded by nearly zero in the adjacent areas. Indeed, this divergence between primary and associative cortical areas nicely foreshadows the discussion in Section 8 as well as the leading sentence in Section 9 Conclusions.

Section 8 expands beyond monosynaptic homotopic cortical projecting neurons to high order integrations brain regions. As one might expect these areas are more difficult for cellular or histologic techniques and rely more on human subject data. The authors discuss split-brain (i.e. post corpus callosotomy) and acallosal (i.e. callosal agenesis) data. Care must be taken in these two populations as

all though both represent lack of a corpus callosum, the split-brain subjects were born and at least initial development occurred with an intact corpus callosum, where the acallosal patients did not develop this way and possibly/likely developed alternative structural connections for interhemispheric communications (see Tovar-Moll, PNAS, 2014, Structural and functional brain rewiring clarifies preserved interhemispheric transfer in humans born without the corpus callosum). You also present similar supporting information in Section 9 by reference to Ptito and Lepore, 1983. I have no objections to the data summarized in this section, I do recommend including a brief cautionary statement when interpreting these two similar, yet sufficiently different, human subject populations.
Section 9 concludes the discussion. The introduction of a new concept to consider callosal connections as logic gates is not well supported by the rest of the review paper. If you feel this is indeed important, I recommend you develop the concept further with better explanation of the supporting evidence. Otherwise I would recommend removing it. As written this concept appears in a single sentence in the Conclusions and also in the Abstract therefore not in line with the rest of this otherwise well organized and fluid presentation of concepts.

Figures
Figure 1 - the inset comparing steepness of EPSP between callosal and peripheral stimuli would be more readily interpretable with units displayed on the respective axis.

Figure 5 - The SS "Sylvian sulcus" labels in panels A3 and B3 are pointing to the insula, not the Sylvian fissure / sulcus.
Furthermore, the midline has significant overlap of in the peripheral nervous system. I find it difficult to ascertain that bilateral cortical fMRI activations from near-midline tactile stimulation provides reliable data on the function of the CC as opposed to bilateral input to each hemisphere arriving from bilateral peripheral sensory nerves that overlap near and reaching across the midline. Indeed, you refer to such a phenomenon in section 6.2.

Reviewer #2: Main comments:

The review article "The functional characterization of callosal connections" by Innocenti et al. is a well written, comprehensive review about features of interhemispheric communication via axons forming the corpus callosum. This is a thought provoking article; most of the evidence presented supports the broader rationale that a key feature of callosal function includes modulation of target's responses in a context-dependent manner, which I found very innovative and of potential interest to a wide range of neuroscientists. This is presented from several perspectives that include anatomy, physiology, and lesions in humans and other mammals. Most of the evidenced reviewed comes from studies in primates (including humans) and carnivores, which is a strength from a point of view relevant to humans. However, in my opinion, many of the authors' claims about callosal structure and function could have been strengthened by including more examples from rodents given the wealth of very relevant and detailed literature (see below). Having said that, I also appreciate that the number of references is already very large (>250), so this might be hard to accommodate unless the authors are willing to update some of the older/less relevant citations. This latter point could be very beneficial, as some parts of the manuscript refer to recent advanced methods, such as single-neuron reconstructions or optogenetic circuit mapping, which have provided more precise details than classical methods. For example, the statement that "less than 1% of callosal cells bifurcate and project to ipsilateral areas" is based on methods from over 30 years ago, but more recent findings have shown that in rodents a much larger proportion of callosal neurons also project ipsilaterally (see Allen Atlas and references PMID: 26796534, PMID: 31555081, PMID: 29379878, PMID: 32405029 as examples).

The paragraphs starting in lines 222, 316, 342, 354, 457, 506, 530, amongst others, refer to callosal projections in area 17/18 of the visual cortices (mostly in primates/carnivores; "vertical meridian", "midline rule"), yet the fact that a homologous projection from V1 to contralateral border between V1/V2 is also present in rodents is briefly mentioned, if at all. Most importantly, there is a similar convergence of callosal axons from S1 to the contralateral S1/S2 border that is very prominent in rodents (PMID: 24945772, PMID: 33661095, PMID: 17942728, PMID: 20105242, PMID: 32166131) and its inclusion might help strengthen the article's points.

Finally, a concluding paragraph synthesizing the main ideas put forward about callosal function (e.g., the highlights of first page) could help bring closure to the article.

Minor comments:
Comment on 'lacking functional characterization' in line 39 of abstract is then contradicted in line 104.
Line 96 suggests that the CC arose at the same time as the mammalian neocortex, however this

occurred much later in evolution (see PMID: 25071525).
In section 4.3, optogenetic stimulation has also shown that callosal axons synapse onto pyramidal neurons and interneurons across layers and not just L1 (PMID: 17435752, PMID: 24945772). This is again relevant in lines 801-803.
In line 518, similar findings of PMID: 30658859 include callosal spines cluster with non-callosal ones of similar orientation preference.
Similarly, in line 826 (as mentioned above), somatosensory disruptions also affect callosal projections to S1/S2 in rodents (PMID: 24945772; PMID: 17942728).
Line 821, different to what?

