© 2020. Published by The Company of Biologists Ltd | Development (2020) 147, dev189738. doi:10.1242/dev.189738

REVIEW

Development and plasticity of the corpus callosum

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ABSTRACT

The corpus callosum (CC) connects the cerebral hemispheres and is the major mammalian commissural tract. It facilitates bilateral sensory integration and higher cognitive functions, and is often affected in neurodevelopmental diseases. Here, we review the mechanisms that contribute to the development of CC circuits in animal models and humans. These species comparisons reveal several commonalities. First, there is an early period of massive axonal projection. Second, there is a postnatal temporal window, varying between species, in which early callosal projections are selectively refined. Third, sensory-derived activity influences axonal refinement. We also discuss how defects in CC formation can lead to mild or severe CC congenital malformations.

KEY WORDS: Agenesis, Axonal guidance and plasticity, Callosal projecting neurons, Corpus Callosum, Cortex, Development, Interhemispheric

Introduction

The corpus callosum (CC) is the major axonal tract of the mammalian brain and the principal path transferring information between hemispheres (Fame et al., 2011; Fenlon and Richards, 2015) (Fig. 1). It facilitates higher-order functions of the cerebral cortex such as multidimensional representation of information, associative and executive tasks, coordination of sensory-motor responses, intellectual processing, and management of social and emotional stimuli (Paul et al., 2003; Brown et al., 1999). It is of particular importance in humans, as several cortical functional areas, like those of language, are not symmetrically represented.

The development of the CC has mostly been inferred from animal models and from individuals with CC anomalies. These studies have revealed that CC development begins at embryonic stages and continues during a protracted period of postnatal life; as such, the CC takes longer than other circuits to develop. First, after the fusion of the hemispheres, midline structures generate a route for interhemispheric axons. Then, cortical neurons of the cingulate cortex are the first ones to elongate their axons and cross along this path following specific guidance cues. These pioneer axons serve to guide the subsequent axons of the bulk of cortical neurons. Although some developmental callosal projections are maintained, a significant amount are refined. Finally, along with increasing myelination, contralateral projections that have invaded the cortical plate branch and remodel even further. Both axonal refinement and myelination are instructed in an activity-dependent manner, demonstrating that CC development is a highly plastic process.

CC alterations and/or altered patterns of interhemispheric activity appear in many neurodevelopmental disorders, including complete or partial CC agenesis (AgCC), autism spectrum disorders (ASD), schizophrenia, visual cortical impairments and epilepsy (Aboitiz and Montiel, 2003). Currently, however, researchers and clinicians are unable to predict exactly how structural defects in the CC impact the life and behavior of an individual. We also understand very little about the origin of these problems, partly because the extremely broad spectrum of phenotypes complicates causal correlations between genetics and connectivity. Moreover, the existence of yet to be understood plastic compensatory mechanisms entangles this task further and prevents any accurate prediction of how individuals, even when bearing the same mutations, will perform. As an example, physicians are unable to explain why the CC appears seemingly dispensable in some individuals with complete AgCC, while its loss has dramatic consequences in others.

Here, we review the development of CC interhemispheric circuits, their plasticity and their involvement in disease. We first discuss classical experiments and current knowledge relating to the molecular programming and selection of those cortical neurons that will become adult callosal projecting neurons (CPNs) and, thus, that will form the adult CC. We then review axonal guidance and activity-dependent mechanisms within CC formation. We also discuss axonal exuberance and refinement as important mechanisms that select CPNs and endow the young cortex with extraordinary plasticity. Finally, we review human CC development and CC-related diseases and disorders.

Early studies of cortical callosal populations

To identify the neurons that constitute interhemispheric connectivity, pioneering investigators in the 1970s injected axonal retrograde tracer molecules into the cortical plate of one hemisphere of mammalian animal models. They reported the distribution of labeled somas in the opposite (contralateral) cortex (Fig. 2A), recording in which of the six cortical layers (L1-6) the labeled cells were found. These experiments revealed that adult CPNs are predominantly located in L2/3 and L5, with fewer in L6, whereas L4 neurons are mostly devoid of labeling (Fame et al., 2011; Suarez et al., 2014b). They also showed that distributions vary among the functional areas of the cortex: whereas some contain few CPNs, others, like secondary visual areas, have higher proportions (Fame et al., 2011; Dehay et al., 1988; Innocenti, 1981). These adult callosal circuits have since been confirmed using various experimental approaches, including genetic tracing, viral injections and electrophysiology (Tagawa et al., 2008; Rodríguez-Tornos et al., 2016; Petreanu et al., 2007; Wang et al., 2007).

The next challenge was to address how adult CPN distribution arises during development. To investigate this, researchers performed retrograde tracer injections in young developing animals. They found that, although there are more CPNs in young animals, they distribute following adult-like patterns; the same areas and layers (e.g. L4) always appear devoid of retrotracing signal (Dehay et al., 1986; Fame

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Development (2020) 147, dev189738. doi:10.1242/dev.189738

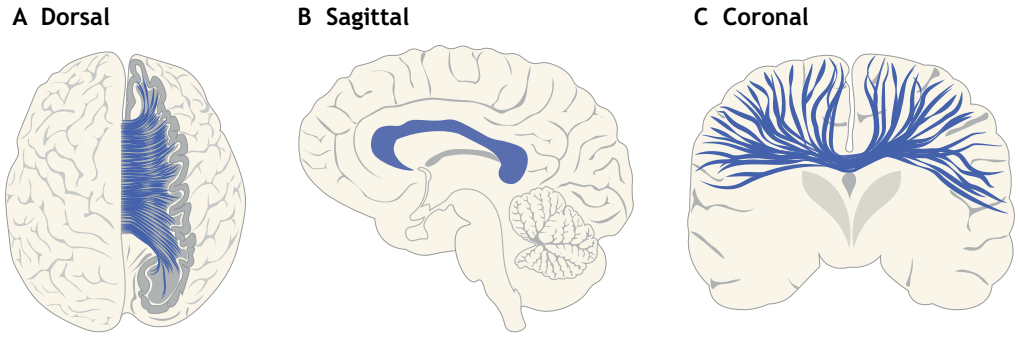

Fig. 1. The general organization of the corpus callosum. (A-C) The structure and location of the human CC (hCC), shown in blue. The CC is the major commissural tract connecting the cortical hemispheres. It is located in the roof of the lateral ventricles, above the diencephalon and beneath the cerebral cortex. (A) Dorsal view of the hCC. (B) Sagittal view of the hCC. This view is broadly used during clinic diagnosis, as the entire rostro-caudal formation is clearly visible. (C) Coronal view of the hCC. Axons of cortical neurons from both hemispheres meet in the midline and cross to the opposite hemisphere in order to target specific contralateral regions. Most of these projections will connect homotopic regions within the brain, and fewer will connect heterotopic regions.

et al., 2011; Innocenti and Clarke, 1983; Innocenti et al., 1977; Meissirel et al., 1991; O'Leary et al., 1981). The logical interpretation was that some neurons never project callosally and that CPNs are born as such. But do developing CPNs project elsewhere? The groups of Dennis O'Leary, Henry Kennedy and others, explored this question using retrograde tracers. They found that the adult and developing L5 contains both CPNs and subcerebral projecting neurons (SCPNs). Importantly, the two subpopulations were clearly segregated at all developmental stages (Stanfield et al., 1982; O'Leary and Koester, 1993). Hence, this suggested that CPNs never reach subcerebral targets, not even transiently. Associative neurons and CPNs were also found as separated subpopulations from early developmental stages (Meissirel et al., 1991). These findings supported a model in which programs determining the cortical projection fates set early major restrictions of axonal development and guidance (Lamantia and Rakic, 1990b; Fame et al., 2011; Fenlon and Richards, 2015; Meissirel et al., 1991; Dehay et al., 1988; Koester and O'Leary, 1993). However, it has been long known that there are cortical neurons with dual contralateral and ipisilateral (cortical or subcortical) projections (Wilson, 1987; Mitchell and Macklis, 2005; Sohur et al., 2014; MacDonald et al., 2018; Economo et al., 2016), indicating that some neurons are able to guide their axons through the midline without disabling subcortical or local wiring fates.

The exuberance and refinement of callosal projections during development

O'Leary, Kennedy, Dehay, Innocenti, Clarke and others not only identified distinct neuronal subpopulations but also uncovered a crucial phenomenon in the central nervous system (CNS): developmental refinement (Stanfield et al., 1982; Innocenti and Price, 2005; Innocenti et al., 1977; O'Leary and Koester, 1993; Meissirel et al., 1991; Dehay et al., 1986; O'Leary, 1987). Injections in different animal models demonstrated that regions containing CPNs always show a higher number of labeled cells at early stages than in adults. This implicated that significant axonal elimination of exuberant callosal projections follows the early pre-sorting of CPNs. This refinement did not involve the death of CPNs (Innocenti et al., 1977; O'Leary et al., 1981; Innocenti and Clarke, 1983, 1984a,b; Clarke and Innocenti, 1986; Meissirel et al., 1991; Dehay et al., 1986; O'Leary and Koester, 1993). Recent work has delved deeper into this refinement process. As explained below, novel studies have further revealed that there is not an early CPN pre-sorting event; on the contrary, sending transient callosal projections is a developmental mechanism that generally involves most neurons in all cortical layers and areas, including L4 neurons (De León Reyes et al., 2019).

