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Contralateral targeting of the corpus callosum in normal and pathological brain function

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The corpus callosum connects the two cortical hemispheres of the mammalian brain and is susceptible to structural defects during development, which often result in significant neuropsychological dysfunction. To date, such individuals have been studied primarily with regards to the integrity of the callosal tract at the midline. However, the mechanisms regulating the contralateral targeting of the corpus callosum, after midline crossing has occurred, are less well understood. Recent evidence suggests that defects in contralateral targeting can occur in isolation from midline-tract malformations, and may have significant functional implications. We propose that contralateral targeting is a crucially important and relatively under-investigated event in callosal development, and that defects in this process may constitute an undiagnosed phenotype in several neurological disorders.

Development of the corpus callosum

The formation of precise connections between the two hemispheres of the brain is essential for many aspects of neural function, including integration of lateralised sensory input and regulation of higher-order cognitive, social, and emotional processing [1]. Such bilateral integration between the two cortical hemispheres is largely mediated by axons forming the corpus callosum, the largest fibre tract in the human brain. The development of the corpus callosum involves a precise sequence of events [2,3] (Figure 1). First, newborn cortical neurons committed to a callosal-projection fate extend an axon medially. Next, aided by a complex interaction of secreted and contact-mediated axon-guidance cues, axons extend towards, across, and then away from the midline, defined as midline crossing. Finally, callosal axons continue coursing through the contralateral intermediate zone and turn into the cortical plate, ultimately arborising and stabilising to form functional connections in a layer- and cortical area-specific manner. We will refer to these later stages of contralateral callosal axon innervation and stabilisation as contralateral targeting. Thus, callosal development involves numerous stages, and it is necessary to understand the progression and mechanisms underlying all of these to fully comprehend this process and the errors that may arise in it.

Callosal malformations

Once callosal neurons and axons are formed, several different outcomes could result from coordinated or isolated misregulation of the subsequent stages during development. For instance, failure of midline crossing often results in callosal dysgenesis, a congenital brain malformation that includes complete (agenesis) or partial absence of the corpus callosum as well as callosal hypoplasia [4–6]. This may lead to an absence of contralateral connectivity

Glossary

Axon-tracing techniques: can include anterograde (marker taken up by the cell body and transported to the axon terminal) or retrograde (marker taken up by terminals and transported back to the cell body) transport. Includes methodologies such as:

Molecular tracing: includes degeneration-based techniques and injectable molecular dyes [anterograde and retrograde methods, such as horseradish peroxidase, injection of tritiated amino acids (autoradiography), cholera toxin subunit B, or DiI]. Limitations include variable injection site/size and inability to transfect specific populations of neurons.

Genetically targeted tracing: includes viral tracing (anterograde, retrograde, and *trans*-synaptic methods, involving infection of a population of neurons with a viral vector) and *in utero* electroporation (anterograde, involving the transfection of a genetic construct encoding a fluorescent protein). Has the advantage of labelling cells in a developmentally and/or genetically constrained manner.

Callosal dysgenesis: a group of disorders of the corpus callosum that includes its absence (agenesis), partial absence, and hypoplasia (global decrease in size).

Diffusion magnetic resonance imaging (dMRI): an MRI method that detects the diffusion of water molecules in biological structures and consequently provides information about the structure and organisation of the internal tissue. It is commonly used to evaluate white-matter tracts in the brain non-invasively.

Functional anisotropy: a value extracted from dMRI data that describes the directionality of water diffusion in a given structure, with a high value suggesting a single ordered direction (i.e., along a single axis) and a low value suggesting unrestricted diffusion in all directions.

Functional magnetic resonance imaging (fMRI): a non-invasive method of MRI of living brains that measures the presence and extent of neuronal activation in different brain regions via changes in blood flow over time.

Neuropsychological assessment: non-invasive tests performed on human subjects to assess factors such as intelligence, sensory processing, motor functioning, and speed of processing.

Tractography: a technique that uses dMRI data to create 3D maps of neuronal tracts.

Transcranial magnetic stimulation: non-invasive method often applied to humans that involves the application of a constrained magnetic field to the outside of the head, allowing the specific activation of underlying brain regions.

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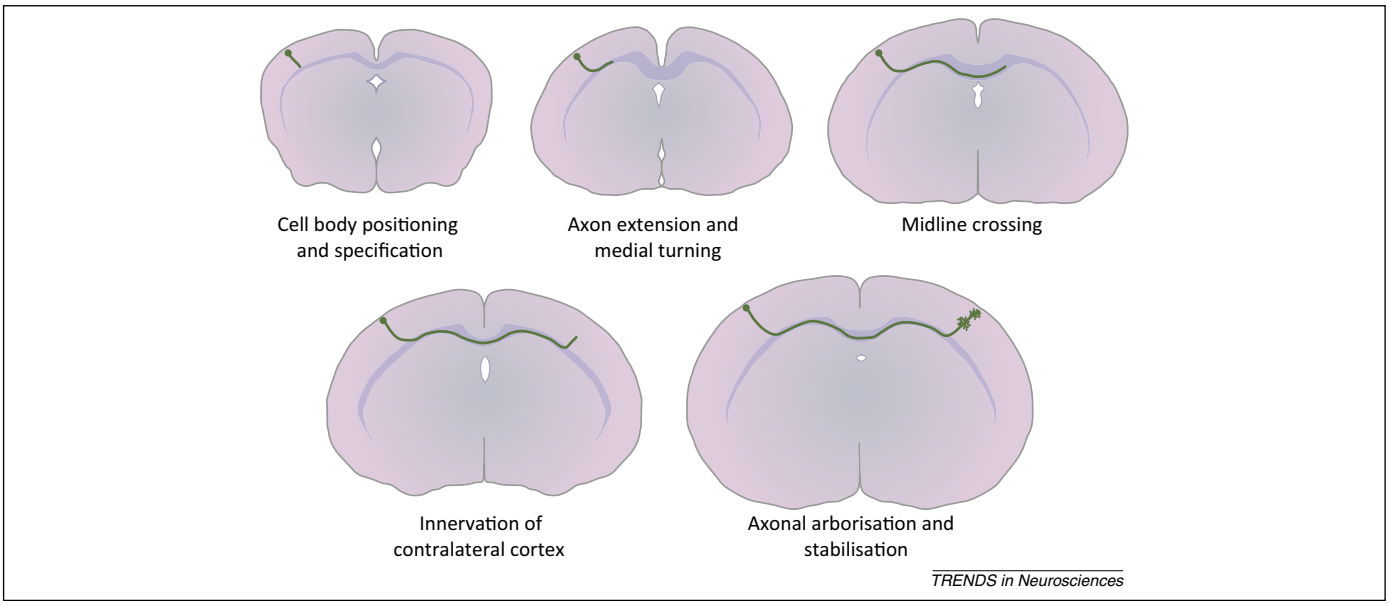