Typos in lines: 222 (italics in "iv"), 238 (un/an), 388 (space), 519 (properties of), 826 (space), 1787 (reversed order middle-right?), 1789 (brain 'temporal?' slices), 1809-1810 ('A' and 'D' not shown; also mention that in this figure rostral is to the right).

Reviewer #3: This is an excellent review paper that provides a timely and considered overview of the anatomy and physiology of the corpus callosum. The team of authors should be commended for their efforts. However, there are some aspects of the presentation that could potentially be improved.

First, although I suspect the authors want to "lay out the facts", I think that the paper would benefit from more a general synthesis, perhaps with a potentially organizing model or construct set out in the beginning and then summaries at the end of each section that refer back to those ideas. To me, one of the most interesting ideas that emerges from the paper was that of the callosum being a "conditional" driver that is doing something quite different than classic models of the callosum as being engaged in simply shuttling information back and forth between the hemispheres so as to allow for the perceptual world to be stitched together across the midline. To the degree that this idea could be presented initially (and contrasted with classic models), and them could be integrated into the summary of each of the major subsections (1,2,etc.), I think it would give the paper more of an impact. For example, in section 2, there could be a concluding section that points out that the (mainly) glutamatergic properties of callosal neurons makes them well suited to drive assemblies of neuronal activity, and that the variety of callosal diameters, with varying conduction times, and both homotopic and heterotopic connections, enable the effects to be conditional.

Second, in terms of writing style, I think it would be helpful to make clear when one is talking about neuronal connections/anatomy in general, versus callosal connections/anatomy more specifically. There were places in which I was not sure initially to which the author was referring. For example, at the beginning of section 2.2. ii. the first section reads "The position of the cell body of the neuron of origin…..". The prior sentence was about CC axons, so it wasn't clear whether this sentence referred to callosal axons or axons in general. I think it would help to flag to which you are referring. "Across all neurons, the position of the cell body….." and then clearly tag when you are talking about the callosum "Callosal axons…"

Third, another really interesting point raised in the paper is the comment made on the top of page 12, which involves the discrepancy between findings from reversible lesions and those of split-brain patients and animal models with fiber tract lesions. However, there is a dual caveat here. On the bottom of page 11, it is stressed that reversible lesions affect more than callosal cells, but then it is stated on the top of page 12 that one must be cautious in drawing conclusions from split-brain human and animal studies. Since the comment on lesions came second, it made it seem as if more weight was being given to the reversible lesion results. Is that what the author intended? If not, then one might say that each of these methods have their limitations in the same sentence. Furthermore, I think a sentence or two about the causes and potential resolutions of this discrepancy would be helpful (even if reviewed elsewhere).

Fourth, there are additional papers that the authors might find of use to include in their review, which come more from the human (than animal) literature.

With regards to the anatomical connections of the corpus callosum:
De Benedictis, A., Petit, L., Descoteaux, M., Marras, C. E., Barbareschi, M., Corsini, F., et al. (2016). New insights in the homotopic and heterotopic connectivity of the frontal portion of the human corpus callosum revealed by microdissection and diffusion tractography. Human Brain Mapping, 37(12), 4718-4735. http://doi.org/10.1002/hbm.23339 [this paper especially speaks to an issue raised on page 20 of the manuscript]

With regards to functional connectivity of the corpus callosum:
Stark, D. E., Margulies, D. S., Shehzad, Z. E., Reiss, P., Kelly, A. M., Uddin, L. Q., Gee, D. G., Roy, A. K., Banich, M. T., Castellanos, F. X., & Milham, M. P. (2008). Regional variation in interhemispheric coordination of intrinsic hemodynamic fluctuations. The Journal of neuroscience : the official journal of the Society for Neuroscience, 28(51), 13754-13764. https://doi.org/10.1523/JNEUROSCI.4544-08.2008

Gee, D. G., Biswal, B. B., Kelly, C., Stark, D. E., Margulies, D. S., Shehzad, Z., Uddin, L. Q., Klein, D. F., Banich, M. T., Castellanos, F. X., & Milham, M. P. (2011). Low frequency fluctuations reveal integrated and segregated processing among the cerebral hemispheres. NeuroImage, 54(1), 517-527. https://doi.org/10.1016/j.neuroimage.2010.05.073

With regards to connectivity after the split-brain procedure:
Roland, J. L., Snyder, A. Z., Hacker, C. D., Mitra, A., Shimony, J. S., Limbrick, D. D., Raichle, M. E., Smyth, M. D., & Leuthardt, E. C. (2017). On the role of the corpus callosum in interhemispheric functional connectivity in humans. Proceedings of the National Academy of Sciences of the United States of America, 114(50), 13278-13283. https://doi.org/10.1073/pnas.1707050114

With regards to modulation of callosal connectivity:
Preisig, B. C., Riecke, L., Sjerps, M. J., Kösem, A., Kop, B. R., Bramson, B., et al. (2021). Selective modulation of interhemispheric connectivity by transcranial alternating current stimulation influences binaural integration. Proceedings of the National Academy of Sciences of the United States of America, 118(7). http://doi.org/10.1073/pnas.2015488118