In the adult cortex, L4 neurons represent the paradigm of local-only connected neurons. They are the main target of thalamocortical innervation (Feldmeyer et al., 1999). In the primary somatosensory cortex (S1), L4 neurons are 'hubs' of intracolumnar connectivity and their wiring is largely restricted to the barrel column (Feldmeyer, 2012). Early studies of cortical injections using retrograde tracers indicated that L4 neurons never project outside their cortical hemisphere (Fame et al., 2011; Innocenti and Clarke, 1983; Innocenti et al., 1977; Meissirel et al., 1991; O'Leary et al., 1981), which led to the assumption that they harbor intrinsically locally constrained connectivity. However, recent investigations demonstrated that all L4 neurons develop transient interhemispheric axons early in development. Their local connectivity emerges only after postnatal refinement of their early callosal projections (De León Reyes et al., 2019). The key point that led to the discovery of these transient projections was a change in the injection strategy: retrograde tracers were injected directly into the CC instead of targeting the gray matter (Fig. 2B). These injections in the white matter (WM) ensure the labeling of all callosal axons, independently of their behavior in the contralateral hemisphere. Thus, while developing axons that never invade the gray matter or those that remain there for a short time are unlikely to be detected by cortical injections, they are efficiently labeled by CC injections. In fact, the early studies of Innocenti already noticed that more neurons were labeled when injections were placed closer to the WM (Innocenti and Clarke, 1984b; Clarke and Innocenti, 1986). Notably, L4 transient callosals could also be visualized by *in utero* electroporation (IUE) (Fig. 2C) in a mouse transgenic line in which these neurons are specifically marked with green fluorescence protein (GFP). The combination of both techniques allowed the developmental dynamics of L4 refinement to be described. At postnatal day 3 (P3), all L4 neurons display a callosal axon. These axons are gradually eliminated from P3 until P21, when adult local connectivity is achieved and less than 3% of S1L4 remain as CPNs. This refinement is due to the elimination of transient axons and does not involve the death of CPNs. Distinct refinement ratios are also observed, leading to different proportions of callosal L4 neurons in separated functional areas, such as in the secondary somatosensory (S2), primary and secondary visual (V1 and V2), and auditory areas (A1 and A2). Remarkably, CC retrotracing showed that most neurons in the rest of the cortical layers also develop transient exuberant callosal projections (De León Reyes et al., 2019).

Hence, the majority of cortical neurons appear to acquire their adult connectivity based on a common developmental mechanism: sending exuberant callosal projections that are differentially refined

Development (2020) 147, dev189738. doi:10.1242/dev.189738

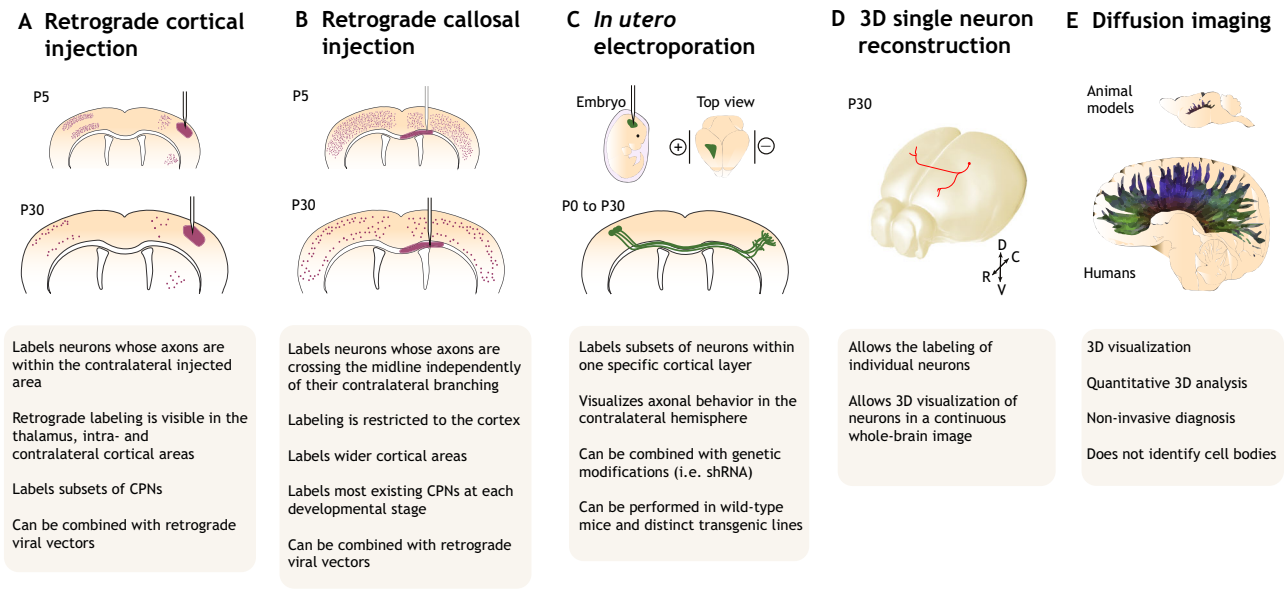

Fig. 2. Methodological approaches for identifying callosal projecting neurons (CPNs) and their axonal behavior. (A) Retrograde cortical injections can be used to label neurons whose axons are present within the area contralateral to that injected. This approach revealed that the number of CPNs is higher in younger animals (e.g. at P5) compared with adults (P30). It also showed that the layer distribution of CPNs is similar at all developmental stages, and that L4 always appears devoid of retrograde labeling. (B) In retrograde callosal injections, the retrograde molecules are injected directly into the CC. This labeling revealed that most cortical neurons project callosally at early stages independently of their laminar identity. Adult connectivity is then acquired after selective axonal refinement of these projections. (C) *In utero* electroporation allows *in vivo* genetic tracing and manipulation of specific cell types. GFP labeling allows the developmental behavior of transient or mature axons to be monitored throughout the midline and contralateral territories. (D) 3D single neuron reconstructions allow the visualization of neuronal projections within the whole brain. (E) Diffusion imaging (DI) can be used to visualize callosal tracts in animal models and humans.

at postnatal stages (De León Reyes et al., 2019). This argues against the early sorting of callosal versus non-callosal projecting fates. Recent work demonstrating the reprogramming of L4 neurons into L2/3-like neurons relied on their acquired ability to project callosally (Hou et al., 2019; Vitali et al., 2018). However, transient callosal axons are shared by both developing L2/3 and L4 subpopulations, and the presence of callosal axons as proof of an identity shift might not be an adequate criterion for demonstrating layer reprogramming. The existence of transient contralateral axons indicates that an early callosal projection program coexists with the potential for local connectivity. This apparent contradiction, which impacts our understanding of early identities and reprogramming, might simply reflect the intrinsic molecular plasticity of young developing neurons.

Activity-dependent refinement: the choice between elimination or terminal connectivity

It is known that sensory cortices are shaped by activity-dependent mechanisms and CPNs are no exception (Antón-Bolaños et al., 2019; Moreno-Juan et al., 2017). The refinement of callosal axons can be influenced by activity-dependent changes at three different levels: (1) sensory alterations from the periphery; (2) alterations in thalamic inputs; and (3) alterations in the firing response or synaptic transmission of pre- and postsynaptic CPNs. In all cases, the severity of the effect strongly depends on the temporal windows of these manipulations; these temporal windows during which callosal connections demonstrate sensitivity to sensory alterations are known as ‘critical periods’.

Sensory alterations from the periphery

Studies of the visual cortex have revealed that postnatal visual experience largely influences CPN selection. For example, in monocularly deprived cats and rats, or in squinted kittens, there is an increase in CPNs (Fig. 3A). These ectopic CPNs are found in V1 (area 17), which is normally a non-callosal area (Innocenti and Frost, 1980, 1979; Olavarria et al., 1987). In contrast, bilateral eye enucleation leads to a reduction of CPNs in the typically callosal-rich area, the border between V1 and V2 (17/18 border) (Fig. 3A) (Innocenti and Frost, 1980, 1979; Olavarria et al., 1987).