Figure 1. Stages of callosal development. Schematic illustrating the different stages of callosal targeting, using callosal neurons from the L2/3 somatosensory neocortex of a mouse as an example. First, callosal cell bodies migrate from the ventricular zone where they are born to their cortical layer and are specified as callosal projection neurons at around postnatal day (P) 0 in the mouse. Next, axons extend and turn medially in the intermediate zone towards the midline (P2). Axons then cross the midline and continue to follow the white-matter tract in the other hemisphere (P4). Projections turn to innervate the contralateral cortical plate (P6) and finally arborise and stabilise in their final contralateral locations (beginning at P8). These last two stages of innervation, arborisation and stabilisation, are collectively termed contralateral callosal targeting.

because callosal axons that are unable to cross the midline stall in the ipsilateral hemisphere (for instance, forming Probst bundles [7,8]). Interhemispheric cortical axons that are unable to cross the midline may also reroute through alternate commissures and correctly innervate their contralateral targets. Evidence for this has come from studies showing that some human patients with callosal agenesis display little/no interhemispheric disconnection in neuropsychological tasks [9–15], as well as an enlarged anterior commissure [9,10]. A recent report has also provided direct evidence of rerouting of cortical axons through the anterior and posterior commissures that positively correlates with functional interhemispheric connectivity [13]. The majority of callosal research has focused on midline crossing [2], and all human callosal disorders are clinically diagnosed based solely on the detection of gross structural defects at the sagittal midline. However, midline callosal dysgenesis represents the most severe form of callosal malformation, and many other neurological disorders are likely to involve more subtle defects in this commissure.

Recent research in mice indicates that the final stages of callosal targeting in the contralateral hemisphere may be more important than previously thought. For instance, several genetic and environmental manipulations can result in severe alteration of contralateral callosal targeting without any structural changes at the midline [16–21]. This suggests that, under a midline-based diagnostic paradigm, callosal defects arising from isolated errors in contralateral targeting may remain undiagnosed in humans. Contralateral callosal targeting is therefore a significant research topic given its importance in the development of correct functional connections and its potentially isolated disruption in humans.

Process of contralateral callosal targeting

Although contralateral callosal targeting is a significant event in the accurate functional wiring of cortical regions, the developmental processes involved are still poorly understood. Nevertheless, it has been shown that once callosal axons arrive at the contralateral white matter, there are dynamic waiting periods lasting a few days before they innervate the cortical plate in the visual [16,22], motor [23], frontal, and parietal [24] systems of rodents, as well as the cat visual system [25]. However, no waiting period has been found in the rodent somatosensory cortex [17], suggesting that this phenomenon may differ between cortical areas. Next, axons innervate the cortical plate, possibly using radial glial processes as a scaffold [26,27]. Callosal projections then arborise and form synapses with neurons located in cortical layers 2/3 and 5, and to a lesser extent 6 in rodents [28]. It is largely accepted that callosal axons innervate similar (homotopic) contralateral regions to those in which their cell bodies are located [29]. However, there is also evidence that callosal axons branch and project to other regions within the same hemisphere [30,31], as well as to contralateral heterotopic areas such as other cortical areas [19,32–34], secondary sensory regions, and the contralateral striatum [35,36].

There is a general consensus in the literature that there is an initial exuberance of callosally projecting neurons during development, and the cell bodies of these neurons frequently occupy cortical areas that are not callosally projecting in the adult ([37] for review). Further, it seems that these neurons cease to be contralaterally connected during development by retracting their contralateral axon, rather than via apoptosis [38–40]. However, the contralateral destination(s) of these transient axons

before retraction, and thus the mechanisms regulating specificity of callosal connectivity in the adult, are unclear. Three possible scenarios could account for the initial exuberance of callosally projecting neurons (Figure 2). The first is that the transient axons innervate the contralateral cortical plate in the same pattern as those destined for functional integration (Figure 2B). This is supported by early studies reporting a lack of developmental exuberance outside adult callosally innervated cortical regions [41–44]. However, these tracing studies (using horseradish peroxidase and tritiated amino acids; see Glossary) did not permit consistent quantification of axon numbers targeting different regions throughout development. Thus, an alternative scenario is that these excess

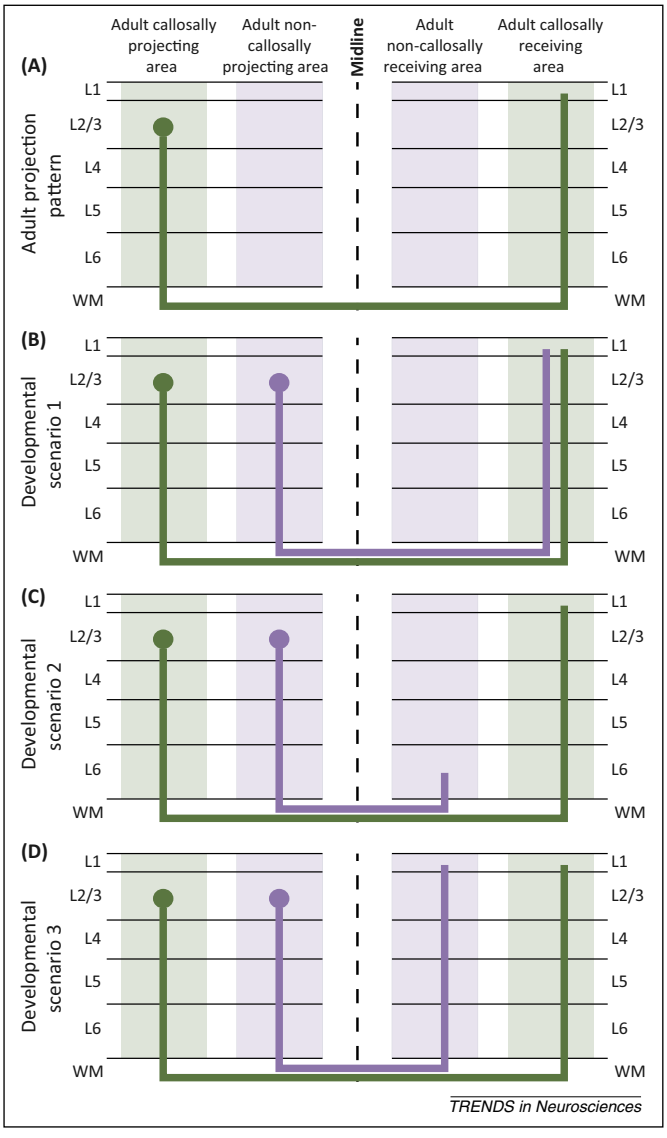

Figure 2. Three possible scenarios of developmental callosal axonal exuberance in the contralateral hemisphere. **(A)** A schematic illustrating the final, stable callosal wiring pattern in the adult (green), where terminating axons are confined to a small area in the contralateral cortical plate (shaded green) whereas other areas do not contain any callosal axons (shaded purple). A developmental exuberance of callosal cell bodies and axons is known to invade contralaterally (purple), but it is unclear whether these axons **(B)** terminate in the same location as those that will stabilise in the adult (green), or if they **(C)** terminate before invading the full contralateral cortical plate (for instance in lower layer 6 of future non-callosal regions). Finally, these axons could also **(D)** terminate within the cortical plate in future non-callosal regions of the adult. It is possible that a combination of these mechanisms operates in different species and brain regions.

callosal neurons only advance their axons into the white matter underlying the cortical plate, or project into the subplate before retracting (Figure 2C), possibly 'sampling' the cortex in a manner akin to thalamocortical axons [45]. This is supported by studies in cats showing that developing callosal axons, particularly those that originate from acallosal adult regions, innervate and branch in the white matter/lower layer 6 in contralateral areas that lack callosal innervation in the adult, and few or no axons innervate the cortical plate outside the mature projection pattern [25,46,47]. Conversely, additional studies in cats and rodents have provided evidence in support of a third scenario: cortical regions that receive little or no callosal input in adults are callosally innervated during earlier developmental stages [48–56] (Figure 2D). Once axons innervate the cortical grey matter, they may also form exuberant branches and synaptic contacts which are later refined [57].