Finally, I appreciate the authors including some of my work in their review and their comments on it (bottom page 19/top of page 20). They have interpreted my work in someone of a different light than initially conceived, which I found interesting, and as such, thought it would be worth commenting on. Our studies show that as task difficulty increases, there is an increasing bilateral advantage, i.e., better performance when critical information is divided between the hemisphere as compared to directed to one. The authors have interpreted this finding as suggesting that different callosal connections or their effects are engaged by different stimuli or by level of task difficulty. This indeed may be the case but was not a possibility that we had considered. Rather, we had assumed that callosal transmission was uniform, automatic and obligatory. We presumed, when tasks are easy, one hemisphere can handle the task adequately and dividing the information ensues additional (time) costs of callosal transmission. In contrast, when tasks are more difficult, dividing and distributed across two processors, i.e., the hemispheres, provides an advantage that outweighs the cost of callosal transmission. I have no problem with the author's alternative interpretation, but thought that I would mention the original one.

With regards to this behavioral effect, there is some nice work by groups other than my own tying the behavioral effect to the morphology and function of the callosum:

Davis, S. W., Kragel, J. E., Madden, D. J., & Cabeza, R. (2012). The architecture of cross-hemispheric communication in the aging brain: linking behavior to functional and structural connectivity. Cerebral Cortex (New York, NY : 1991), 22(1), 232-242. http://doi.org/10.1093/cercor/bhr123

Davis, S. W., & Cabeza, R. (2015). Cross-hemispheric collaboration and segregation associated with task difficulty as revealed by structural and functional connectivity. The Journal of Neuroscience : the Official Journal of the Society for Neuroscience, 35(21), 8191-8200. http://doi.org/10.1523/JNEUROSCI.0464-15.2015

Höller-Wallscheid, M. S., Thier, P., Pomper, J. K., & Lindner, A. (2017). Bilateral recruitment of prefrontal cortex in working memory is associated with task demand but not with age. Proceedings of the National Academy of Sciences of the United States of America, 114(5), E830-E839. http://doi.org/10.1073/pnas.1601983114

Marie Banich

1st Author Response Letter

THE FUNCTIONAL CHARACTERIZATION OF CALLOSAL CONNECTIONS

Point-to-point Reply to the reviewer's comments

We are very grateful to the reviewers of our manuscript for appreciating our effort and for the sharp, constructive, and inspiring comments they have made. We have accepted them all, as detailed below in our point-to-point reply. Please notice that all text modifications are highlighted in yellow. The new references are also highlighted in yellow in the body of the text but not in the references list.

Reviewer #1: This is a well written review that thoroughly discusses the breadth and depth of the field to date. I congratulate the authors on an excellent manuscript that expertly guides the reader through the published literature in a well-organized manner. I greatly appreciated how each section built on the prior and smoothly transitioned to the following section. I suggested very few small revisions in Sections 7, 8, and 9, as well as some minor edits to a few of the figures. Otherwise, I think this work is appropriate for publication and will add value to the current literature.

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The authors here make a mistake in their reading of Roland, et al, 2017. They describe this as a review of results obtained from several groups. That manuscript is not a review of data from other groups, it is novel data of 22 unique subjects at a single institution who had a complete or partial callosotomy for medically refractory epilepsy. The unique advantage of studying complete and partial callosotomies is these results showed effectively a dose response where the complete callosotomies affected the entire brain, yet the partial callosotomy affected primarily the portion implicated by the structure connectivity of the anterior two-thirds of the corpus callosum. This is what one would expect if the interhemispheric FC (functional connectivity) was primarily mediated by structural connectivity through the corpus callosum. Furthermore, that data diverged slightly from the results published by O'Reilly, et al, in 2013 in 3 monkeys where 2 had anterior commisurotomies and 1 did not. O'Reilly showed a large impact of the anterior commissure, which when spared was associated with significant interhemispheric functional connectivity remaining. In the Roland, et al, 2017 data, none of the human subjects had the anterior commissure sectioned yet the overwhelming majority of the interhemispheric functional connectivity was lost after complete callosotomy. All regions showed a decrease in interhemispheric functional connectivity, most to near zero. The motor and vision areas decreased, although less so than more associative areas. These are the relatively focal areas where interhemispheric, and more specifically homotopic, functional connectivity was hypothesized to have either additional polysynaptic (post-synaptic?) input non from callosal projecting neurons or possibly through subcortical structures (e.g. thalamocortical circuits). Figure 4A shows this well, where the pre/post central and primary visual areas show a small residual homotopic functional connectivity while surrounded by nearly zero in the adjacent areas. Indeed, this divergence between primary and associative cortical areas nicely foreshadows the discussion in Section 8 as well as the leading sentence in Section 9 Conclusions.