Studies of the rodent somatosensory (SS) cortex confirm the importance of sensory-derived activity for CPN selection but with some opposite outcomes (Fig. 3B). Early unilateral whisker cauterization or sectioning of the infraorbital nerve (ION) eliminates about half of the L2/3 callosal contralateral branches (Suarez et al., 2014a; Huang et al., 2013). CC retrotracing injections revealed that this is because only 15% of L2/3 neurons remain as CPNs in this context (De León Reyes et al., 2019). Interestingly, the number of L2/3 CPNs, or their contralateral columns, is not altered if manipulations of the whiskers or ION are performed bilaterally (De León Reyes et al., 2019; Suarez et al., 2014a; Huang et al., 2013; Koralek and Killackey, 1990). However, S1L4 CPN number increases up to 20%, resembling the increases in visual CPNs observed upon unilateral deprivations (Fig. 3B) (De León Reyes et al., 2019).

Altogether, any attempt to establish a unidirectional relationship between sensory activity and refinement, or to extract general rules for callosal decisions, appears simplistic. For example, while balanced activity between hemispheres is a crucial requirement for some callosal neurons, it seems dispensable for others, such as visual CPNs or the remaining S1L2/3 populations of unilaterally cauterized mice (De León Reyes et al., 2019; Suarez et al., 2014a; Huang et al., 2013; Koralek and Killackey, 1990). In addition, none of the studies discussed above provide the precise mechanisms by which activity influences the selection of CPNs. Thus, understanding how the thalamus orchestrates callosal circuit assembly requires further investigation.

Development (2020) 147, dev189738. doi:10.1242/dev.189738

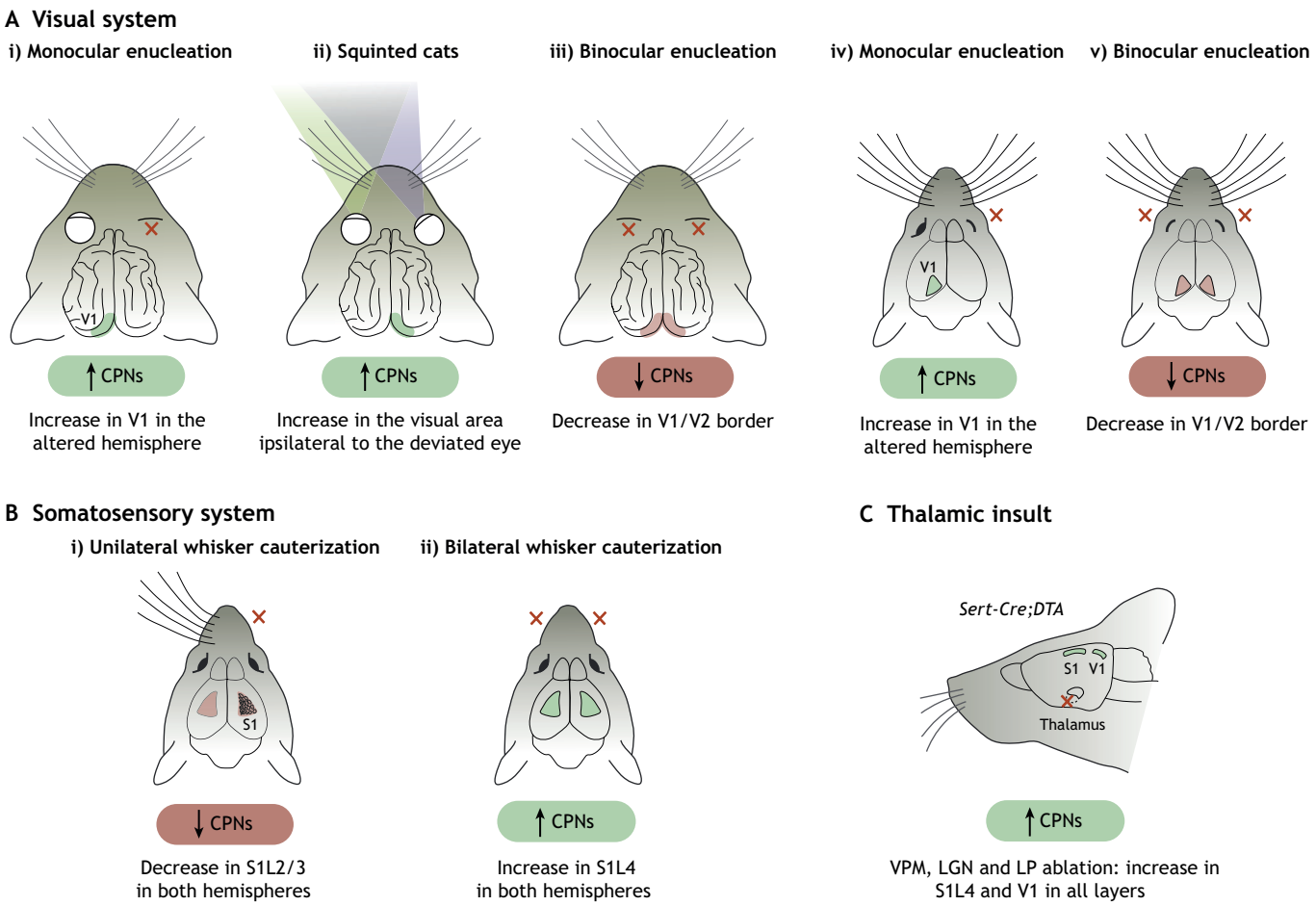

Fig. 3. Early sensory alterations influence the stabilization of callosal neurons. (A-C) Representative examples from the visual system (A), the somatosensory system (B) and the thalamus (C) are shown. (A) Early monocularly deprived cats exhibit an increased number of callosal projecting neurons (CPNs) in the primary visual cortex (V1) contralateral to the removed eye (i). Surgically induced strabismus (squinting) in cats augments L3 CPNs in the visual cortex (ii). Binocular enucleation in cats results in a decrease in CPNs in the V1/V2 border, which is a typically callosal-rich area (iii). Monocular enucleation in rodents leads to an increase in V1 CPNs in the hemisphere contralateral to the removed eye (iv). Binocular enucleation in rats produces a reduction in CPNs in V1/V2 (v). (B) Unilateral whisker cauterization in mice disrupts the balanced activity between S1 areas and decreases the number of L2/3 CPNs in both hemispheres (i). Bilateral whisker cauterization in mice eliminates whisker-derived inputs. Preserving balanced activity and leading to increased stabilization of S1L4 CPNs (ii). (C) Genetic ablation of specific thalamic nuclei using *Sert-Cre;DTA* mice increases CPN numbers. LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; VPM, ventral posterior nucleus.

Sensory thalamic alterations

Functional areas of the cortex are each innervated by distinct thalamic nuclei and they also exhibit very different proportions of CPNs. S1L4, which is principally innervated by the ventral posterior nucleus (VPM), contains almost no CPNs in adult mice. In contrast, many S2L4 neurons, innervated by the posteromedial nucleus (Pom), display callosal projections (23% of S2L4) (De León Reyes et al., 2019). These differences appear to be due to different refinement dynamics that depend on distinct thalamic inputs. The genetic ablation of VPM, which causes an innervation switch of S1L4 by ectopic Pom thalamocortical axons (Pouchelon et al., 2014), reduces refinement and increases the number of S1L4 CPNs (20% of S1L4) (De León Reyes et al., 2019) (Fig. 3C). An independent study that aspirated the dorsal thalamus of rats at birth also reported ectopic callosal branches innervating S1 (Koralek and Killackey, 1990). In the visual system, it was found that the ablation of thalamic axons innervating V1 duplicates the number of CPNs in all cortical layers, causing functional interhemispheric hyperconnectivity (De León Reyes et al., 2019) (Fig. 3C). This agrees with previous thalamic lesion studies performed in rats with expanded callosal innervation of V1 (Cusick and Lund, 1982). Thus, thalamic inputs determine at least three aspects of callosal assembly: (1) refinement rates and CPN numbers; (2) CPN distribution across layers; and (3) contralateral postsynaptic targets.

The firing response and synaptic transmission modulate CPN projection

The relevance of the firing response of a neuron during the establishment of callosal connections has been examined using Kir2.1, an inward rectifying potassium channel that lowers the resting membrane potential of the neuron. In the mouse visual system, overexpressing Kir2.1 in L2/3 neurons, and thus lowering their firing threshold (i.e. making it less likely to fire upon a given input), decreases contralateral innervation (Mizuno et al., 2007). Kir2.1 overexpression affects callosal axons but not neuronal identity, migration or the general capacity to project axons (Mizuno et al., 2010, 2007). Similar results were obtained in the mouse SS cortex, where the overexpression of Kir2.1 in L2/3 was shown to reduce the contralateral axonal column formed at the S1/S2 border without impairing ipsilateral branching (Suarez et al., 2014a;

Development (2020) 147, dev189738. doi:10.1242/dev.189738

Wang et al., 2007; Hand et al., 2015). Interestingly, the combination of Kir2.1 expression together with the blockage of synaptic transmission in the presynaptic CPN –via tetanus toxin expression – causes a more severe reduction in contralateral projections than Kir2.1 expression alone (Mizuno et al., 2007). This demonstrates the importance of synaptic vesicle release for axonal connectivity. It was further shown that, in L2/3 callosal axons, mitochondrial capture dictates the points of axonal branching, and mitochondrial size determines presynaptic release during callosal refinement (Courchet et al., 2013; Lewis et al., 2018).