In addition to generalised transient callosal exuberance, several discrete heterotopic callosal projections have been found during development, which are lost by adulthood [38,39,58–60]. However, it is currently unclear whether these two processes are related or if they are governed by different wiring rules. The process of callosal targeting is likely to comprise some combination of all of these mechanisms and may differ between species and cortical regions. Nevertheless, elucidating the mechanisms involved could be important for understanding whether the formation of transient callosal connections is a requisite for normal development or whether it is a byproduct of axon guidance/stabilisation strategies. Careful quantification of callosal axons in a region- and age-specific manner will be necessary to begin answering these questions.

Developmental mechanisms

Since the 1970s, studies employing injectable molecular and degeneration-dependent tracing techniques have revealed that the normal contralateral targeting of callosal axons is highly dependent on the type and magnitude of postnatal sensory experience in the somatosensory [61] and visual [62–69] systems. More recently, the technique of *in utero* electroporation has significantly advanced the field of cortical development by allowing cell type- and layer-specific transfection of genetic constructs (Box 1). Application of this technique in mice has revealed that genetically silencing the electrical activity of callosal neurons or their contralateral targets unilaterally disrupts the formation of a stereotypical dense callosal projection at the border between the primary and secondary sensory regions in both the somatosensory [17,19,70] and visual [16,71] systems. A better understanding of this process was recently gained by the discovery that a spatially-symmetric balance of either sensory-driven or endogenous activity between the two cortical hemispheres is required for the correct formation of this projection in the somatosensory system [19,70] (Figure 3). Furthermore, the formation of other contralateral projections from the primary somatosensory cortex is insensitive to any activity-repressing manipulations, suggesting that different mechanisms drive contralateral targeting in a region-specific manner [19].

Box 1. Methodological advances

(i) *Cell-specific transfection via* in utero *electroporation (IUE)*
Involves the injection of a DNA construct into the brain of a rodent embryo. Electrodes are then placed across the uterus to attract negatively charged DNA to the positive electrode to be incorporated into newly born neurons in a precise location (Figure I) [115]. Targeted IUE of mouse neocortex at embryonic day (E)15.5 transfects layer 2/3 cortical neurons, which are the primary callosal projection neurons. This has been the primary methodology used to investigate contralateral targeting of the corpus callosum in mice over the past decade [16–20,27,70,71,116–118]. IUE is a particularly powerful technological advance in this area because it allows a specific subpopulation of callosal neurons (and their contralateral innervation pattern) to be consistently and precisely labelled as well as transfected with a wide array of genetic constructs, which may include:

- Fluorescent proteins for precise and consistent visualisation.
- Sequences of shRNA or siRNA to silence expression of specific gene(s) within these neurons.
- Overexpression constructs.
- Constructs that increase or decrease long-term electrical activity (some of which can be pharmacologically induced).
- Optogenetic constructs that can activate or silence neurons in a precise temporal window using a light stimulus.
- Fluorescent markers that indicate dynamic changes in neuronal electrical activity.

(ii) *High-resolution diffusion magnetic resonance imaging (dMRI)*
Exploits the anisotropic movement of water molecules along ordered structures to visualise the extent and directionality of white matter tracts of the brain. dMRI has been used to image white-matter tracts for several years and has already provided many insights regarding normal and pathological contralateral targeting in humans [7,8,73]. Basic dMRI measurements such as fractional anisotropy and mean diffusivity provide sensitive, but non-specific, information about white-matter organisation. However, modelling of white matter tracts using tractography programs allows spatially specific analysis of axonal pathways and can be used to generate representative measurements of connectivity between cortical regions. Similarly, the development of higher-order models describing white matter, such as constrained spherical deconvolution employed by MRTrix [119], facilitates the identification of crossing intravoxel fibres and therefore a more biologically accurate representation of tracts, particularly at the white–grey matter interface. A recently developed technique termed neurite orientation dispersion and density imaging also holds much potential for the characterisation of microstructural differences in white-matter tracts [120].
Experiments using these techniques in mice and humans have already resulted in significant progress in our understanding of the mechanisms that regulate contralateral targeting, and provide promise for further advances in the future.

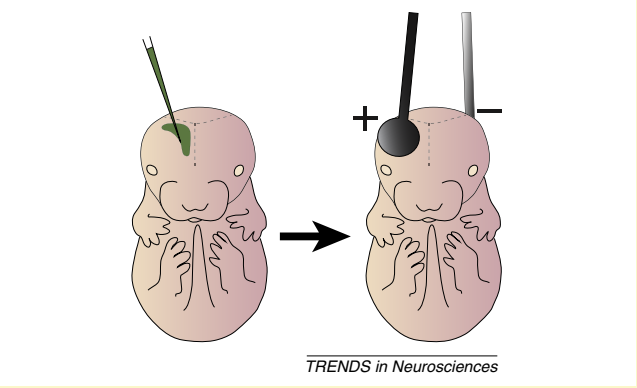

Figure I. *In utero* electroporation.