Reply: Thank you for the suggestions. We are sorry for the mistake about Roland et al. 2017 study. The error has been corrected and this section has been rearranged. Furthermore, in section 7.3 we have additionally clarified the limitations of inferring structural connectivity from fMRI-based functional connectivity as well as the difference between fMRI- and EEG-based functional connectivity by adding the following sentence:

"In contrast to fMRI-based functional connectivity dominated by low-frequency (< 0.1 Hz) oscillations of BOLD signal (see 7.1), EEG coherence reflects synchronized fluctuations of excitability in distributed neuronal ensembles on a millisecond time scale inherent to cognitive processes. "

Section 8 expands beyond monosynaptic homotopic cortical projecting neurons to high order integrations brain regions. As one might expect these areas are more difficult for cellular or histologic techniques and rely more on human subject data. The authors discuss split-brain (i.e. post corpus callosotomy) and acallosal (i.e. callosal agenesis) data. Care must be taken in these two populations as all though both represent lack of a corpus callosum, the split-brain subjects were born and at least initial development occurred with an intact corpus callosum, where the acallosal patients did not develop this way and possibly/likely developed alternative structural connections for interhemispheric communications (see Tovar-Moll, PNAS, 2014, Structural and functional brain rewiring clarifies preserved interhemispheric transfer in humans born without the corpus callosum). You also present similar supporting information in Section 9 by reference to Ptito and Lepore, 1983. I have no objections to the data summarized in this section, I do recommend including a brief cautionary statement when interpreting these two similar, yet sufficiently different, human subject populations.

Reply: Thank you for the important suggestion, a cautionary note has been introduced by quoting the study by Tovar-Moll et al PNAS 2014:

“It is important to stress that caution is needed when comparing the results obtained from spilt- brain patients vs callosal agenesis, since in the latter condition the absence of the CC from birth can promote the formation of anomalous white-matter pathways, such as those connecting homotopic region of the inferior parietal lobules (Brodmann areas 39), via the anterior and posterior commissures (Tovar-Moll et al., 2014). This form of long-distance plasticity is probably responsible for the preservation of the interhemispheric transfer of tactile object recognition found in this study”.

Section 9 concludes the discussion. The introduction of a new concept to consider callosal connections as logic gates is not well supported by the rest of the review paper. If you feel this is indeed important, I recommend you develop the concept further with better explanation of the supporting evidence. Otherwise I would recommend removing it. As written this concept appears in a single sentence in the Conclusions and also in the Abstract therefore not in line with the rest of this otherwise well organized and fluid presentation of concepts.

Reply: We agree with the reviewer, the logical gates issue is a complex one and was not sufficiently developed in the text, thus we have removed it completely from the manuscript.

Figures

Figure 1 - the inset comparing steepness of EPSP between callosal and peripheral stimuli would be more readily interpretable with units displayed on the respective axis.
Reply: Calibrations in the X and Y axis of Figure 1 have been added, based on the original paper.

Figure 5 - The SS "Sylvian sulcus" labels in panels A3 and B3 are pointing to the insula, not the Sylvian fissure / sulcus.
Reply: The figure has been modified according to the reviewer's suggestion.

Furthermore, the midline has significant overlap in the peripheral nervous system. I find it difficult to ascertain that bilateral cortical fMRI activations from near-midline tactile stimulation provides reliable data on the function of the CC as opposed to bilateral input to each hemisphere arriving from bilateral peripheral sensory nerves that overlap near and reaching across the midline. Indeed, you refer to such a phenomenon in section 6.2.
Reply: *This is correct, thank you for the comment. We have added the sentence "Bilateral cortical fMRI activation from near-midline tactile stimulation observed also in completely callosotomized patients does indicate that bilateral input can reach each hemisphere through subcortical pathways. In this case the function of the CC in intact brain subjects could be modulatory (see Fabri et a., 2006)".*

Reviewer #2:
Main comments:

The review article "The functional characterization of callosal connections" by Innocenti et al. is a well written, comprehensive review about features of interhemispheric communication via axons forming the corpus callosum. This is a thought provoking article; most of the evidence presented supports the broader rationale that a key feature of callosal function includes modulation of target's responses in a context-dependent manner, which I found very innovative and of potential interest to a wide range of neuroscientists. This is presented from several perspectives that include anatomy, physiology, and lesions in humans and other mammals. Most of the evidenced reviewed comes from studies in primates (including humans) and carnivores, which is a strength from a point of view relevant to humans. However, in my opinion, many of the authors' claims about callosal structure and function could have been strengthened by including more examples from rodents given the wealth of very relevant and detailed literature (see below). Having said that, I also appreciate that the number of references is already very large (>250), so this might be hard to accommodate unless the authors are willing to update some of the older/less relevant citations. This latter point could be very beneficial, as some parts of the manuscript refer to recent advanced methods, such as single-neuron reconstructions or optogenetic circuit mapping, which have provided more precise details than classical methods. For example, the statement that "less than 1% of callosal cells bifurcate and project to ipsilateral areas" is based on methods from over 30 years ago, but more recent findings have shown that in rodents a much larger proportion of callosal neurons also project ipsilaterally (see Allen Atlas and references PMID: 26796534, PMID: 31555081, PMID: 29379878, PMID: 32405029 as examples).

Reply: Thank you for these comments.