Callosal connectivity also appears to be coupled with the acquisition of mature and specific firing modes through the expression of certain transcription factors (TFs). In L2/3, the TF Cux1 facilitates the upregulation of the ion channel Kv1 (initiated at P8 and preserved later), which is responsible for producing the typical reliable L2/3 firing patterns. The acquisition of such mature firing patterns is necessary for callosal stabilization; downregulation of either Cux1 or Kv1 in L2/3 CPNs causes the elimination of contralateral branches (Rodríguez-Tornos et al., 2016). Another element that has a strong influence on the development of callosal connections is the activity of their postsynaptic neuronal counterparts in the opposite hemisphere. Non-altered CPNs are unable to stabilize their callosal projections when Kir2.1 is overexpressed in their postsynaptic targets (Mizuno et al., 2010). Moreover, postsynaptic activity regulates local versus long-range circuits. In L2/3 neurons, sensory activity upregulates the TF Mef2c, diminishing their long-range contralateral inputs, which in turn boosts local connectivity. Contrary, postnatal deletion of Mef2c increases contralateral S1 long-range responses and decreases local afferents (Rajkovich et al., 2017). This highlights the importance of neuronal-intrinsic activity and input processing in both the pre- and postsynaptic neurons for proper callosal connectivity.

Guidance cues during CC formation

During CC development, cortical neurons route their projections through the callosal pathway towards precisely defined contralateral areas and layers. CPN axons and cells located along their way express a variety of guidance ligands and receptors, ensuring proper navigation. The role of several of these guidance cues has been addressed using knock out (KO) and transgenic animal models (summarized in Table 1). From these data, we can extract some general conclusions. First, the most frequent phenotype among all models is one in which cortical axons approach the midline but fail to cross to the opposite hemisphere; or if they do, they follow aberrant interhemispheric routes, as in the case of Slit2 or Robo KO (Bagri et al., 2002; Lopez-Bendito et al., 2007; Unni et al., 2012). Second, most KO mice models are lethal at birth, and E17 or P1 are the latest stages analyzed. As callosal axons from upper cortical layers do not reach the midline until postnatal stages (De León Reyes et al., 2019), the effect of these guidance cues in the later-crossing axons remains to be addressed. Third, many genes described as necessary for callosal axon guidance, such as those encoding Slit2, netrin 1 or Eph/ephrin, are also implicated in other biological processes such as the proper development of the cells forming midline structures (Unni et al., 2012; Shu and Richards, 2001; Andrews et al., 2007). This has to be taken into account when interpreting phenotypes of KO models.

As an example, take the guidance molecules Slit2 and Robo1. Slit2 is a chemorepulsive molecule secreted by glial cells at the midline and its receptor, Robo1, is located on callosal axon growth cones. Slit2-Robo1 interaction causes cortical axons to avoid both ventral and dorsal territories, forcing them to cross the midline (Shu and Richards, 2001). In Slit or Robo KO models, some CPN axons cross to the contralateral hemisphere, but the majority turn to navigate into the septum or within the rostrocaudal plane next to the midline, forming the so-called Probst bundles (Bagri et al., 2002; Andrews et al., 2007; Shu and Richards, 2001). Interestingly, in these and other KO models (such as netrin 1 or DCC KOs) an ectopic ventral commissural pathway appears (Bagri et al., 2002; Fazeli et al., 1997; Serafini et al., 1996; Lopez-Bendito et al., 2007; Unni et al., 2012). This raises the hypothesis that callosal guidance cues do not act as a midline barrier for cortical axons; rather, they might have appeared as an evolved mechanism favoring midline crossing at the corticoseptal boundary (Suarez et al., 2014b).

A closer look at these studies also reveals that only a few have tested the cell-autonomous effect of guidance molecules. For example, neurogenin 2 (Ngn2) KO, which is lethal at birth, causes partial to severe AgCC. However, when an shRNA-Ngn2 is electroporated into L2/3 neurons, no defects in midline crossing are seen. Instead, electroporated L2/3 neurons project ectopically to cortico-cortical and subcortical targets (Hand and Polleux, 2011). Similarly, in the KO of the neuropilin 1 subunit that mediates semaphorin interaction, AgCC with incomplete penetrance is observed (Gu et al., 2003), but the specific downregulation of this receptor in L2/3 does not impair midline crossing (Wu et al., 2014). Hence, many guidance-related phenotypes in KO models might not be caused by cell-autonomous restrictions of midline crossing. Indeed, the primary etiology of complete AgCC is due to defects in interhemispheric remodeling by midline glial populations that provide a substrate for callosal axons to cross the midline (Gobius et al., 2016). In addition to the requirement for a fused interhemispheric glial substrate, the timing of callosal axon crossing is regulated non-cell-autonomously (Choe et al., 2012).

In agreement with all of the above, a molecule that acts specifically as a stop signal for CC axons at the midline has not yet been identified. It was thought that the acquisition of CPN identity was governed by the capacity of a neuron to cross the midline following specific guidance cues. However, previous and recent findings demonstrate that most cortical neurons cross over to the contralateral hemisphere, supporting the lack of axonal restrictions for midline crossing (De León Reyes et al., 2019; Innocenti and Price, 2005). As such, it is likely that guidance cues are not responsible for the selection of commissural neurons but fundamental for proper innervation of specific targets.

The molecular identity of CPNs and the plasticity of young cortical neurons

Cortical neurons have traditionally been classified according to their morphology, laminar location, connectivity and neurotransmitter expression (Peters and Jones, 1984; Ramón y Cajal, 1995; Rakic, 1995). More recently, the '-omics' revolution has refined this taxonomy by revealing genes, transcriptomes and proteins that identify neuronal subpopulations even at single-cell resolution (Nowakowski et al., 2017; Molyneaux et al., 2015; Lodato and Arlotta, 2015; Pouchelon et al., 2014; Azim et al., 2009a; Mayer et al., 2018; Joshi et al., 2008; Frangeul et al., 2016). However, despite these breakthroughs, we are still unable to define the precise molecular identity of CPNs.

A significant number of studies have investigated TFs as potential identity determinants by reporting connection failures after their loss of function (Molyneaux et al., 2007). Yet only a few genes have demonstrated themselves to be sufficient to promote specific connectivity, i.e. their gain of function implies the acquisition of a given projection pattern. Such is the case of Fezf2, the overexpression of which in L2/3 is sufficient to generate ectopic subcortical