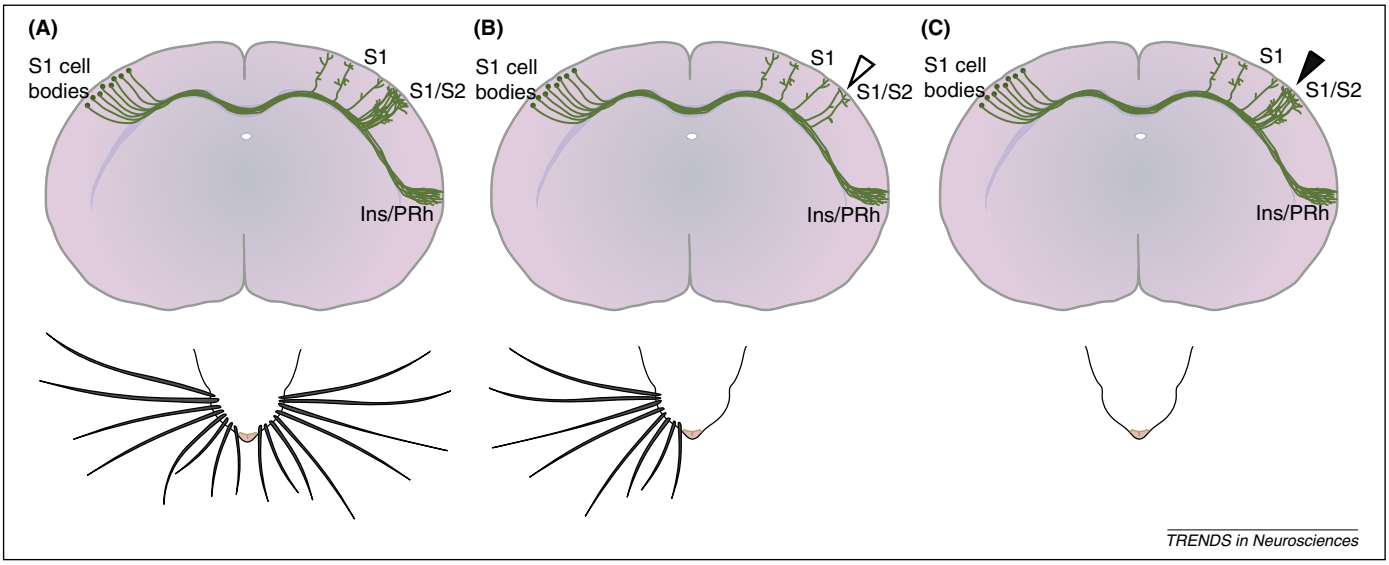

Figure 3. Role of activity in contralateral callosal targeting. Schematic illustrating the recent finding that contralateral callosal targeting is dependent on a balance of activity between homotopic cortical regions. **(A)** Mice that have no alteration to their sensory activity (control) have a stereotypic pattern of contralateral callosal projections arising from L2/3 primary somatosensory (S1) cortical neurons: diffuse projections to contralateral S1, a dense projection to the border region between S1 and the secondary somatosensory cortex (S2), and a heterotopic projection to the insular/perirhinal cortex (Ins/PRh). **(B)** When the facial whiskers are cauterised unilaterally, the S1/S2 projection is disrupted (open arrowhead), whereas the other projections remain intact. **(C)** When whiskers on both sides of the face are cauterised there is a partial rescue of the S1/S2 projection (closed arrowhead), indicating that this process is reliant on a balance of cortical activity between the two hemispheres. Adapted from [19].

Although this work further implicates activity in contralateral targeting, the molecular mechanisms regulating this process remain largely unknown. However, there is some evidence suggesting that the precise location and extent of callosal terminal arborisation relies on genes responsible for metabolic/structural processes [18,20]. It has also recently been shown that position within the callosal tract (and not cortical origin) determines the site of contralateral targeting, and that tract-sorting is regulated by the interaction between neuropilin 1 and semaphorin 3A [21]. Conditional knockdown of neuropilin 1 in callosal neurons also results in ectopic contralateral targeting mediated through interactions with the Rab5 protein [72]. Thus, the mechanism whereby axons enter the cortical plate in order of tract position is likely to broadly regulate contralateral targeting, with other unknown cues probably regulating the cortical area-specific guidance and stabilisation of axons into their discrete contralateral regions. Conditional cell/area-specific knockdown of specific gene candidates and/or sequencing of mRNA expressed by callosal neurons during contralateral axon targeting may help to elucidate the molecular mechanisms driving this process.

Contralateral callosal targeting in humans

Despite previous evidence showing that contralateral callosal mistargeting can be affected in isolation from midline defects in mice [16–21], no studies have thus far assessed the behavioural consequence of disrupted contralateral targeting in isolation. However, using diffusion magnetic resonance imaging (dMRI) and tractography (Box 1), it has been demonstrated that contralateral callosal targeting can be highly variable and misregulated in humans with gross structural malformations of the corpus callosum at the midline [7,8,73]. These defects in contralateral targeting appear to be highly variable across individuals with similar callosal remnants at the sagittal midline (Figure 4); however preliminary observations suggest that those with abnormally heterotopic contralateral targeting have the most severe neurological impairments [7]. This indicates that disrupted contralateral targeting (concomitant with other malformations or in isolation) could have functional consequences in humans.

The potential causes of disrupted contralateral targeting as well as its functional implications are particularly difficult to assess in humans because very little is understood about its developmental progression and regulatory mechanisms. dMRI and classic anatomical studies in foetal and early neonatal brains indicate that the callosal tract forms at around 13–14 gestational weeks [74] and continues to elaborate both anteriorly and posteriorly at the midline until at least 20 gestational weeks [75,76]. After this, the entire callosal tract enlarges with the rest of the brain until adulthood, although some callosal subregions seem to grow at differing rates during this time [76]. Myelination of the corpus callosum reaches maturity after birth [77]; however, the age at which callosal elaboration ceases and the time-course of retraction/stabilisation remains uncertain. Up to 70% of callosal axons are reported to be eliminated in the 4 months after birth in the rhesus monkey [78], and the cross-sectional area of the human corpus callosum decreases during late gestation and early neonatal life [79], which likely reflects an overall reduction in axon number [37,80]. Thus, the final stages of contralateral targeting (arborisation and retraction/stabilisation of innervating axons) are likely to continue after birth in humans. Given

