nature portfolio

Peer Review File

Asynchronous Development of the Mouse Auditory Cortex is Driven by Hemispheric Identity and Sex

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This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have added important data that definitely strengthen the manuscript. I still think that some of the claimed conclusions are not supported by the data in the current version. Please find my concerns below.
1- Figure 2. The addition of mEPSC measurements is most interesting, showing differential changes in mEPSC and mIPSC in both hemispheres of male mice. For a fair comparison however, the same age groups should be used for the display of mEPSC and mIPSC. In particular, the time window P12-P15 should be put forward for mEPSC also (as is done for mIPSC), as it is the time window showing plasticity in the third figure of the manuscript.

2- Figure 3: I value the new data performed for figure 3. However, there are still some open ends. Here are my suggestions:
a. I would appreciate to see the data that shows no plasticity in the left hemisphere following exposure between p15-18 and p16-19. Do these results mean that there is no plasticity in the left hemisphere of male mice at all? If this is the case, it should be clearly stated. Adding the data on female mice does not address it. Also, how does that fit with the results of Fig 1 and 2 showing changes in the left hemisphere of male mice?
b. For female mice, the data shows plasticity in the left hemisphere between p12-p15, but not in the right. Does that mean that the right hemisphere of female shows no plasticity at all, or is it just shifted? (same question as above, but for females)
c. To my understanding, the data in Figure 3 is only for exposed mice. What about control mice? Do they have asymmetries between left and right in terms of neurons responding to 7 kHz tone? Such a baseline would allow us to interpret the data more clearly.
3- What is the p value for Reviewer Fig 1. Why not show this data in the manuscript, maybe as a supplementary figure?

Reviewer #2

(Remarks to the Author)
The authors have done a great job responding to the previous critiques from myself and the other review, and have improved the organization of the manuscript in terms of how the different experiments fit together. No further comments, very nice work!

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have added important controls that now address all of my comments. I congratulate them on an interesting study and on raising awareness of the potential differences between hemispheres and between sexes in neuroscience research.

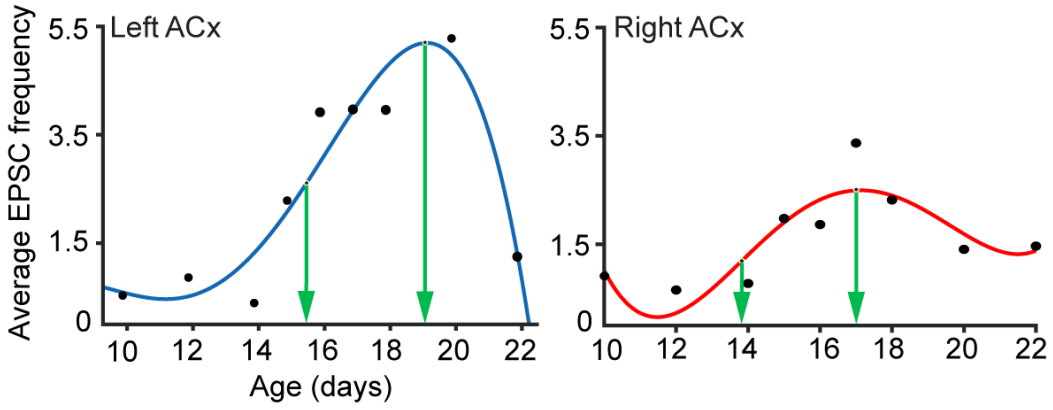
Reviewer #1 (Remarks to the Author):

The authors have added important data that definitely strengthen the manuscript. I still think that some of the claimed conclusions are not supported by the data in the current version. Please find my concerns below.

1- Figure 2. The addition of mEPSC measurements is most interesting, showing differential changes in mEPSC and mIPSC in both hemispheres of male mice. For a fair comparison however, the same age groups should be used for the display of mEPSC and mIPSC. In particular, the time window P12-P15 should be put forward for mEPSC also (as is done for mIPSC), as it is the time window showing plasticity in the third figure of the manuscript.