We have introduced and discussed results from several rodent studies, especially those providing information not available from other species. To acknowledge the reviewer's comment about rodent studies we have quoted the work by Economo et al eLIfe (2016), MacDonald et al eNeuro (2018); Zhang et al Front Neurosci (2019). At the same time to confirm the paucity of callosal neuron projecting also to ipsilateral areas in monkeys we have quoted an additional work by Meissirel et al JNeurosci (1991) [https://doi.org/10.1523/JNEUROSCI.11-11-03297.1991]and added a cautionary note on the techniques used in monkeys: *"However, new analyses with more advanced techniques in monkeys might temper this conclusion"*.

The paragraphs starting in lines 222, 316, 342, 354, 457, 506, 530, amongst others, refer to callosal projections in area 17/18 of the visual cortices (mostly in primates/carnivores; "vertical meridian", "midline rule"), yet the fact that a homologous projection from V1 to contralateral border between V1/V2 is also present in rodents is briefly mentioned, if at all.

Reply: Thank you for the suggestion, we have now quoted the studies by Land et al. CerCor 2015; Andelin et al J Comp Neurol 2020 in Section 3.2 and 1, Shimaoka et al eLife 2019 in Section 5, supporting that bilateral correlations increase towards the midline representations in mice sensory systems.

Most importantly, there is a similar convergence of callosal axons from S1 to the contralateral S1/S2 border that is very prominent in rodents (PMID: 24945772, PMID: 33661095, PMID: 17942728, PMID: 20105242, PMID: 32166131) and its inclusion might help strengthen the article's points

Reply: Thank you, the suggested papers are now quoted, and a sentence has been added to discuss them in Section 5.1.

Finally, a concluding paragraph synthesizing the main ideas put forward about callosal function (e.g., the highlights of first page) could help bring closure to the article.

Reply: Section 9 is entirely dedicated to Conclusions and Perspectives.

Minor comments:

Comment on 'lacking functional characterization' in line 39 of abstract is then contradicted in line 104.

Reply: Concerning the status of the functional characterization of callosal connections, we have changed the sentence by removing *"...is usually lacking"*, and by adding *"deserves further exploration"*

Line 96 suggests that the CC arose at the same time as the mammalian neocortex, however this occurred much later in evolution (see PMID: 25071525).

Reply: The reviewer is correct; the sentence has been changed as to consider this criticism and the study by Suarez et al. (2014a) has been quoted in the Introduction:

"It is the largest commissure of the brain in all placental mammals and emerged about 150 millions years ago (Aboitiz and Montiel, 2003; Suárez et al., 2018, 2014b).

In section 4.3, optogenetic stimulation has also shown that callosal axons synapse onto pyramidal neurons and interneurons across layers and not just L1 (PMID: 17435752, PMID: 24945772). This is again relevant in lines 801-803.
Reply: The studies by Petreanu et al (2007) and Suarez et al (2014b) have been quoted.

In line 518, similar findings of PMID: 30658859 include callosal spines cluster with non-callosal ones of similar orientation preference.
Reply: The study by Lee et al. (2019) has been quoted, in addition to a very recent one by Liang et al Cerebral Cortex (2021) [https://doi.org/10.1093/cercor/bhab084], although not requested by the reviewer. Both studies point to the role of callosal connections in the integration of neural networks and brain states.

Similarly, in line 826 (as mentioned above), somatosensory disruptions also affect callosal projections to S1/S2 in rodents (PMID: 24945772; PMID: 17942728).
Reply: The studies by Suarez et al (2014b) and Wang et al (2007) have been quoted

Line 821, different to what?
Reply: different has been removed and "manifold" added in its pace.

Typos in lines: 222 (italics in "iv"), 238 (un/an), 388 (space), 519 (properties of), 826 (space), 1787 (reversed order middle-right?), 1789 (brain 'temporal?' slices), 1809-1810 ('A' and 'D' not shown; also mention that in this figure rostral is to the right).
Reply: Thank you, we have made the suggested corrections. Brain slices are "transversal".

Reviewer #3: This is an excellent review paper that provides a timely and considered overview of the anatomy and physiology of the corpus callosum. The team of authors should be commended for their efforts. However, there are some aspects of the presentation that could potentially be improved.

First, although I suspect the authors want to "lay out the facts", I think that the paper would benefit from more a general synthesis, perhaps with a potentially organizing model or construct set out in the beginning and then summaries at the end of each section that refer back to those ideas. To me, one of the most interesting ideas that emerges from the paper was that of the callosum being a "conditional" driver that is doing something quite different than classic models of the callosum as being engaged in simply shuttling information back and forth between the hemispheres so as to allow for the perceptual world to be stitched together across the midline. To the degree that this idea could be presented initially (and contrasted with classic models), and them could be integrated into the summary of each of the major subsections (1,2, etc.), I think it would give the paper more of an impact. For example, in section 2, there could be a concluding section that points out that the (mainly) glutamatergic properties of callosal neurons makes them well suited to drive assemblies of

neuronal activity, and that the variety of callosal diameters, with varying conduction times, and both homotopic and heterotopic connections, enable the effects to be conditional.
Reply: This is a very good suggestion, not easy to implement, however, given the length of the ms. In spite of this, we have followed what suggested by Dr. Banich as much as we could (please see Introduction, Sections 2, 3, 4, 5, 6, 7.1, 7.2, 7.3, 7.4, 8. Section 9 on Conclusions and Perspectives basically remains as it was.