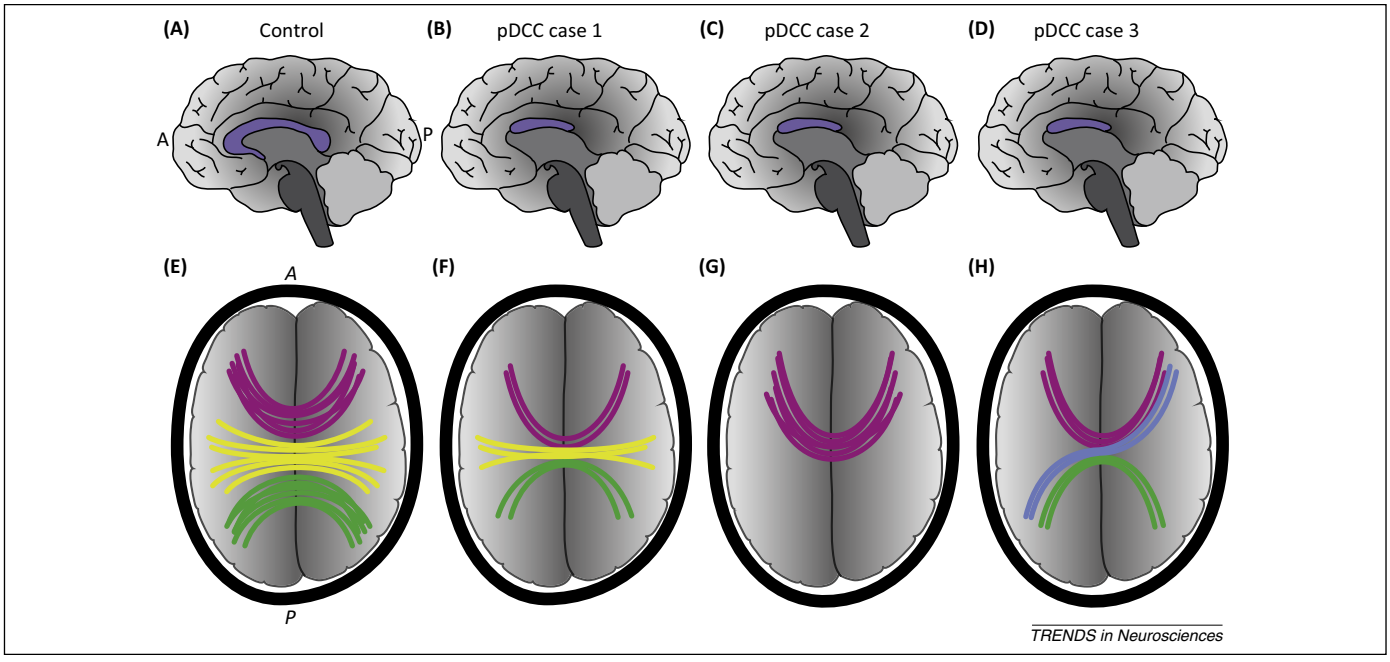

Figure 4. Variable contralateral targeting in individuals with partial dysgenesis of the corpus callosum (pDCC). Schematic sagittal brains of examples of a control **(A)** and three pDCC humans **(B–D)**, showing the corpus callosum in purple. Schematic of corresponding images resulting from diffusion magnetic resonance imaging with tractography in horizontal view, showing examples of callosal tracts that have been discovered in each case **(E–H)**. Whereas control individuals [(A) and (E)] have broadly homotopic and consistent callosal projections, individuals with pDCC can demonstrate severe contralateral mistargeting (blue tracts in (H)]. This outcome is highly variable between individuals [compare (F)–(H)], even when the callosal remnants are of similar size and position at the midline. Abbreviations: *A*, anterior, *P*, posterior. Adapted from [7,73].

the known dependence of contralateral targeting on neuronal activity, postnatal manipulations of sensory input hold the potential to prevent/treat pathological cases of callosal mistargeting. The ability of interhemispherically projecting neurons to innervate their final targets via alternative commissural routes when the corpus callosum is developmentally absent [13] highlights the potential for manipulations of contralateral targeting to produce correct and functional interhemispheric connections, despite concomitant brain malformations. This is of particular significance given the callosal involvement in a wide range of neuropsychiatric disorders with unclear genetic underpinnings that could involve defects in contralateral targeting.

Corpus callosum in neurodevelopmental disorders

There is a wealth of literature suggesting that defects of the corpus callosum are present in many human neurodevelopmental disorders. Neuropsychological, transcranial magnetic stimulation, and functional MRI (fMRI) studies have demonstrated dysfunction of interhemispheric communication in disorders such as autism [81], schizophrenia [82,83], attention deficit hyperactivity disorder (ADHD) [84], developmental language disorder (DLD) [85], and dyslexia [86]. A large number of studies have also shown alterations in the size of the corpus callosum or some of its subregions in these patients. However, the extent of callosal malformations is largely obscured by differences in cohort selection criteria and/or methodological approaches between studies (see meta-analyses summarising these data for autism [87], schizophrenia [88], and ADHD [89], and a review for dyslexia [90]). dMRI studies have shown a somewhat more consistent, although still variable, decrease in functional anisotropy (FA; a measure of tract organisation) of the corpus callosum or its subregions in autism [91–93], schizophrenia [94], ADHD [95], and DLD [96]. Symptom severity and callosal abnormalities are also correlated in several of these disorders, suggesting that callosal defects may predict functional outcome (see examples for autism [92,97–99], schizophrenia [100–102], ADHD [103], and dyslexia [104]). We propose that alterations in contralateral callosal targeting are likely to contribute to these observations. For instance, failure to successfully stabilise axons that are then retracted may lead to a smaller corpus callosum with fewer axons. Similarly, as FA is thought to be a measure of white matter organisation [105], low FA values in dMRI studies could reflect alterations in the precise topographical organisation of the tract, and subsequently the accuracy of contralateral callosal targeting [21], even in the absence of gross differences in overall callosal size at the midline.

The recent discovery that region-specific contralateral callosal targeting in mice relies on a balance of interhemispheric cortical activity [19,70] raises the possibility that callosal defects in humans could also be related to abnormal brain symmetry. Interestingly, gross morphological [106–108] and functional [109–111] abnormalities in hemispheric symmetry have been observed in several of these disorders. Further, in DLD the callosal abnormalities are greatest in those regions that have significantly altered morphological symmetry compared to controls [107], while schizophrenic patients display a correlation between the degree of structurally abnormal brain symmetry and callosal axon number [112]. Although these data suggest a relationship between altered symmetry and callosal integrity, whether cortical changes underlie the callosal alterations or vice versa, as well as the mechanistic and functional implications of each case, remain to be determined. Future studies investigating these questions using fMRI [113] and/or dMRI-based tractography [114] in human foetal and infant brains at risk for these disorders may better elucidate the early differences in structure and/or activity, as well as any causal relationship between the two, that result in altered adult neurological outcome.

Box 2. Outstanding questions

- Is the process of heterotopic callosal targeting distinct from that of homotopic targeting? How does the degree of heterotopic targeting differ between brain regions/species?
- Where do developmentally exuberant callosal axons terminate in the contralateral hemisphere and what is their function?
- How does the brain compare neuronal activity between the two cortical hemispheres to ensure correct wiring? What aspect of activity is important for contralateral callosal targeting?
- What molecular mechanisms are involved in contralateral callosal targeting to specific cortical areas?
- Do isolated defects in contralateral callosal targeting exist in humans? What is the functional consequence of this disruption?
- Is contralateral targeting disturbed in neurodevelopmental disorders that are known to involve the corpus callosum such as autism and schizophrenia? What factors underlie callosal changes in these brains, and what is the behavioural outcome of such disruption?

Concluding remarks

Contralateral callosal targeting is a developmental event of particular importance given the possibility of its disruption in isolation to midline morphology and its dependence on early environmental influences such as sensory input. We propose that defects in contralateral targeting are likely to be of functional significance but may currently remain undiagnosed in humans. Thus, a shift in focus beyond callosal malformations being primarily a midline-crossing disorder and into more subtle connectivity phenotypes may significantly enhance our understanding of human pathologies. We have highlighted the current incomplete understanding of the processes and mechanisms that drive contralateral callosal targeting, and have discussed the promising technological advances that may shed further light on the many unresolved questions (Box 2). A better understanding of the normal and pathological development of contralateral callosal targeting, combining non-invasive human imaging and neuropsychological evaluation techniques with mechanistic studies in animal models, may lead to improved diagnoses and treatments for a variety of human brain dysfunctions.

