Trends in Neurosciences

CellPress

Spotlight

# Early neuronal inhibition sculpts adult cortical interhemispheric connectivity

Míriam Javier-Torrent[1,3], Antonela Bonafina[1,3], and Laurent Nguyen[1,2,*]

**The maturation of cerebral cortical networks during early life involves a major reorganization of long-range axonal connections. In a recent study, Bragg-Gonzalo, Aguilera, *et al*. discovered that in mice, the interhemispheric connections sent by S1L4 callosal projection neurons are pruned via the tight control of their ipsilateral synaptic integration, which relies on the early activity of specific interneurons.**

Higher cognitive processes are computed by the neocortex, the wiring of which undergoes major refinements during early postnatal life, throughout the so-called critical period [1]. In mammals, cortical neurons comprise two major classes: projection neurons and inhibitory interneurons. Projection neurons are born during successive waves of neurogenesis and migrate radially to form the 'inside-out' patterned six-layered neocortex. Among them, the callosal projection neurons project contralaterally via axons, some of which are eliminated postnatally [2]. Yet, the functional relevance of these transient interhemispheric circuits and how they are restructured after birth remains unclear. The functional maturation of callosal projection neurons relies on cortical spontaneous neuronal activity [3], as well as sensory inputs received during the critical period [4]. This goes along with studies suggesting that early neuronal activity must be spatially balanced between somatosensory cortical regions to allow proper interhemispheric callosal axon targeting [5]. In rodents, layer (L) 4 of the S1 region of the somatosensory cortex (aka barrel cortex) contains an array of barrel-like structures, each of which corresponds to a facial whisker. S1L4 is contacted by thalamocortical axons arising from the thalamic ventral posteromedial nucleus. This circuit enables the processing of tactile stimuli [4] and a bilateral disruption of ventral posteromedial nucleus connections results in an increase in the number of S1L4 callosal projection neurons [2]. Concomitant to the formation of their axons, newborn somatostatin (SST) interneurons form transient synapses with S1L4 callosal projection neurons. Both neuron cell types receive excitatory thalamocortical inputs and SST interneurons promote feedback inhibition to S1L4 callosal projection neurons. This circuit is refined in mice after the first postnatal week [6]. Moreover, early elimination of some of the SST interneurons that contact parvalbumin (PV) interneurons, or disrupted maturation of SST interneurons, impairs the PV feedforward inhibition to projection neurons after thalamocortical stimulation [7]. These lines of evidence support a key role for SST interneurons in thalamocortical circuit maturation. Furthermore, a dysfunction of $GABA_A$ receptors ($GABA_AR$) in callosal projection neurons prevents proper pruning of their contralateral projections, leaving their ipsilateral ones intact [8]. This observation suggests that a GABAergic activity modulates the contralateral network integration of callosal projection neurons.

In a recent publication, to test whether the interhemispheric axonal projections of S1L4 callosal projection neurons are directly modulated by the activity of interneurons, Bragg-Gonzalo, Aguilera, *et al*. administered diazepam to mice *in vivo* to potentiate GABAergic activity via its positive allosteric modulation of $GABA_ARs$ [9]. Diazepam was injected during the first 2 weeks after birth and led to an increased number of adult S1L4 callosal projection neurons. Using *in utero* electroporation, the authors traced the axons of these neurons and showed that the diazepam treatment, by changing the balance between excitatory/inhibitory (E/I) inputs received by S1L4 callosal projection neurons, stabilizes their callosal projections that would normally be eliminated postnatally. Altogether, these results suggest that a tight control of the presynaptic activity during the critical period underlies the postnatal remodeling of the long-range projections of S1L4 callosal projection neurons [9].

As discussed earlier, SST interneurons also receive thalamocortical axons and establish transient synaptic contacts with S1L4 callosal projection neurons and PV interneurons during postnatal development [6]. To analyze the contribution of SST interneurons to the postnatal long-range rewiring of S1L4 callosal projection neurons, Bragg-Gonzalo, Aguilera, *et al.* leveraged the SST-Cre:DTA mouse model. The depletion of SST interneurons led to a premature increase in the inhibition of S1L4 callosal projection neurons. Interestingly, SST interneurons contact PV interneurons and contribute to their maturation during development [7]. Using an early enhancer element (E2) to trace immature PV interneurons and their synaptic inputs, the authors revealed that SST-Cre:DTA mice present a premature innervation of their S1L4 callosal projection neurons by the PV interneurons, likely contributing to the enhanced inhibition of S1L4 callosal projection neurons observed in this mouse model. The aforementioned experiments raised two questions: (i) Are mature contralateral S1L4 callosal projection neurons active in SST-Cre:DTA mice? (ii) What would be the functional consequence of maintaining S1L4 callosal projection neuron long-range connectivity in this mouse model? To address these questions, Bragg-Gonzalo, Aguilera, *et al.* performed calcium imaging in control or SST-Cre:DTA adult mouse cortices during object exploration sessions. The authors demonstrated that the callosal axons of S1L4 callosal projection neurons were active during exploration, but

importantly, these mice presented whisker-sensory related deficits.

