Development, evolution and pathology of neocortical subplate neurons

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Abstract | Subplate neurons have an essential role in cortical circuit formation. They are among the earliest formed neurons of the cerebral cortex, are located at the junction of white and grey matter, and are necessary for correct thalamocortical axon ingrowth. Recent transcriptomic studies have provided opportunities for monitoring and modulating selected subpopulations of these cells. Analyses of mouse lines expressing reporter genes have demonstrated novel, extracortical subplate neurogenesis and have shown how subplate cells are integrated under the influence of sensory activity into cortical and extracortical circuits. Recent studies have revealed that the subplate is involved in neurosecretion and modification of the extracellular milieu.

The subplate zone is a transient cortical structure that forms during mammalian brain development. Its early maturity, and location at the interface between the developing cortex and the ingrowing afferent fibres (FIGS 1,2), allows the subplate to have fundamental roles in the establishment of intracortical and extracortical circuitries, and to contribute to the guidance and areal targeting of thalamocortical axons[1].

Subplate neurons are a heterogeneous population of neurons that are among the earliest generated in the cerebral cortex[1–5]. They show advanced maturity at stages of cortical development when most other cortical neurons have not yet migrated to their final positions and some are yet to be born[6,7]. Ablation of subplate cells leads to abnormal formation or disruption of sensory maps in rats, cats and ferrets, and may be a cause of developmental abnormalities in humans[8]. With further development, most subplate cells undergo regulated cell death[3]. The subplate has become a topic of renewed interest because recent advances in molecular and cellular profiling have enabled the identification of different subpopulations of subplate neurons and, as a consequence, their fates can be traced in normal development and in developmental brain disorders[9–11].

In this article, we provide a comprehensive review of the anatomy and molecular identity of the subplate zone in mouse and primate development. We describe how the gross anatomy of and gene expression in this zone change with time and differ between species, but we do not cover in detail the physiology and circuit function of the subplate in this time window (for a review, see REF. 12). We also focus on the individual cellular components of the subplate zone, their molecular identity and their sites of generation. Finally, we touch on how perinatal brain injuries in the subplate in animal models and in humans can lead to subtle cytoarchitectonic and circuit abnormalities that are relevant to autism or schizophrenia.

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Subplate zone versus subplate cells

The subplate zone is generally identified based on cytoarchitectonic distinctions (FIGS 1,2), although not all mammalian species have a distinct subplate zone during brain development[13–15]. It is clear that the subplate zone contains some of the earliest born neurons of the cerebral cortex in all species examined[2,16–18], and that these cells are more mature than the overlying cortex[6,7]. The subplate zone also contains components — such as radial glial processes, radially and tangentially migrating neurons, early developing astrocytes, microglia and oligodendrocyte precursors — that are also found in other fetal compartments, but it is distinguished by an extensive extracellular space that is filled with hydrophilic extracellular matrix and heterogeneous contingents of 'waiting' cortical afferents and transient synapses[19]. Below, we discuss subplate zone development. As there are considerable differences in this process between primates and rodents[20], we deal with each in separate sections.

Subplate stages in primates. Four stages of subplate development, maturation and dissolution are commonly accepted for humans and non-human primates, but different stages are reached at different times in somatosensory and visual cortex[21]. In the 'pre-subplate stage'