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References

1 Aboitiz, F. and Montiel, J. (2003) One hundred million years of interhemispheric communication: the history of the corpus callosum. *Braz. J. Med. Biol. Res.* 36, 409–420
2 Gobius, I. and Richards, L. (2011) Creating connections in the developing brain: mechanisms regulating corpus callosum development. *Colloquium Ser. Dev. Brain* 2, 1–48
3 Suárez, R. *et al.* (2014) Evolution and development of interhemispheric connections in the vertebrate forebrain. *Front. Hum. Neurosci.* 8, 497
4 Edwards, T.J. *et al.* (2014) Clinical, genetic and imaging findings identify new causes for corpus callosum development syndromes. *Brain* 137, 1579–1613
5 Hetts, S.W. *et al.* (2006) Anomalies of the corpus callosum: an MR analysis of the phenotypic spectrum of associated malformations. *Am. J. Roentgenol.* 187, 1343–1348
6 Paul, L.K. *et al.* (2007) Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. *Nat. Rev. Neurosci.* 8, 287–299
7 Tovar-Moll, F. *et al.* (2007) Neuroplasticity in human callosal dysgenesis: A diffusion tensor imaging study. *Cereb. Cortex* 17, 531–541
8 Bénézit, A. *et al.* (2015) Organising white matter in a brain without corpus callosum fibres. *Cortex* 63, 155–171
9 Barr, M.S. and Corballis, M.C. (2002) The role of the anterior commissure in callosal agenesis. *Neuropsychology* 16, 459–471
10 Barr, M.S. and Corballis, M.C. (2003) Redundancy gain in the acallosal brain. *Neuropsychology* 17, 213–220
11 Barr, M.S. *et al.* (2005) Early visual evoked potentials in callosal agenesis. *Neuropsychology* 19, 707–727
12 Brescian, N.E. *et al.* (2013) Case study: a patient with agenesis of the corpus callosum with minimal associated neuropsychological impairment. *Neurocase* 20, 606–614
13 Tovar-Moll, F. *et al.* (2014) Structural and functional brain rewiring clarifies preserved interhemispheric transfer in humans born without the corpus callosum. *Proc. Natl. Acad. Sci. U.S.A.* 111, 7843–7848
14 Sperry, R. (1968) Plasticity of neural maturation. *Dev. Biol.* 2, 306–327
15 Lassonde, M. *et al.* (1991) Absence of disconnexion syndrome in callosal agenesis and early callosotomy: brain reorganization or lack of structural specificity during ontogeny? *Neuropsychologia* 29, 481–495
16 Mizuno, H. *et al.* (2007) Evidence for activity-dependent cortical wiring: formation of interhemispheric connections in neonatal mouse visual cortex requires projection neuron activity. *J. Neurosci.* 27, 6760–6770
17 Wang, C-L. *et al.* (2007) Activity-dependent development of callosal projections in the somatosensory cortex. *J. Neurosci.* 27, 11334–11342
18 Courchet, J. *et al.* (2013) Terminal axon branching is regulated by the LKB1-NUAK1 kinase pathway via presynaptic mitochondrial capture. *Cell* 153, 1510–1525
19 Suárez, R. *et al.* (2014) Balanced interhemispheric cortical activity is required for correct targeting of the corpus callosum. *Neuron* 82, 1289–1298
20 Lu, T. *et al.* (2013) X-linked microtubule-associated protein, Mid1, regulates axon development. *Proc. Natl. Acad. Sci. U.S.A.* 110, 19131–19136
21 Zhou, J. *et al.* (2013) Axon position within the corpus callosum determines contralateral cortical projection. *Proc. Natl. Acad. Sci. U.S.A.* 110, E2714–E2723
22 Fish, S.E. *et al.* (1991) Organization, development and enucleation-induced alterations in the visual callosal projection of the hamster: single axon tracing with *Phaseolus vulgaris* leucoagglutinin and Di-I. *Eur. J. Neurosci.* 3, 1255–1270
23 Halloran, M. and Kalil, K. (1994) Dynamic behaviors of growth cones extending in the corpus callosum of living cortical brain slices observed with video microscopy. *J. Neurosci.* 14, 2161–2177
24 Floeter, M.K. and Jones, E.G. (1985) The morphology and phased outgrowth of callosal axons in the fetal rat. *Dev. Brain Res.* 22, 7–18
25 Aggoun-Zouaoui, D. and Innocenti, G.M. (1994) Juvenile visual callosal axons in kittens display origin- and fate-related morphology and distribution of arbors. *Eur. J. Neurosci.* 6, 1846–1863
26 Norris, C. and Kalil, K. (1991) Guidance of callosal axons by radial glia in the developing cerebral cortex. *J. Neurosci.* 11, 3481–3492
27 Sehara, K. *et al.* (2012) Distinct developmental principles underlie the formation of ipsilateral and contralateral whisker-related axonal patterns of layer 2/3 neurons in the barrel cortex. *Neuroscience* 226, 289–304
28 Petreanu, L. *et al.* (2007) Channelrhodopsin-2-assisted circuit mapping of long-range callosal projections. *Nat. Neurosci.* 10, 663–668
29 Yorke, C.H. and Caviness, V.S. (1975) Interhemispheric neocortical connections of the corpus callosum in the normal mouse: a study based on anterograde and retrograde methods. *J. Comp. Neurol.* 164, 233–245
30 Mitchell, B.D. and Macklis, J.D. (2005) Large-scale maintenance of dual projections by callosal and frontal cortical projection neurons in adult mice. *J. Comp. Neurol.* 482, 17–32
31 Cauller, L.J. *et al.* (1998) Backward cortical projections to primary somatosensory cortex in rats extend long horizontal axons in layer I. *J. Comp. Neurol.* 390, 297–310
32 Veinante, P. and Deschênes, M. (2003) Single-cell study of motor cortex projections to the barrel field in rats. *J. Comp. Neurol.* 464, 98–103
33 Boyd, E.H. *et al.* (1971) Homotopic and nonhomotopic interhemispheric cortical projections in the squirrel monkey. *Exp. Neurol.* 32, 256–274
34 Kretz, R. and Rager, G. (1990) Reciprocal heterotopic callosal connections between the two striate areas in Tupaia. *Exp. Brain Res.* 82, 271–278
35 Wilson, C.J. (1987) Morphology and synaptic connections of crossed corticostriatal neurons in the rat. *J. Comp. Neurol.* 263, 567–580
36 Sohur, U.S. *et al.* (2014) Anatomic and molecular development of corticostriatal projection neurons in mice. *Cereb. Cortex* 24, 293–303
37 Innocenti, G.M. and Price, D.J. (2005) Exuberance in the development of cortical networks. *Nat. Rev. Neurosci.* 6, 955–965
38 Ivy, G.O. and Killackey, H.P. (1982) Ontogenetic changes in the projections of neocortical neurons. *J. Neurosci.* 2, 735–743
39 O'Leary, D.D.M. *et al.* (1981) Evidence that the early postnatal restriction of the cells of origin of the callosal projection is due to the elimination of axonal collaterals rather than to the death of neurons. *Dev. Brain Res.* 1, 607–617
40 Chalupa, L.M. and Killackey, H.P. (1989) Process elimination underlies ontogenetic change in the distribution of callosal projection neurons in the postcentral gyrus of the fetal rhesus monkey. *Proc. Natl. Acad. Sci. U.S.A.* 86, 1076–1079
41 Ivy, G.O. *et al.* (1979) Differential distribution of callosal projection neurons in the neonatal and adult rat. *Brain Res.* 173, 532–537
42 Ivy, G.O. and Killackey, H.P. (1981) The ontogeny of the distribution of callosal projection neurons in the rat parietal cortex. *J. Comp. Neurol.* 195, 367–389
43 Olavarria, J. and van Sluyters, R.C. (1985) Organization and postnatal development of callosal connections in the visual cortex of the rat. *J. Comp. Neurol.* 239, 1–26
44 Cornwell, P. *et al.* (1984) Extrinsic visual and auditory cortical connections in the 4-day-old kitten. *J. Comp. Neurol.* 229, 97–120
45 Ghosh, A. *et al.* (1990) Requirement for subplate neurons in the formation of thalamocortical connections. *Nature* 347, 179–181
46 Innocenti, G. (1981) Growth and reshaping of axons in the establishment of visual callosal connections. *Science* 212, 824–827
47 Innocenti, G.M. and Clarke, S. (1984) The organization of immature callosal connections. *J. Comp. Neurol.* 230, 287–309
48 Ding, S-L. and Elberger, A.J. (1994) Confirmation of the existence of transitory corpus callosum axons in area 17 of neonatal cat: an anterograde tracing study using biotinylated dextran amine. *Neurosci. Lett.* 177, 66–70
49 Ding, S-L. and Elberger, A.J. (2001) Postnatal development of biotinylated dextran amine-labeled corpus callosum axons projecting from the visual and auditory cortices to the visual cortex of the rat. *Exp. Brain Res.* 136, 179–193
50 Elberger, A.J. (1993) Distribution of transitory corpus callosum axons projecting to developing cat visual cortex revealed by DiI. *J. Comp. Neurol.* 333, 326–342
51 Elberger, A.J. (1994) Transitory corpus callosum axons projecting throughout developing rat visual cortex revealed by DiI. *Cereb. Cortex* 4, 279–299