Overall, the results from this novel study reveal that interfering with early postnatal ipsilateral connectivity impairs later pruning of interhemispheric S1L4 callosal projections. Indeed, enhancement of local inhibition during the critical period prevents the remodeling of S1L4 projections. This work raises additional questions. For example, what is the pruning mechanism at play that progressively reshuffles these interhemispheric projections? Is it a cell-autonomous driven mechanism (e.g., destabilization of the axonal cytoskeleton) or do other cellular actors (e.g., microglia, astrocytes) play key functions during this dynamic process? This work also describes an unexpected increase in number of S1L4 callosal projection neurons in both SST-Cre:DTA mice and those who received postnatal diazepam treatment. Where do these supernumerary neurons arise from? Future experiments should test whether changes in contralateral circuit integration promote longer-term survival of the callosal projection neurons.

Initially, the projection neurons that wire contralaterally share corresponding transcriptomic features [10]. However, gene expression is modified by activity-dependent mechanisms and may drive the mature projection pattern of these neurons. Along with this concept, Bragg-Gonzalo, Aguilera, *et al.* highlighted a key role for SST and PV interneurons in shaping this maturation process. However, whether these inhibitory inputs also sculpt the molecular fingerprint of the callosal projection neurons remains to be explored. The identification of various cortical interneuron subtypes by scRNAseq raises the question as to whether the axonal pruning of the S1L4 callosal projection neurons is controlled by specific subpopulations of SST and PV interneurons. This work also paves the way to investigate further specific perturbations of the E/I balance at the callosal projection neurons that in humans, may contribute to neurodevelopmental conditions such as autism spectrum disorder and schizophrenia.

## Acknowledgments

The work performed in the Nguyen laboratory is supported by ULiège (Crédit Classique), the F.R.S.-F.N.R.S. (PDR T.0185.20; EOS 0019118F-RG36), WELBIO (CR-2022A-12), the Fonds Leon Fredericq, the Fondation Simone et Pierre Clerdent, the ERANET Neuron (STEM-MCD and NeuroTalk), the Win2Wal (ChipOmics; #2010126), and the ERC-Synergy (UNFOLD).

## Declaration of interests

The authors have no competing interest in relation to this work.

[1]Laboratory of Molecular Regulation of Neurogenesis, GIGA Institute, University of Liège, Liège 4000, Belgium
[2]WELBIO department, WEL Research Institute, Avenue Pasteur, 6, 1300 Wavre, Belgium
[3]Co-first authors contributed equally to this work

*Correspondence:
Lnguyen@uliege.be (L. Nguyen).

https://doi.org/10.1016/j.tins.2024.08.002



## References

1. Jabaudon, D. (2017) Fate and freedom in developing neocortical circuits. *Nat. Commun.* 8, 16042
2. De León Reyes, S. *et al.* (2019) Transient callosal projections of l4 neurons are eliminated for the acquisition of local connectivity. *Nat. Commun.* 10, 4549
3. Tezuka, Y. *et al.* (2022) Developmental stage-specific spontaneous activity contributes to callosal axon projections. *eLife* 11, e72435
4. Diamond, M.E. *et al.* (2008) 'Where' and 'what' in the whisker sensorimotor system. *Nat. Rev. Neurosci.* 9, 601–612
5. Suárez, R. *et al.* (2014) Balanced interhemispheric cortical activity is required for correct targeting of the corpus callosum. *Neuron* 82, 1289–1298
6. Marques-Smith, A. *et al.* (2016) A transient translaminar GABAergic interneuron circuit connects thalamocortical recipient layers in neonatal somatosensory cortex. *Neuron* 89, 536–549
7. Tuncdemir, S.N. *et al.* (2016) Early somatostatin interneuron connectivity mediates the maturation of deep layer cortical circuits. *Neuron* 89, 521–535
8. Babij, R. *et al.* (2023) Gabrb3 is required for the functional integration of pyramidal neuron subtypes in the somatosensory cortex. *Neuron* 111, 256–274.e10
9. Bragg-Gonzalo, L. *et al.* (2024) Early cortical GABAergic interneurons determine the projection patterns of L4 excitatory neurons. *Sci. Adv.* 10, eadj9911
10. Di Bella, J. *et al.* (2021) Molecular logic of cellular diversification in the mouse cerebral cortex. *Nature* 595, 554–559