Table 1. Penetrance of partial or severe AgCC in knockout mouse models

Mutant	Age at analysis	CC phenotype	Penetrance	Other commissural phenotypes	References
Slit2$^{-/-}$	E17	Few axons cross the midline; some are stacked at the midline forming Probst bundles. Most reach the midline but ectopically enter the septum or rostral regions.	Full (*n*=5)	Ectopic HC projections to rostral, ventral and lateral targets. HC and CC axons are mixed. Presence of an ectopic ventral commissure located above the AC.	Bagri et al. (2002), Unni et al. (2012)
Slit3$^{-/-}$	E17	Rostral and medial: few axons cross the midline, most ectopically enter the septum or form Probst bundles Caudal: no visible defects	33% (*n*=4/12)	-	Unni et al. (2012)
Slit1$^{-/-}$ *Slit3*$^{-/-}$	E17	Rostral: Probst bundles Medial: few axons cross, most form Probst bundles Caudal: no visible defects	42% (*n*=5/12)	-	Unni et al. (2012)
Robo1$^{-/-}$	E17-E18	Few axons cross the midline. Most reach the midline but ectopically enter the septum.	Full (*n*=7)	Ectopic HC projections to rostral, ventral and lateral targets. HC and CC axons are mixed.	Andrews et al. (2007), Unni et al. (2012)
Robo1$^{-/-}$ *Robo2*$^{-/-}$	E18	Most reach the midline but ectopically grow into the septum or form Probst bundles	Full (*n*=3)	Presence of an ectopic ventral commissure located above the AC. The AC is ventrally displaced.	Lopez-Bendito et al. (2007)
Ntn1$^{-/-}$	E17-E18	Most reach the midline but ectopically grow into the septum or form Probst bundles	Full (*n*=11)	Absence of the HC. The AC is reduced. A large aberrant commissure is found in the roof of the fourth ventricle.	Serafini et al., (1996), Fothergill et al., (2014)
Dcc1$^{-/-}$	E17	Most reach the midline but ectopically grow into the septum or form Probst bundles	Full (*n*=3)	Absence of the HC. The AC is reduced. A large aberrant commissure is found in the functional region between hindbrain and midbrain.	Fazeli et al. (1997), Fothergill et al. (2014)
Epha5$^{-/-}$	Adult	Partial AgCC in frontal, visual and secondary somatosensory cortex. Motor CC is unaffected.	48% (*n*=15/31)	Hippocampal contralateral projections are reduced	Yue et al. (2002), Hu et al. (2003)
Ephb1$^{-/-}$	P1	Variable phenotypes: mild to severe AgCC	87% (*n*=47)	-	Yokoyama et al. (2001), Mendes et al. (2006)
Ephb2$^{-/-}$	P1	Variable phenotypes: mild to severe AgCC	61% (*n*=23)	Reduced AC	
Ephb3$^{-/-}$	P1	No effect on CC	0% (*n*=22)	Reduced AC	
Ephb3$^{-/-}$	P0-P1	AgCC with variable severity	37.5% (*n*=3/8)	-	Orioli et al. (1996)
Ephb2$^{-/-}$ *Ephb3* $^{-/-}$	P0-P1	AgCC with variable severity	89% (*n*=8/9)	Reduced AC	Orioli et al. (1996)
Ephb1$^{-/-}$ *Ephb2*$^{-/-}$	P1	AgCC with variable severity	88% (*n*=22)	-	Yokoyama et al. (2001), Mendes et al. (2006)
Ephb1$^{-/-}$ *Ephb3*$^{-/-}$	P1	AgCC with variable severity	90% (*n*=20)	-	
Ephb2$^{-/-}$ *Ephb3*$^{-/-}$	P1	AgCC with variable severity	Full (*n*=7)	-	
Efnb1$^{-/-}$	E17	AgCC. Axons ectopically project entering the ipsilateral septum.	Full (*n*=4)	-	Bush and Soriano (2009)
Efnb3$^{-/-}$	P1	AgCC with variable severity	84% (*n*=37)	-	Yokoyama et al. (2001), Mendes et al. (2006)
Ephb1$^{-/-}$ *Efnb3*$^{-/-}$	P1	AgCC with variable severity	Full (*n*=23)	-	
Ephb2$^{-/-}$ *Efnb3*$^{-/-}$	P1	AgCC with variable severity	89% (*n*=16)	-	
Fzd3$^{-/-}$	E18	AgCC with variable severity	-	Loss of the thalamocortical and corticothalamic tracts, and the AC	Wang et al. (2002)
App$^{-/-}$ *Aplp2*$^{-/-}$	P0	AgCC with variable severity	-	-	Wang et al. (2017)
Draxin$^{-/-}$	P0-P1	AgCC with variable severity	Severe (*n*=7/12) Mild (*n*=5/12)	Reduced HC and AC. Variable severity.	Islam et al. (2009)
Plxna1$^{-/-}$	P0	AgCC in the anterior part of the CC.	76% (*n*=10/13)	-	Hossain et al. (2019)
Sema3c$^{-/-}$	E18	Partial to severe AgCC in the anterior part of the CC. Reduced elongation of post-crossing callosal axons.	-	-	Niquille et al. (2009, Mire et al. (2018)
Sema3a$^{-/-}$	E17	Slight reduction of pioneer axons Disrupted axon order in the CC and in the contralateral hemisphere	-	-	Catalano et al. (1998), Piper et al. (2009), Zhou et al. (2013)
Npn1 Sema$^{-/-}$	E17	AgCC with variable severity	-	-	Gu et al. (2003)
Ngn2$^{-/-}$	E18	AgCC with variable severity	Severe (*n*=2/13) Partial (*n*=6/13)	-	Hand and Polleaux (2011)
Ryk$^{-/-}$	E18	Axons cross the midline but are unable to reach the contralateral hemisphere. After midline crossing some axons grow back towards the midline.	Severe (*n*=5/22) Partial (*n*=17/22)	No visible alterations in other commissures	Keeble and Cooper (2006)
Draxin$^{-/-}$; *Tsku*$^{-/-}$	P1	AgCC with variable severity	Weak (60% *n*=3/5) Severe (40% *n*=2/5)	Agenesis of the AC	Hossain et al. (2013)

AC, anterior commissure; AgCC, agenesis of the CC; CC, corpus callosum; E, embryonic day; HC, hippocampal commissure; P, postnatal day; -, not analyzed or not mentioned. Rostral refers to the anterior part of the CC. Medial refers to the medial part of the CC. Caudal refers to the caudal part of the CC. When not specified, the defects in the CC are present all along the rostro-caudal axis.

Development (2020) 147, dev189738. doi:10.1242/dev.189738

Development (2020) 147, dev189738. doi:10.1242/dev.189738

projections (Rouaux and Arlotta, 2013), or Tbr1, the expression of which in L5 converts corticospinal into cortico-thalamic neurons (McKenna et al., 2011). However, many other TFs do not fulfill the criteria for being master instructors. For example, Cux1 deletion in L2/3 CPN impairs callosal stabilization but Cux1 is also expressed in other non-callosal populations and its overexpression does not drive callosal fate (De León Reyes et al., 2019; Rodríguez-Tornos et al., 2016).

Perhaps the most important example of a TF often mistaken as a definitive callosal determinant is Satb2. E18.5 Satb2 KO brains show very few axons crossing the midline and exhibit increased subcortical projections, which led to the conclusion that Satb2 loss reprograms CPNs into subcortical neurons (Alcamo et al., 2008; Britanova et al., 2008). However, as Satb2 KO mice die at birth, conditional deletions of Satb2 in the cortex were generated to overcome this lethality and test its role in upper layers (Zhang et al., 2019; Leone et al., 2015). Surprisingly, a significant number of neurons have callosal connections in these mice. Furthermore, retrograde injections in the thalamus and peduncle revealed that ectopic corticothalamic and corticospinal axons originate exclusively from deep layers. This argued against the conversion of Satb2-deficient L2/3 CPNs to a deep layer fate, as proposed by the initial investigations (Zhang et al., 2019; Leone et al., 2015). More recent studies showed that IUE-based elimination of Satb2 in L2/3 does not impair callosal connectivity. Nevertheless, L2/3 axons are visible in the internal capsule, indicating that they also develop subcortical projections. The same experiment in either L5 or L6 induces a significant reduction of contralateral connections (Paolino et al., 2020). Molecularly, Satb2 regulates distinct gene networks in layer- and time-dependent manners (Paolino et al., 2020; Leone et al., 2015). In summary, it seems that Satb2 expression is involved in the establishment of callosal projections of deep layers but has a different role in L2/3. Indeed, cumulative reports conclusively show that Satb2 expression, although developmentally regulated, is broad and not restricted to CPN or late-born upper layer neurons (Alcamo et al., 2008; De León Reyes et al., 2019; Britanova et al., 2008; Harb et al., 2016; Huang et al., 2013; Jaitner et al., 2016).

Therefore, so far there is no common molecular program regulating all CPNs, nor a 'master gene' whose expression commits neurons to a callosal fate. But why is it so difficult to find a CPN molecular fingerprint? This could be due to the fact that CPNs locate in all layers and are born from different precursors during an extended developmental window (from E12 to E15); however, this is just a partial explanation. It has been demonstrated that CPNs are a much more heterogeneous population than anticipated, and that their RNA expression profiles change dynamically during development (Arlotta et al., 2005; Molyneaux et al., 2009, 2015; Lodato and Arlotta, 2015). If we combine this information with the notion of early transient callosal projections and cortical epigenetic modifications, it seems plausible that an initially unspecified molecular program allowing axonal exuberance is subsequently overwritten by molecular instructions added gradually during postnatal differentiation (Rouaux et al., 2012; De León Reyes et al., 2019; Molyneaux et al., 2007, 2015; Lodato and Arlotta, 2015; Pouchelon et al., 2014; Azim et al., 2009b; Mayer and Fishell, 2018; Joshi et al., 2008; Frangeul et al., 2016; Nowakowski et al., 2017). Context-dependent postnatal genetic editing might be responsible for discarding a default callosal fate, triggering refinement. In agreement with the idea of such a non-committed early program, it has been shown that young neurons can co-express TFs that are always segregated in the adult, and there are also reports of some adult 'hybrid' subpopulations, such as those with dual interhemispheric and subcortical axons that co-express Sox5 and Lmo4 (Sohur et al., 2014; Azim et al., 2009b).

Overall, axonal exuberance could be the natural phenotypic consequence of early-unspecified molecular programs that are gradually crafted during development (Fig. 4). The elusive molecular identity of CPNs might simply reflect the existence of multiple developmental molecular trajectories that drive stabilization of callosal axons. This permissive scenario might explain the plasticity of young callosal neurons and their potential to generate diverse CC circuits in context- and activity-dependent manners (Pouchelon et al., 2014; Su et al., 2017; Yap and Greenberg, 2018; West and Greenberg, 2011).