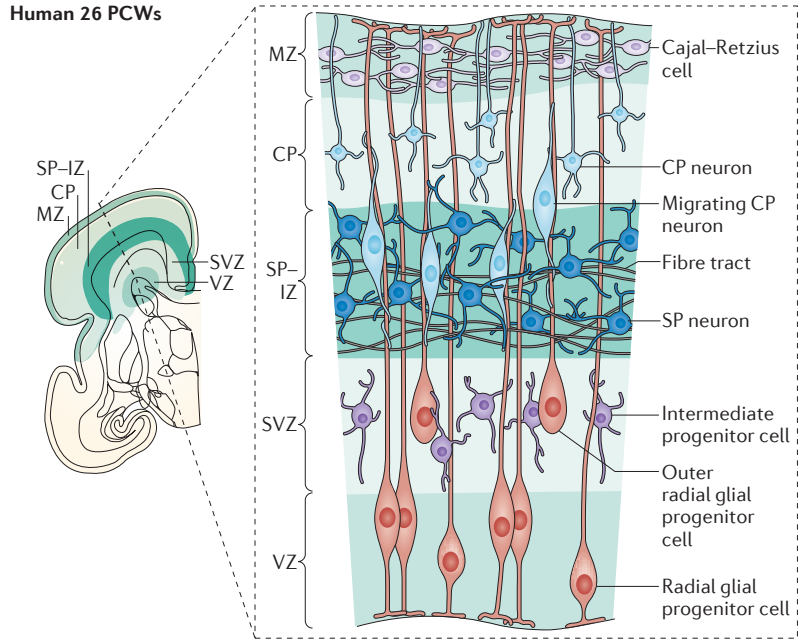

Figure 1 | **Compartments and zones of the developing human cerebral cortex.** Schematic coronal section showing the relative location and size of the major compartments within the developing human dorsal cortex at 26 post-conception weeks (PCWs), at the peak of neurogenesis and cell migration[19,147]. The germinal zone consists of layers (ventricular zone (VZ) and subventricular zone (SVZ)) in which cell divisions take place. The subplate (SP) and the intermediate zone (IZ) lie between the SVZ and the cortical plate (CP). The outermost layer is the marginal zone (MZ). The inset box provides a higher-powered view of the cellular make-up of the transient developmental zones within this developing cortical region. Radial glial progenitors divide asymmetrically within the VZ while in contact with the ventricular surface, whereas intermediate progenitors divide symmetrically in the SVZ. The SP and IZ boundary cannot be always distinguished on the basis of simple cytoarchitecture. The IZ contains more fibre tracts, whereas the SP is more abundant in postmitotic SP neurons with well-developed cellular processes. Synapses form in this layer. The CP contains migrating and immature CP neurons that are densely packed with only rudimentary cell processes, whereas the MZ contains fibre bundles and Cajal–Retzius cells as well as distal dendrites of cells below the MZ. Figure is modified, with permission, from REF. 19 © 1999-2015 John Wiley & Sons, Inc. All Rights Reserved. Figure is also modified from Molnár, Z. & Rutherford, M. Brain maturation after preterm birth. *Sci. Transl. Med.* **5**, 168ps2 (2013). Reprinted with permission from AAAS.

(12 post-conception weeks (PCWs) in humans and embryonic day 50 (E50) in the monkey *Macaca mulatta*), the cerebral wall contains a thin, cell-sparse, pre-subplate zone between the intermediate zone and the cortical plate. Through use of immunohistochemistry, the subplate can be distinguished from the intermediate zone as early as 8.5 PCWs[22]. The pre-subplate has higher levels of synaptophysin and growth-associated protein 43 (GAP43; also known as neuromodulin) than the intermediate zone, and the distribution of glial fibrillary acidic protein (GFAP) looks qualitatively different between the two compartments[22]. Electron microscopy has revealed the existence of synapses in the pre-subplate from 8 PCWs, whereas axons are more concentrated in the intermediate zone[21]. At 12–15 PCWs in humans and E54–E59 in monkeys, the deep cortical plate becomes much less cell dense and transforms into the subplate[21]. This is known as the 'subplate formation stage' and, at this point, a lower subplate (which is thin and cell sparse) and an upper subplate (which is thick and rich in cells) can be distinguished from each other and from the cortical plate (which is very cell dense). In MRI studies of human post-mortem material, the subplate becomes identifiable from 14.5 PCWs[22–24].

During the 'subplate stage', which occurs at 15–35 PCWs in humans and E59–E120 in monkeys, the subplate itself becomes the most prominent embryonic zone of the telencephalon. The expansion in size is partly due to invasion by various ingrowing fibre systems. The subplate zone represents a dynamic 'waiting compartment' for ingrowing thalamocortical afferents[25–27], basal forebrain cholinergic afferents[28] and callosal and ipsilateral corticocortical afferents[29–31]. Fibres are most densely packed just below the cortical plate. An additional thin, compact, cell-dense layer within the subplate zone is present in putative visual cortex in the macaque at E70–E80 (FIG. 2) and humans at 17 PCWs[21,32]. A human *in vivo* imaging study between 15 and 24 PCWs revealed area-specific temporal changes in subplate volume, with the fastest growth rates observed along the superior and lateral aspects of the temporal lobe[33].