Response: **We thank the reviewer for their feedback on these data. The revised manuscript now shows mEPSC data grouped the same way as the mIPSC data (see Fig 2). Similar trends are observed, but using the IPSC grouping does obscure some important dynamics, which we now highlight with a figure panel depicting the data collected on a continuous age graph. (see below and Fig 2k in the paper). This day-by-day age panel was in a supplementary figure in the previous submission, and now we feature it in the main manuscript. This panel shows that the age to both half-peak and peak maturational trajectory for mEPSCs in the Right ACx occurs earlier than in the Left ACx. This observation is not apparent when the EPSC data is grouped like the IPSC data.**

We would also like to emphasize that Figs 1 and 2 show maturational trajectories rather than plasticity windows. See below for additional clarification on this point.



2- Figure 3: I value the new data performed for figure 3. However, there are still some open ends. Here are my suggestions:
a. I would appreciate to see the data that shows no plasticity in the left hemisphere following exposure between p15-18 and p16-19. Do these results mean that there is no plasticity in the left hemisphere of male mice at all? If this is the case, it should be clearly stated. Adding the data on female mice does not address it.

Response: **We added an additional figure (Fig. 4) to the manuscript with two additional cohorts of tone reared males to address the reviewer's question on plasticity. Briefly, we are adding:**

- ***Late tone reared male data (p16-19, in Fig. 4h-j)***
- ***Late tone reared male data (p18-21, in Fig. 4k-m)***

These data show the surprising result that the tonotopic map of the Left ACx in males is less responsive to tone rearing history compared to both the Right ACx of males and Right and Left ACx in females.

Also, how does that fit with the results of Fig 1 and 2 showing changes in the left hemisphere of male mice?

Response: **We thank the reviewer for this point which we now clarify throughout the manuscript. In Figs 1 and 2 we show indications of temporally offset cortical maturation, rather than plasticity windows. In the manuscript, we have clarified that although a cortical area may be maturing, this does not automatically mean that the circuit mechanisms needed for the overrepresentation of a tone frequency is active (see e.g. lines 253-258). Maturation refers to many developmental processes and stabilization of adult neural circuits, whereas critical period plasticity involves the capacity for change and adaptation to the sensory environment within those circuits. The data shown in Figures 3 and 4 speak to one functional implication of Right and Left ACx maturing at different ages - in this case, the sensory plasticity generating hemisphere-specific topographic representations of tone frequency in ACx.**

b. For female mice, the data shows plasticity in the left hemisphere between p12-p15, but not in the right. Does that mean that the right hemisphere of female shows no plasticity at all, or is it just shifted? (same question as above, but for females)

Response: **We added one additional cohort of tone reared females to address the reviewer's question on plasticity.**

- ***Early tone reared female data (p9-12, in Fig. 4)***

This data set shows that the Right ACx in female mice does undergo a critical period for tonotopic plasticity and that the timing of this plasticity occurs during an earlier age window than the Left ACx in female mice.

c. To my understanding, the data in Figure 3 is only for exposed mice. What about control mice? Do they have asymmetries between left and right in terms of neurons

responding to 7 kHz tone? Such a baseline would allow us to interpret the data more clearly.

Response: **We show data from naïve animals with no history of tone rearing in Supplementary Fig 2. These data show no naturally occurring hemisphere-dependent overrepresentation of 7kHz tones. This finding has been well-documented in previous literature ((Barkat *et al*, 2011; Cisneros-Franco & de Villers-Sidani, 2019; de Villers-Sidani *et al*, 2007; Han *et al*, 2007; Zhou *et al*, 2008; Zhou *et al*, 2011)).**

3- What is the p value for Reviewer Fig 1. Why not show this data in the manuscript, maybe as a supplementary figure?

Response: **This plot is now in Supplementary Fig 2 and we added the corresponding p-value.**

Reviewer #2: The authors have done a great job responding to the previous critiques from myself and the other review, and have improved the organization of the manuscript in terms of how the different experiments fit together. No further comments, very nice work!

Response: **We thank the reviewer for their assessment and for appreciating our work and revisions!**

References

Barkat TR, Polley DB, Hensch TK (2011) A critical period for auditory thalamocortical connectivity. *Nat Neurosci* 14: 1189-1194

Cisneros-Franco JM, de Villers-Sidani E (2019) Reactivation of critical period plasticity in adult auditory cortex through chemogenetic silencing of parvalbumin-positive interneurons. *Proc Natl Acad Sci U S A* 116: 26329-26331

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