Second, in terms of writing style, I think it would be helpful to make clear when one is talking about neuronal connections/anatomy in general, versus callosal connections/anatomy more specifically. There were places in which I was not sure initially to which the author was referring. For example, at the beginning of section 2.2. ii. the first section reads "The position of the cell body of the neuron of origin.....". The prior sentence was about CC axons, so it wasn't clear whether this sentence referred to callosal axons or axons in general. I think it would help to flag to which you are referring. "Across all neurons, the position of the cell body....." and then clearly tag when you are talking about the callosum "Callosal axons..."
Reply: Thank you for noticing this problem, the ambiguity has been resolved wherever it occurred, as suggested.

Third, another really interesting point raised in the paper is the comment made on the top of page 12, which involves the discrepancy between findings from reversible lesions and those of split-brain patients and animal models with fiber tract lesions. However, there is a dual caveat here. On the bottom of page 11, it is stressed that reversible lesions affect more than callosal cells, but then it is stated on the top of page 12 that one must be cautious in drawing conclusions from split-brain human and animal studies. Since the comment on lesions came second, it made it seem as if more weight was being given to the reversible lesion results. Is that what the author intended? If not, then one might say that each of these methods have their limitations in the same sentence. Furthermore, I think a sentence or two about the causes and potential resolutions of this discrepancy would be helpful (even if reviewed elsewhere).
Reply: Thank you for noticing these caveats, we have rearranged this part of the ms, as suggested, and included a sentence on the discrepancy of the results between the consequences of transitory vs permanent lesions, the latter extensively discussed in clinical and experimental neurology. For this we were forced to quote four additional studies:

- Turrigiano GG. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci*. 1999;22(5):221-227. doi:10.1016/s0166- 2236(98)01341-1
- Marder E, Goaillard JM. Variability, compensation and homeostasis in neuron and network function. *Nat Rev Neurosci.* 2006;7(7):563-574. doi:10.1038/nrn1949
- Keck, T., Keller, G. B., Jacobsen, R. I., Eysel, U. T., Bonhoeffer, T., & Hübener, M. (2013). Synaptic scaling and homeostatic plasticity in the mouse visual cortex in vivo. *Neuron,* 80(2), 327–334. https://doi.org/10.1016/j.neuron.2013.08.018
- Carrera E, Tononi G. Diaschisis: past, present, future. *Brain.* 2014;137(Pt 9):2408-2422. doi:10.1093/brain/awu101

Fourth, there are additional papers that the authors might find of use to include in their review, which come more from the human (than animal) literature.

With regards to the anatomical connections of the corpus callosum:

De Benedictis, A., Petit, L., Descoteaux, M., Marras, C. E., Barbareschi, M., Corsini, F., et al. (2016). New insights in the homotopic and heterotopic connectivity of the frontal portion of the human corpus callosum revealed by microdissection and diffusion tractography. Human Brain Mapping, 37(12), 4718-4735. http://doi.org/10.1002/hbm.23339 [this paper especially speaks to an issue raised on page 20 of the manuscript]
Reply: This study has been quoted and discussed in section 8.

With regards to functional connectivity of the corpus callosum:
Stark, D. E., Margulies, D. S., Shehzad, Z. E., Reiss, P., Kelly, A. M., Uddin, L. Q., Gee, D. G., Roy, A. K., Banich, M. T., Castellanos, F. X., & Milham, M. P. (2008). Regional variation in interhemispheric coordination of intrinsic hemodynamic fluctuations. The Journal of Neuroscience: the official journal of the Society for Neuroscience, 28(51), 13754-13764. https://doi.org/10.1523/JNEUROSCI.4544- 08.2008
Reply: This study has been quoted and discussed in section 7.1

Gee, D. G., Biswal, B. B., Kelly, C., Stark, D. E., Margulies, D. S., Shehzad, Z., Uddin, L. Q., Klein, D. F., Banich, M. T., Castellanos, F. X., & Milham, M. P. (2011). Low frequency fluctuations reveal integrated and segregated processing among the cerebral hemispheres. NeuroImage, 54(1), 517- 527. https://doi.org/10.1016/j.neuroimage.2010.05.073
Reply: This study has been quoted and discussed in section 7.1.