During the subplate stage, acetylcholinesterase staining highlights a clear distinction between the subplate and the intermediate zones. From 16 PCWs, the distribution of neuropeptide Y-expressing cells and K^+/Cl^- co-transporter KCC2 (also known as SLC12A5)-expressing cells can be used to further divide the subplate into an upper (outer) zone near the border with the cortical plate and a lower (inner) zone near the border with the intermediate zone[22,34]. No data exist to resolve whether the immunohistochemical subdivision into upper and lower subplate compartments forms a neat divide at the transient compact band in the visual cortex, and in fact there is no evidence of what cell types comprise this band (FIGS 1,2).

In the final, 'subplate dissolution' stage, which occurs in humans at >35 PCWs and in monkeys at >E120, subplate neurons decline in number and the volume of subplate decreases. This stage is concurrent with the secondary gyrification of the brain[21]. The reduction in volume is driven primarily by a decrease in extracellular space and fewer axon bundles within the subplate zone. The rate of decline varies between brain regions and is faster underneath sulci than underneath gyri. MRI also suggests that there is a regional difference in the loss of subplate because it appears 'patchy' in late gestation, both regionally and in relation to the cortical folds, eventually remaining only at the tops of gyri[35]. A distinct subplate zone is no longer identifiable by about 6 months post birth in humans[21], but large cells embedded in white matter are thought to be the remaining subplate cells, now referred to as interstitial white matter cells[21,36–38].

Subplate development in rodents. In contrast to the well-defined histological criteria for subplate identification in human brains, the rodent subplate is not easily identifiable in early development (E13–E16 in mice) because it lacks a clear boundary with the intermediate zone. From E16 until birth, a fairly clear boundary between the

Callosal
Cells projecting across the corpus callosum or located within the corpus callosum, a fibre tract connecting the two cerebral hemispheres.