The behavior of transient callosal axons

Transient axons sample multiple territories that will not be integrated into adult circuits under normal conditions, but that can be innervated if context changes occur, generating non-canonical circuits (Olavarria et al., 1987; Huttenlocher and Raichelson, 1989; Uematsu et al., 1996; De León Reyes et al., 2019; Innocenti and Frost, 1979). Neurons with broader exploratory behavior likely harbor a greater plasticity (Fig. 5), and there seem to be differences in the behavior of exuberant projections and refinement processes in different brain territories. In the thalamus, exuberant efferent collaterals from first-order (FO) thalamic nuclei, such as the LGN, VPM or the ventral part of the MGN, confine their projections to the cortical area that they will innervate in the adult (de Venecia and McMullen, 1994; Naegele et al., 1988; Rebsam et al., 2002; Catalano et al., 1996). In contrast, young axons of higher-order (HO) thalamic nuclei, such as Pom

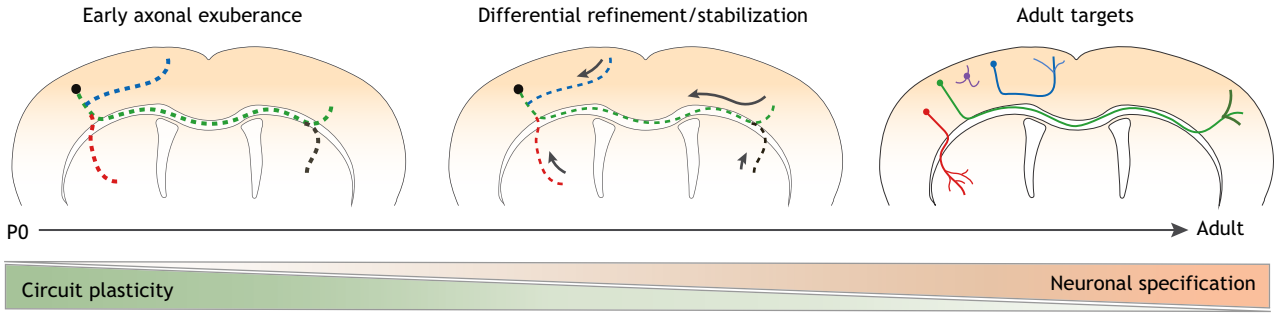

Fig. 4. Schematic illustration of the refinement of early axonal projections. During the early stages of CC development (left panel), cortical neurons send transient axons to distinct territories. At these stages, cortical neurons are not fully specified, they harbor more plasticity and circuits can be reshaped in a context-dependent manner. Transient exuberant projections are then selectively refined or stabilized during postnatal stages (middle panel). During this time, cortical neurons gradually acquire their adult identities and the capacity to reshape callosal circuits decreases. Adult connectivity is ultimately achieved after selective axonal stabilization or elimination within the explored territories (right panel).

Development (2020) 147, dev189738. doi:10.1242/dev.189738

thalamocortical projections, navigate broadly throughout non-adult territories. They non-specifically invade barrel columns, supplementary SS and motor areas (Deschenes et al., 1998; Lu and Lin, 1993), which may explain why VPM ablation results in an expansion of Pom axons within S1L4 (Pouchelon et al., 2014). The HO visual nucleus (lateral posterior nucleus; LP) also innervates more territories than the LGN; it targets not only V1 and V2 but also A2 (Ji et al., 2016). Adult corticospinal (CS) neurons also explore multiple territories prior to refinement (Stanfield et al., 1982; Ribeiro Gomes et al., 2020; Stanfield and O'Leary, 1985; Bates and Killackey, 1984; Cabana and Martin, 1984). In the pyramidal tract, most adult CS axons decussate contralaterally and only minor populations project ipsilaterally. In young cats and monkeys, however, the proportion of ipsilateral and contralateral projections is similar (Ribeiro Gomes et al., 2020; Li and Martin, 2000). Importantly, these ipsilateral projections can survive into the adult when lesions in the sensorimotor cortex or CS tract occur early (Reinoso and Castro, 1989; Huttenlocher and Raichelson, 1989; Hicks and D'Amato, 1970; Uematsu et al., 1996).

In the CC, both transient and to be terminal callosal axons cross the midline and follow indistinguishable paths through the WM. However, the behavior of transient axons once they reach the contralateral hemisphere is more obscure, and potential targets of plastic connectivity are more difficult to predict. It was reported that, in the cat visual cortex, only some of the V1 transient axons traced at P9 barely invade the homotopic V1L6 (Innocenti, 1981; Aggoun-Zouaoui and Innocenti, 1994). However, similar experiments performed at P6 revealed a significant amount of V1 axons climbing to upper layers (Ding and Elberger, 1994). Hence, the WM might act as a barrier for some transient axons, but it is not a common impediment. Interestingly, both studies described that V1 transient CC axons preferentially explore the contralateral V1/V2. Somatosensory transient callosal axons invade the gray matter but only navigate contralateral homotopic regions (De León Reyes et al., 2019; Chalupa and Killackey, 1989). In contrast, in the auditory cortex of newborn cats (P1-P4), transient callosals behave less restrictedly. They not only innervate the contralateral auditory but also transiently scan V1 and V2 cortices (Innocenti and Clarke, 1984a), which perhaps explains why auditory circuits are expanded in visually impaired individuals (Collignon et al., 2011, 2013). In sum, the fact that cortical neurons explore non-adult targets gives them the possibility to generate non-canonical circuits under altered situations. The exploratory capacity of CC transient exuberant projections is yet to be characterized but it seems to vary between areas and CPN subpopulations.

The biological significance of axonal exuberance in the cortex

Refinement of transient projections is a common developmental process within the CNS (Sohur et al., 2014; Catalano et al., 1996; Naegele et al., 1988; Rebsam et al., 2002; O'Leary, 1987; De León Reyes et al., 2019; Innocenti et al., 1988; O'Leary et al., 1981; Chalupa and Killackey, 1989; Stanfield and O'Leary, 1985; Stanfield et al., 1982; Galea and Darian-Smith, 1995; O'Leary and Stanfield, 1989, 1985; Innocenti and Price, 2005). Moreover, the overproduction of neuronal cells and synaptic densities is commonly observed in the nervous system (Denaxa et al., 2018; Wong et al., 2018; Duan et al., 2020). Yet spending cellular energy in producing massive numbers of transient axons that will be later eliminated might seem as an apparent contradiction. Such initial overabundance may have simply evolved as one of the viable mechanisms for building connections. Alternatively, it might have offered selective advantages during brain evolution. In this section, we elaborate on the possible benefits of developmental axonal exuberance.

When discussing the biological significance of axonal exuberance in the CC, it is useful to compare it with other systems. As mentioned above, not all neuronal types enjoy the 'permissibility' to explore multiple targets in both hemispheres. In the retina, for example, differential Zic2 expression in RGCs directs axons exclusively to the ipsi- or contralateral hemisphere (Drager, 1985; Sretavan, 1990; Erskine and Herrera, 2014). Young vertebrate motor neurons also exhibit high muscle group specificity (Lance-Jones and Landmesser, 1981; Tosney and Landmesser, 1984, 1985). In these stereotyped systems, early molecular identities restrict axonal pathfinding. However, the cortex is built of highly complex networks that initiate their wiring according to *a priori* unknown environmental stimuli. If young cortical neurons were to project exclusively to their final targets, they would require very complex initial genetic codes that, in turn, could impair the adaptability of cortical networks. Axonal overproduction might therefore be the evolutionarily selected mechanism for wiring circuits that optimally respond to the external world, without requiring the early and complex genetic coding of their future connections (Cowan et al., 1984). In support of this hypothesis, theoretical modeling has shown that, for distributed networks like the brain, algorithms based on hyper-connectivity followed by pruning provide a more efficient and robust way of building circuits than a model of increasingly growing networks (Navlakha et al., 2015). For the CC, it allows the activity-dependent sculpting of specific callosal circuits required in each functional area. In summary, it is likely that the advantages – reduced initial coding, efficiency, robustness and plasticity – outweigh the energy spent in building non-definitive projections. Thus, axonal exuberance and refinement is likely the reflection of self-assembling, context-instructed and highly plastic wiring of complex networks.

Corpus callosum development in humans

The study of the human CC (hCC) relies on noninvasive imaging approaches that allow structural analysis, such as PET, MRI or DI (Fig. 2E), and fMRI, which measures neuronal activity within brain regions of living subjects (Herve et al., 2013; Dennis and Thompson, 2013). However, these techniques do not have the resolution to determine the number and specific location of CPNs. Hence, current knowledge about the number, layer location and refinement dynamics of human CPNs is mostly inferred from postmortem analyses or primate models (LaMantia and Rakic, 1990a; Chalupa and Killackey, 1989; Chalupa et al., 1989; Dehay et al., 1986, 1988; Meissirel et al., 1991). General descriptions of the hCC, both developmental and pathological, are also based on postmortem analyses, as well as non-invasive MRI and tractography (Fig. 2E). However, although gross callosal dysgenesis such as partial or complete AgCC can be reasonably easily diagnosed by imaging techniques, subtle CC circuit defects might be underestimated.