With regards to connectivity after the split-brain procedure: Roland, J. L., Snyder, A. Z., Hacker, C. D., Mitra, A., Shimony, J. S., Limbrick, D. D., Raichle, M. E., Smyth, M. D., & Leuthardt, E. C. (2017). On the role of the corpus callosum in interhemispheric functional connectivity in humans. Proceedings of the National Academy of Sciences of the United States of America, 114(50), 13278-13283. https://doi.org/10.1073/pnas.1707050114
Reply: This study was already described and commented in section 7.1

With regards to modulation of callosal connectivity:
Preisig, B. C., Riecke, L., Sjerps, M. J., Kösem, A., Kop, B. R., Bramson, B., et al. (2021). Selective modulation of interhemispheric connectivity by transcranial alternating current stimulation influences binaural integration. Proceedings of the National Academy of Sciences of the United States of America, 118(7). http://doi.org/10.1073/pnas.2015488118
Reply: This study has been quoted at the end of section 4.1.

Finally, I appreciate the authors including some of my work in their review and their comments on it (bottom page 19/top of page 20). They have interpreted my work in someone of a different light than initially conceived, which I found interesting, and as such, thought it would be worth commenting on. Our studies show that as task difficulty increases, there is an increasing bilateral advantage, i.e., better performance when critical information is divided between the hemisphere as compared to directed to one. The authors have interpreted this

finding as suggesting that different callosal connections or their effects are engaged by different stimuli or by level of task difficulty. This indeed may be the case but was not a possibility that we had considered. Rather, we had assumed that callosal transmission was uniform, automatic and obligatory. We presumed, when tasks are easy, one hemisphere can handle the task adequately and dividing the information ensues additional (time) costs of callosal transmission. In contrast, when tasks are more difficult, dividing and distributed across two processors, i.e., the hemispheres, provides an advantage that outweighs the cost of callosal transmission. I have no problem with the author's alternative interpretation but thought that I would mention the original one.
Reply: Thank you for the interesting point, which has been fully accepted and discussed at the beginning of a new section (7.4), which was also inspired by your request to quote additional studies on behavioral effects (please see your next comment).

With regards to this behavioral effect, there is some nice work by groups other than my own tying the behavioral effect to the morphology and function of the callosum:

Davis, S. W., Kragel, J. E., Madden, D. J., & Cabeza, R. (2012). The architecture of cross-hemispheric communication in the aging brain: linking behavior to functional and structural connectivity. Cerebral Cortex (New York, NY: 1991), 22(1), 232-242. http://doi.org/10.1093/cercor/bhr123

Davis, S. W., & Cabeza, R. (2015). Cross-hemispheric collaboration and segregation associated with task difficulty as revealed by structural and functional connectivity. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 35(21), 8191-8200. http://doi.org/10.1523/JNEUROSCI.0464-15.2015

Höller-Wallscheid, M. S., Thier, P., Pomper, J. K., & Lindner, A. (2017). Bilateral recruitment of prefrontal cortex in working memory is associated with task demand but not with age. Proceedings of the National Academy of Sciences of the United States of America, 114(5), E830- E839. http://doi.org/10.1073/pnas.1601983114
Reply: Thank you for the excellent suggestion, all above papers have been source of inspiration for a small new section (7.4) entitled *"Behavioral effects on callosal architecture and communication"*, where they are quoted.

2nd Decision Letter

Dear Prof. Caminiti,

Thank you for submitting your manuscript to Progress in Neurobiology. We have received comments from reviewers on your manuscript. Your paper should become acceptable for publication pending suitable minor revision and modification of the article in light of the appended reviewer comments.

When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made point by point, and provide suitable rebuttals for any comments not addressed.

To submit your revised manuscript go to https://www.editorialmanager.com/proneu/ and log in as an Author where you will see a menu item called 'Submission Needing Revision'.

Please resubmit your manuscript by Dec 31, 2021.

We look forward to receiving your revised manuscript.

Kind regards,
Sabine Kastner, MD, PhD
Editor-in-Chief
Progress in Neurobiology

Comments from the Editors and Reviewers:

Reviewer #1: The authors have appropriately responded to all my recommendations I have no further concerns or reservations.

Reviewer #2: The authors have done a good job in addressing my previous comments, and I would be happy to endorse this review for publication, as it summarizes important aspects of human brain function that would be of interest to a wide range of neuroscientists.
I only have very small comments and suggestions that the authors may wish to address, in agreement with the Editor, which could potentially strengthen the scope and impact of the main message of this article:

In particular, I appreciate the inclusion of data from more non-human mammals, which in my opinion strengthen the point of the conservation of callosal functions. Similarly, the overall message of callosal connections involved in conditional/modulatory context is much clearer now, and it is explicitly mentioned throughout the text (highlights, abstract, introduction and conclusion). Perhaps the title could also reflect this message more directly, as it currently might read too all-encompassing, but I leave this to the authors/editor as I don't feel too strongly either way.

I do, however, recommend that the authors rewrite the abstract to better indicate such message in a more structured manner, as it currently reads (to me) mostly ambiguous and aspirational (except for the last sentence, which is good but seems disconnected). For example, contrasting notions could be presented with a short synthesis on the types of evidence for each, and a brief synthesis that supports the authors' main hypothesis of callosal function.