52 Elberger, A.J. (1994) The corpus callosum provides a massive transitory input to the visual cortex of cat and rat during early postnatal development. *Behav. Brain Res.* 64, 15–23
53 Chow, K.L. *et al.* (1981) Callosal projections of the striate cortex in the neonatal rabbit. *Exp. Brain Res.* 42, 122–126
54 Lund, R.D. *et al.* (1984) The development of callosal projections in normal and one-eyed rats. *Dev. Brain Res.* 14, 139–142
55 Miller, M.W. and Vogt, B.A. (1984) The postnatal growth of the callosal connections of primary and secondary visual cortex in the rat. *Dev. Brain Res.* 14, 304–309
56 Dehay, C. *et al.* (1988) Characterization of transient cortical projections from auditory, somatosensory, and motor cortices to visual areas 17, 18, and 19 in the kitten. *J. Comp. Neurol.* 272, 68–89
57 Aggoun-Zouaoui, D. *et al.* (1996) Growth of callosal terminal arbors in primary visual areas of the cat. *Eur. J. Neurosci.* 8, 1132–1148
58 Hedin-Pereira, C. *et al.* (1999) Morphogenesis of callosal arbors in the parietal cortex of hamsters. *Cereb. Cortex* 9, 50–64
59 Innocenti, G. *et al.* (1986) Interchange of callosal and association projections in the developing visual cortex. *J. Neurosci.* 6, 1384–1409
60 Innocenti, G.M. and Clarke, S. (1984) Bilateral transitory projection to visual areas from auditory cortex in kittens. *Dev. Brain Res.* 14, 143–148
61 Koralek, K.A. and Killackey, H.P. (1990) Callosal projections in rat somatosensory cortex are altered by early removal of afferent input. *Proc. Natl. Acad. Sci. U.S.A.* 87, 1396–1400
62 Lund, R.D. *et al.* (1978) Squint-induced modification of callosal connections in cats. *Brain Res.* 144, 169–172
63 Frost, D.O. and Moy, Y.P. (1989) Effects of dark rearing on the development of visual callosal connections. *Exp. Brain Res.* 78, 203–213
64 Zufferey, P.D. *et al.* (1999) The role of pattern vision in the development ofcortico-cortical connections. *Eur. J. Neurosci.* 11, 2669–2688
65 Lund, R.D. and Mitchell, D.E. (1979) The effects of dark-rearing on visual callosal connections of cats. *Brain Res.* 167, 172–175
66 Olavarria, J.F. and Van Sluyters, R.C. (1995) Overall pattern of callosal connections in visual cortex of normal and enucleated cats. *J. Comp. Neurol.* 363, 161–176
67 Shatz, C.J. (1977) Anatomy of interhemispheric connections in the visual system of Boston Siamese and ordinary cats. *J. Comp. Neurol.* 173, 497–518
68 Dehay, C. *et al.* (1989) Maturation and connectivity of the visual cortex in monkey is altered by prenatal removal of retinal input. *Nature* 337, 265–267
69 Frost, D.O. *et al.* (1990) Effects of alternating monocular occlusion on the development of visual callosal connections. *Exp. Brain Res.* 83, 200–209
70 Huang, Y. *et al.* (2013) Sensory input is required for callosal axon targeting in the somatosensory cortex. *Mol. Brain* 6, 53
71 Mizuno, H. *et al.* (2010) Pre-synaptic and post-synaptic neuronal activity supports the axon development of callosal projection neurons during different post-natal periods in the mouse cerebral cortex. *Eur. J. Neurosci.* 31, 410–424
72 Wu, K-Y. *et al.* (2014) Semaphorin 3A activates the guanosine triphosphatase Rab5 to promote growth cone collapse and organize callosal axon projections. *Sci. Signal.* 7, ra81
73 Wahl, M. *et al.* (2009) Variability of homotopic and heterotopic callosal connectivity in partial agenesis of the corpus callosum: a 3T diffusion tensor imaging and Q-ball tractography study. *Am. J. Neuroradiol.* 30, 282–289
74 Ren, T. *et al.* (2006) Imaging, anatomical, and molecular analysis of callosal formation in the developing human fetal brain. *Anat. Rec. A: Discov. Mol. Cell. Evol. Biol.* 288A, 191–204
75 Huang, H. *et al.* (2006) White and gray matter development in human fetal, newborn and pediatric brains. *Neuroimage* 33, 27–38
76 Rakic, P. and Yakovlev, P.I. (1968) Development of the corpus callosum and cavum septi in man. *J. Comp. Neurol.* 132, 45–72
77 Dubois, J. *et al.* (2014) The early development of brain white matter: a review of imaging studies in fetuses, newborns and infants. *Neuroscience* 276, 48–71
78 LaMantia, A. and Rakic, P. (1990) Axon overproduction and elimination in the corpus callosum of the developing rhesus monkey. *J. Neurosci.* 10, 2156–2175
79 Clarke, S. *et al.* (1989) Forms and measures of adult and developing human corpus callosum: is there sexual dimorphism? *J. Comp. Neurol.* 280, 213–230
80 Kostović, I. and Jovanov-Milošević, N. (2006) The development of cerebral connections during the first 20-45 weeks' gestation. *Semin. Fetal Neonatal Med.* 11, 415–422
81 Just, M.A. *et al.* (2007) Functional and anatomical cortical underconnectivity in autism: evidence from an fMRI study of an executive function task and corpus callosum morphometry. *Cereb. Cortex* 17, 951–961
82 Mohr, B. *et al.* (2000) Interhemispheric cooperation during word processing: evidence for callosal transfer dysfunction in schizophrenic patients. *Schizophr. Res.* 46, 231–239
83 Hoptman, M.J. *et al.* (2012) Decreased interhemispheric coordination in schizophrenia: a resting state fMRI study. *Schizophr. Res.* 141, 1–7
84 Buchmann, J. *et al.* (2003) Disturbed transcallosally mediated motor inhibition in children with attention deficit hyperactivity disorder (ADHD). *Clin. Neurophysiol.* 114, 2036–2042
85 Fabbro, F. *et al.* (2002) A callosal transfer deficit in children with developmental language disorder. *Neuropsychologia* 40, 1541–1546
86 Dhar, M. *et al.* (2010) Reduced interhemispheric coherence in dyslexic adults. *Cortex* 46, 794–798
87 Frazier, T.W. and Hardan, A.Y. (2009) A meta-analysis of the corpus callosum in autism. *Biol. Psychiatry* 66, 935–941
88 Arnone, D. *et al.* (2008) Meta-analysis of magnetic resonance imaging studies of the corpus callosum in schizophrenia. *Schizophr. Res.* 101, 124–132
89 Valera, E.M. *et al.* (2007) Meta-analysis of structural imaging findings in attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 61, 1361–1369
90 Sun, Y-F. *et al.* (2010) Brain imaging findings in dyslexia. *Pediatr. Neonatol.* 51, 89–96
91 Travers, B.G. *et al.* (2012) Diffusion tensor imaging in autism spectrum disorder: a review. *Autism Res.* 5, 289–313
92 Alexander, A.L. *et al.* (2007) Diffusion tensor imaging of the corpus callosum in Autism. *Neuroimage* 34, 61–73
93 Aoki, Y. *et al.* (2013) Comparison of white matter integrity between autism spectrum disorder subjects and typically developing individuals: a meta-analysis of diffusion tensor imaging tractography studies. *Mol. Autism* 4, 1–17
94 Patel, S. *et al.* (2011) A meta-analysis of diffusion tensor imaging studies of the corpus callosum in schizophrenia. *Schizophr. Res.* 129, 149–155
95 Cao, Q. *et al.* (2010) The macrostructural and microstructural abnormalities of corpus callosum in children with attention deficit/hyperactivity disorder: A combined morphometric and diffusion tensor MRI study. *Brain Res.* 1310, 172–180
96 Kim, J. *et al.* (2006) Diffusion-tensor magnetic resonance imaging in children with language impairment. *Neuroreport* 17, 1279–1282
97 Hardan, A.Y. *et al.* (2009) Corpus callosum volume in children with autism. *Psychiatry Res. Neuroimaging* 174, 57–61
98 Pryweller, J.R. *et al.* (2014) White matter correlates of sensory processing in autism spectrum disorders. *Neuroimage Clin.* 6, 379–387
99 Hahamy, A. *et al.* (2015) The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder. *Nat. Neurosci.* 18, 302–309
100 Innocenti, G.M. *et al.* (2003) Schizophrenia, neurodevelopment and corpus callosum. *Mol. Psychiatry* 8, 261–274
101 Nakamura, K. *et al.* (2012) Reduced white matter fractional anisotropy and clinical symptoms in schizophrenia: a voxel-based diffusion tensor imaging study. *Psychiatry Res. Neuroimaging* 202, 233–238
102 Whitford, T.J. *et al.* (2010) Corpus callosum abnormalities and their association with psychotic symptoms in patients with schizophrenia. *Biol. Psychiatry* 68, 70–77
103 Giedd, J.N. *et al.* (1994) Quantitative morphology of the corpus callosum in attention deficit hyperactivity disorder. *Am. J. Psychiatry* 151, 665–669
104 Odegard, T.N. *et al.* (2009) Brain connectivity in non-reading impaired children and children diagnosed with developmental dyslexia. *Neuropsychologia* 47, 1972–1977