The human cortex is organized into neuronal layers and, as in other mammals, the CC establishes bilateral connections between cortical areas (Wahl et al., 2007, 2009). The adult hCC is thought to contain more than 200,000,000 fibers (Aboitiz et al., 1992; Tomasch, 1954) and can be subdivided into seven regions from rostral to caudal: the rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium (Fig. 6A). As most of the CC fibers connect homotopic regions, the organization of callosal fibers correlates with the rostro-caudal distribution of functional areas (Fig. 6B) (Caminiti et al., 2013).

In regard to human CC development, postmortem immunostaining of coronal sections from human fetal brains revealed that, as in mice (Ren et al., 2006; Koester and O'Leary, 1994; Rash and Richards,

Development (2020) 147, dev189738. doi:10.1242/dev.189738

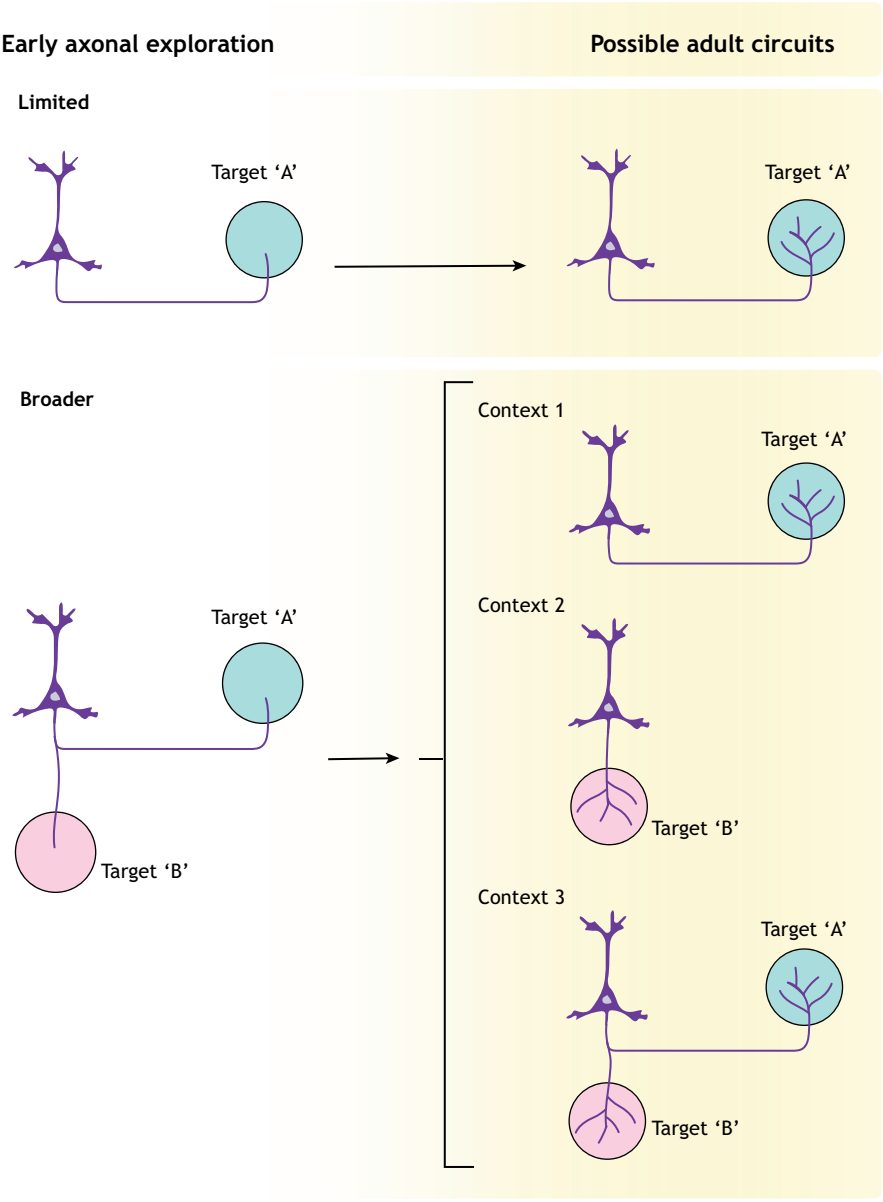

Fig. 5. Early axonal exploration and possible adult circuits. Early in development, neurons project their axons to distinct targets. Neurons with limited exploration capacity (top panel) can only target specific territories, thereby limiting the possible adult circuits they can acquire. By contrast, neurons with broader exploration capacity (bottom panel) can innervate multiple territories and are able to stabilize or refine transient projections in a context-dependent manner. These neurons would therefore generate a greater diversity of possible adult circuits (contexts 1-3).

2001; Piper et al., 2009), human pioneer axons originate from the cingulate cortex (Ren et al., 2006). Following this, CC formation is thought to occur from rostral (genu) to caudal (splenium) regions, except for the rostrum, which is the last to appear (Ren et al., 2006; Rakic and Yakovlev, 1968; Byrd et al., 1978; Hewitt, 1962; Barkovich and Norman, 1988). Although some studies argue that the CC might not follow such a rigorous rostro-caudal developmental dynamic (Paul, 2011; Kier and Truwit, 1996; Huang et al., 2006; Huang, 2009), there is consensus that all CC structures are visible at gestational week (GW) 20 (Ren et al., 2006; Rakic and Yakovlev, 1968; Clarke et al., 1989; Kazi et al., 2013; Raybaud, 2010) (Fig. 6C). After GW20, CC thickness increases until GW30 (Barkovich and Norman, 1988; Clarke et al., 1989). Then, during the second postnatal month, approximately 21% of the total cross-sectional thickness of the CC is reduced (Clarke et al., 1989). This indicates that, as in other mammals, the human CC exhibits significant refinement (Fig. 6C). From this point on, developmental myelination causes CC thickening, which complicates addressing the successive amount and temporal dynamics of axonal refinement.

Myelination occurs in a gradual and spatially organized manner from the second postnatal month until 9 years of life (Krupa and Bekiesinska-Figatowska, 2013). Contrary to axonal elongation, myelination occurs from caudal to rostral territories (Krupa and Bekiesinska-Figatowska, 2013). Interestingly, its progression correlates with the functional maturation of neuronal circuits (Pujol et al., 2006; Nickel and Gu, 2018). Indeed it has been described that vocabulary acquisition in 1-year-old children is related to rapid myelination in language areas (Pujol et al., 2006). Therefore, the CC caudal to rostral myelination likely reflects earlier bilateral processing in visual and auditory regions, compared with frontal areas (social skills).

Functional changes continue to occur until adulthood, placing the human CC among the last structures to complete postnatal maturation (Keshavan et al., 2002; Pujol et al., 1993). Growing evidence suggests that activity-dependent CC maturation also plays a role in cognitive learning (Sampaio-Baptista and Johansen-Berg, 2017). During this process, environmental factors can produce long-lasting changes in the human CC. As an example,