I'm happy with the rest of the manuscript and figures, and would only recommend to check for style/typos/grammar, including:
-consider a colon (:) in L192 or briefly mention each subtitle here.
- so/thus instead of 'this' in line 226.
-line 275, move or remove comma after 'divergence'.
-line 293, to enable or that enables.
-line 499, the following… 'section' or 'discussed below'?
-line 513 define EP here (instead of or as well as, in line 575).
-line 759, terminals.
-line 897, humans instead of man.
-line 923, generated. Also consider splitting this paragraph into two sentences (and all others where possible).
-line 976, both hemispheres.
-line 997, occurred 'with/using' the existing.
-line 1078, agenesis studies. Consider splitting this paragraph into shorter sentences.
-line 1207, consider adding Tovar-Moll et al 2014 here too.
-line 2276 (fig 7 legend), consider semicolons after 'baseline' and 'deactivation'.

2nd Author Response Letter

Point-to-Point Reply to Reviewer #2:

We are very grateful to Reviewer for the detailed reading and scrutiny of our manuscript. As suggested, we have made all editing changes proposed (please see *yellow highlight) and rewritten the Abstract, as to make it more stringent, please see it at the end of reply.*

The authors have done a good job in addressing my previous comments, and I would be happy to endorse this review for publication, as it summarizes important aspects of human brain function that would be of interest to a wide range of neuroscientists.
I only have very small comments and suggestions that the authors may wish to address, in agreement with the Editor, which could potentially strengthen the scope and impact of the main message of this article:

In particular, I appreciate the inclusion of data from more non-human mammals, which in my opinion strengthen the point of the conservation of callosal functions. Similarly, the overall message of callosal connections involved in conditional/modulatory context is much clearer now, and it is explicitly mentioned throughout the text (highlights, abstract, introduction and conclusion). Perhaps the title could also reflect this message more directly, as it currently might read too all-encompassing, but I leave this to the authors/editor as I don't feel too strongly either way.

Reply: As for the title, we elected to maintain the actual one, since in a simple way and by using a few words, it adheres to the content of the review.

I do, however, recommend that the authors rewrite the abstract to better indicate such message in a more structured manner, as it currently reads (to me) mostly ambiguous and aspirational (except for the last sentence, which is good but seems disconnected). For example, contrasting notions could be presented with a short synthesis on the types of evidence for each, and a brief synthesis that supports the authors' main hypothesis of callosal function.

Reply: We are grateful to the reviewer for "forcing" us to rewrite an otherwise weak Abstract, please see below the new version:

ABSTRACT

The brain operates through the synaptic interaction of distant neurons within flexible, often heterogeneous, distributed systems. Histological studies have detailed the connections between distant neurons, but their functional characterization deserves further exploration. Studies performed on the corpus callosum in animals and humans are unique in that they capitalize on results obtained from several neuroscience disciplines. Such data inspire a new interpretation of the function of callosal connections and delineate a novel road map, thus paving the way toward a general theory of cortico-cortical connectivity. Here we suggest that callosal axons can drive their post-synaptic targets preferentially when coupled to other inputs endowing the cortical network with a high degree of conditionality. This might depend on several factors, such as their pattern of convergence-divergence, the excitatory and inhibitory operation mode, the range of conduction velocities, the variety of homotopic and heterotopic projections and, finally, the state-dependency of their firing. We propose that, in addition to direct stimulation of post-synaptic targets, callosal axons often play a conditional *driving or modulatory role, which depends on task contingencies, as documented by several recent studies.*

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I'm happy with the rest of the manuscript and figures, and would only recommend to check for style/typos/grammar, including:

-consider a colon (:) in L192 or briefly mention each subtitle here.
Reply: colon has been added

- so/thus instead of 'this' in line 226.
Reply: "this far" has been changed into "so far"

-line 275, move or remove comma after 'divergence'.
Reply: comma has been removed after divergence

-line 293, to enable or that enables.
Reply: "to" has been changed into "that"

-line 499, the following… 'section' or 'discussed below'?
Reply: "section" has been added

-line 513 define EP here (instead of or as well as, in line 575).
Reply: EPs is now defined as evoked potentials, and used as acronym in line 576

-line 759, terminals.
Reply: "terminal" as been changed into "terminals"

-line 897, humans instead of man.
Reply: "humans" has been added instead of "man"

-line 923, generated. Also consider splitting this paragraph into two sentences (and all others where possible).

Reply: This paragraph is now divided in three parts:

"In summary, the analysis of the speed of information transfer has provided intriguing results. Behavioral response can be generated by the hemisphere that receives the information through peripheral or transcallosal inputs. In the latter case, the longer temporal delays of such motor responses are more compatible with transcallosal transfer addressed to subcortical structures, such as contralateral striatum, rather than to contralateral areas".

-line 976, both hemispheres.
Reply: "the" has been deleted

-line 997, occurred 'with/using' the existing.
Reply: occurred "using simple stimuli" has been added

-line 1078, agenesis studies. Consider splitting this paragraph into shorter sentences.
Reply: the first sentence of this section (lines 1078-1079) consists now of two different parts.

-line 1207, consider adding Tovar-Moll et al 2014 here too.
Reply: Tovar-Moll et al 2014 has been added.

-line 2276 (fig 7 legend), consider semicolons after 'baseline' and 'deactivation'.
Reply: This has been done