105 Putnam, M.C. *et al.* (2008) Structural organization of the corpus callosum predicts the extent and impact of cortical activity in the nondominant hemisphere. *J. Neurosci.* 28, 2912–2918
106 Hynd, G.W. *et al.* (1990) Brain morphology in developmental dyslexia and attention deficit disorder/hyperactivity. *Arch. Neurol.* 47, 919–926
107 Herbert, M.R. *et al.* (2005) Brain asymmetries in autism and developmental language disorder: a nested whole-brain analysis. *Brain* 128, 213–226
108 Sommer, I. *et al.* (2001) Handedness, language lateralisation and anatomical asymmetry in schizophrenia: meta-analysis. *Br. J. Psychiatry* 178, 344–351
109 Philip, R.C.M. *et al.* (2012) A systematic review and meta-analysis of the fMRI investigation of autism spectrum disorders. *Neurosci. Biobehav. Rev.* 36, 901–942
110 Oertel-Knöchel, V. and Linden, D.E.J. (2011) Cerebral asymmetry in schizophrenia. *Neuroscientist* 17, 456–467
111 Hale, T.S. *et al.* (2014) Visual network asymmetry and default mode network function in ADHD: an fMRI study. *Front. Psychiatry* 5, 81
112 Chance, S.A. *et al.* (2008) Auditory cortex asymmetry, altered minicolumn spacing and absence of ageing effects in schizophrenia. *Brain* 131, 3178–3192
113 Schöpf, V. *et al.* (2012) Watching the fetal brain at 'rest'. *Int. J. Dev. Neurosci.* 30, 11–17
114 Ouyang, A. *et al.* (2015) Spatial mapping of structural and connectional imaging data for the developing human brain with diffusion tensor imaging. *Methods* 73, 27–37
115 Wang, C. and Mei, L. (2013) In utero electroporation in mice. In *Neural Development* (Zhou, R. and Mei, L., eds), pp. 151–163, Humana Press
116 Assimacopoulos, S. *et al.* (2012) Fibroblast growth factor 8 organizes the neocortical area map and regulates sensory map topography. *J. Neurosci.* 32, 7191–7201
117 Zhang, L. *et al.* (2015) DSCAM and DSCAML1 regulate the radial migration and callosal projection in developing cerebral cortex. *Brain Res.* 1594, 61–70
118 Mizuno, M. *et al.* (2015) Role of an adaptor protein Lin-7B in brain development: possible involvement in autism spectrum disorders. *J. Neurochem.* 132, 61–69
119 Tournier, J.D. *et al.* (2012) MRtrix: Diffusion tractography in crossing fiber regions. *Int. J. Imaging Syst. Technol.* 22, 53–66
120 Zhang, H. *et al.* (2012) NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. *Neuroimage* 61, 1000–1016