Development (2020) 147, dev189738. doi:10.1242/dev.189738

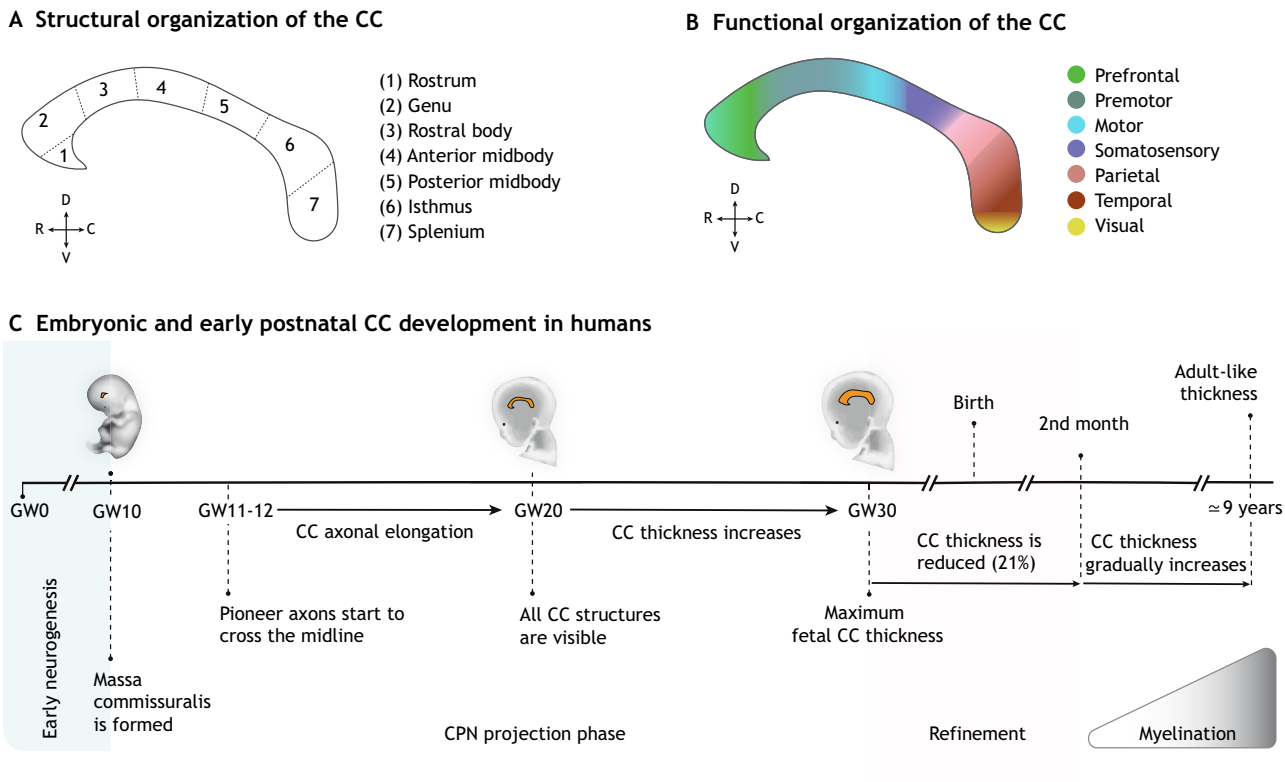

Fig. 6. The human corpus callosum. (A) Structural organization of the human corpus callosum (hCC) represented from rostral to caudal. (B) Functional organization of the hCC represented from rostral to caudal. (C) Embryonic and early postnatal development of the hCC. First, callosal projecting neurons (CPNs) project their axons through the midline. All CC structures are visible by gestation week (GW) 20. CC axonal elongation continues until GW30. Then, 21% of the total CC cross-sectional area is reduced. During the second postnatal month, myelination begins and CC thickness gradually increases. Adult-like thickness is achieved by around 9 years.

musicians that begin musical instruction before the age of 7 have an enlargement in the CC posterior midbody and perform better on sensorimotor synchronization tasks (Steele et al., 2013; Schlaug et al., 1995).

Neurodevelopmental defects in the human corpus callosum

During development, defects in neuronal/glial proliferation and migration, axonal growth, guidance cues, refinement or myelination can interrupt CC formation and lead to mild or severe CC congenital malformations, which are generally classified as dysgenesis. Such defects can be caused by genetic mutations (Edwards et al., 2014) or by early environmental insults, such as alcohol exposure or preterm deliveries (Riley et al., 1995; Sepulveda et al., 2011; Spadoni et al., 2007; Barnes-Davis et al., 2020). Insults during the first stages of CC development (GW11) can lead to the complete absence of CC (cAgCC), while insults occurring later (from GW12 to GW30) can cause a partial loss of the CC (pAgCC) or a reduction in the size of the fully formed CC (hypoplasia).

There is an extensive list of gene mutations related to cAgCC. These mutations show different penetrance and great variability with respect to cognitive and neurological outcomes (Edwards et al., 2014; O'Driscoll et al., 2010). cAgCC commonly appears with the concomitant absence of the hippocampal commissure (HC) and, in 50% of individuals, also with absence of the AC (Raybaud, 2010). In 80% of cases, cAgCC appears associated with distinct brain developmental syndromes (Krupa and Bekiesinska-Figatowska, 2013) and rarely as an isolated defect (Paul et al., 2007; Raybaud, 2010). Sixty-five to 75% of isolated cAgCC cases show no apparent neurological defects (Santo et al., 2012; Bayram et al., 2020). When defects are present, they are frequently related to motor difficulties, deficits in problem solving, social skills or language problems (Marsh et al., 2018; Edwards et al., 2014; Hearne et al., 2019). Interestingly, individuals with developmental cAgCC demonstrate better bilateral processing than individuals that experience surgical sectioning of the CC as adults (Edwards et al., 2014). This likely reflects the plasticity of the developing circuits and the appearance of alternative bilateral processing routes (Monteiro et al., 2019; Paul et al., 2007).

Partial AgCC consists of the absence of specific rostro-caudal regions of the CC due to developmental defects. Neuropsychological performance among individuals with complete and partial AgCC can overlap significantly (D'Antonio et al., 2016). Imaging techniques have revealed aberrant ectopic axonal bundles in these individuals (Edwards et al., 2014) but their function remains largely unknown (Lazarev et al., 2016).

Hypoplasia – the underdevelopment of the CC – is caused by insults or developmental defects occurring after GW20. CC hypoplasia can be associated with vascular defects in GW23 or can occur as a phenotypic manifestation of Anderman, Fryns and DeMorsier syndromes (Krupa and Bekiesinska-Figatowska, 2013; Edwards et al., 2014). Interestingly, animal models show that early developmental insults can result in a reduced number of callosal projections due to an imbalance in the refinement/stabilization ratio (De León Reyes et al., 2019; Innocenti and Frost, 1979; Olavarria et al., 1987), raising this as a possible cause in humans.

CC malformations are frequently related to reduced CC size. However, in rare instances, individuals show thickening of this

structure. CC enlargement has been reported in individuals with neurofibromatosis type I or as an associated clinical feature of other syndromes, such as Cohen and MMC (megalencephaly, mega CC) (Agarwal et al., 2013; Bindu et al., 2010). A recent study reported that mutations in the gene encoding MAST1 cause mega CC due to an increased number of callosal fibers and not because of an increased myelination. Such findings point to a reduction in refinement as a possible origin for mega CC (Tripathy et al., 2018).

CC defects are also present in individuals with schizophrenia, ASD, bipolar disorder and with social function impairments (Chinnasamy et al., 2006; Symington et al., 2010; Motomura et al., 2002; Kumar et al., 2010; Barnea-Goraly et al., 2009; Koshiyama et al., 2020, 2018). The neuropsychiatric features are usually described as overlapping, and neurological screening shows that they can be intermingled (8.5% of individuals with AgCC also have autism) (Edwards et al., 2014).

Conclusions

As we have highlighted here, there are two major stages of CC development. First, there is an early period of growth of non-restricted, massive callosal projections. Following this, sensory-dependent refinement occurs during specific and restricted temporal windows in each layer and area. Thus, acquiring an adult callosal identity seems to be dictated by the capacity to stabilize an early immature callosal projection, possibly via postnatal editing of neuronal-specific molecular programs. Importantly, exuberance appears to be a major contributor to plasticity. It helps to explain circuit diversity, and how genetic malformations and insults can lead to non-canonical circuits. Axonal exuberance might also have conferred an evolutionary advantage for the development of higher-order complex circuits.

The CC is fundamental for proper human cognition and is affected in multiple neurodevelopmental disorders. However, while current imaging techniques can be used to characterize gross CC structure, they likely underestimate more subtle circuit alterations. We therefore require further development of non-invasive imagining and tractography techniques in order to obtain a deeper understanding of CC structures and the mechanisms of its wiring, and to allow for earlier and more accurate diagnosis and prognosis. These will lead to a better understanding of neurotypical CC development, inter-individual variability, and the causes and consequences of CC dysgenesis. Overall, these approaches will hopefully promote the development of treatments that might exploit the intrinsic early plasticity of the CC.

Acknowledgements

We are grateful to L. J. Richards, L. Fenlon and IRC5 members, and to J. García-Marques for critical reading.

Competing interests

The authors declare no competing or financial interests.

Funding

N.S.D.L.R. holds a Severo Ochoa fellowship from the Spanish Ministerio de Economía y Competitividad (BES-2015-071690). L.B.G. holds a fellowship from 'la Caixa' Foundation (LCF/BQ/IN17/11620044 under the Marie Sklodowska-Curie grant 713673 from the European Union Horizon 2020 research and innovation program). This work was funded by grants from ERA-Net Neuron, the European Union and the Ministerio de Economía, Industria y Competitividad de España (ERA-Net Neuron/PCIN-2015-176-C02-02); from the Ministerio de Ciencia, Innovación y Universidades/Agencia Estatal de Investigación/Fondo Europeo de Desarrollo Regional, European Union (SAF2017-83117-R and RED2018-102553T); and from the European Union, FLAG-ERA-Human Brain Project, Ministerio de Ciencia, Innovación y Universidades/Agencia Estatal de Investigación/Fondo Europeo de Desarrollo Regional, European Union (PCI2019-111872-2).

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