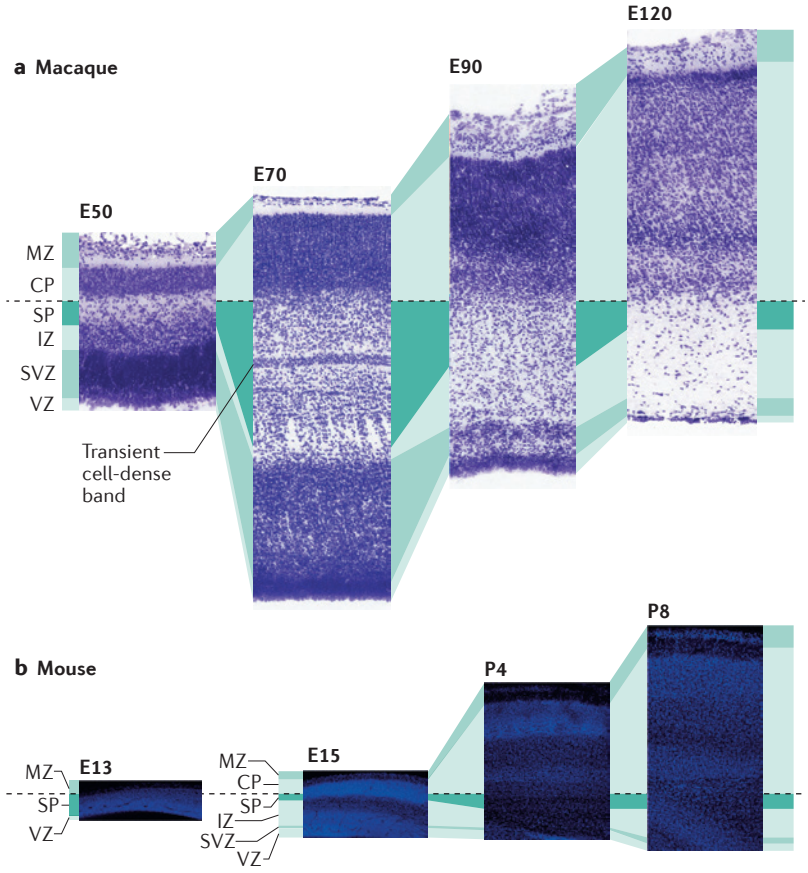

Figure 2 | **Compartments and zones of the developing cerebral cortex in macaques and mice.** **a** | Schematic illustrations and Nissl-stained histological sections of macaque visual cortex showing the relative sizes of different cortical compartments during development (age given as embryonic days (E) post conception). The left-hand side of each panel indicates the subdivision into different compartments (shown in different shades of blue). These are based on the cytoarchitecture of the Nissl-stained sections. The dashed line indicates the boundary between the subplate (SP) and the cortical plate (CP). Total cortical depth does not increase much after E70, whereas the relative sizes of its constituent compartments change drastically in the next 7 weeks of development. The size of the SP peaks (both in absolute size and relative to other compartments) at around E70. In addition, there is a transient cell-dense band visible within the SP layer in the visual cortex at around E70. **b** | Schematic illustration and histological sections of mouse primary sensory cortex stained with DAPI (4′,6-diamidino-2-phenylindole; a nuclear counterstain used to reveal cells) showing the relative sizes of different cortical compartments during development. The mouse SP compartment does not increase much in size during embryonic development and is not always distinct in histological sections. IZ, intermediate zone; MZ, marginal zone; P, postnatal days; SVZ, subventricular zone; VZ, ventricular zone. Images in part **a** courtesy of the Allen Institute for Brain Science, Seattle, Washington, USA ©2014 Allen Institute for Brain Science. NIH Blueprint Non-Human Primate (NHP) Atlas [Internet]. Available from: http://www.blueprintnhpatlas.org/.

Lissencephalic
'Smooth' brains; that is, those without ridges (gyri) or crevices (sulci) on the surface.

Cajal–Retzius cells
Early-born neurons of the cortical anlage that express a secreted molecule called reelin, which is essential for normal cortical lamination to occur.

intermediate zone and the much more cell dense overlying subplate can be identified, but the boundary with layer 6a is indistinct because cortical plate becomes less dense with development. From around the time of birth, the subplate is clearly distinct from both the overlying layer 6a and the underlying intermediate zone (emerging white matter), and remains visible as a distinct layer 6b or layer 7 into adulthood. Some have called this zone the 'upper compact subplate' and include scattered cells below this region in the 'lower subplate'[12]. In rodents, these distinctions are easier to make in somatosensory than in visual cortex (FIG. 2), but the use of molecular markers or transgene-expressing mouse lines can make the boundaries easier to discern.

Diversity of subplate cells

Origin of subplate neurons. Initially, it was thought that all cortical neurons were derived from the ventricular zone and subsequently migrated radially to populate the preplate and, later, the cortical plate[2]. An additional subventricular zone (SVZ) of cell division away from the ventricular wall had been recognized since the meeting of the Boulder Committee (1969)[39,40], but only a subsequent detailed analysis of the monkey cortex showed that this zone can be further subdivided into inner and outer compartments[41]. To date, all proliferative regions and modes of cell division identified in primate brains have also been documented in rodent brains and other large and small, lissencephalic and gyrencephalic brains[42], although the SVZ is disproportionately smaller in mice than in large primates[43]. The onset of cell division in SVZ occurs towards the end of subplate neurogenesis, and was therefore thought not to contribute to the subplate cell population. However, the SVZ contains two types of progenitors, namely intermediate progenitor cells (IPCs) and outer radial glia[44], and it was recently demonstrated that dividing IPCs in mice can give rise to T-box brain protein 2 (TBR2; also known as eomesodermin homologue)-expressing subplate neurons. In fact, more than one-quarter of subplate neurons might be generated from IPCs[45].

Early evidence that the subplate is unlikely to be generated purely from radially migrating cells came from an X-linked LacZ inactivation study[46]. Clusters of cells derived from the same neuroepithelial progenitor labelled radial columns in the cortex, but a mixed band of labelled and unlabelled cells was visible in the subplate region. The mixture of LacZ-positive and LacZ-negative cells specifically in layer 6b suggested the occurrence of tangential mixing within this population. It was subsequently discovered that non-pyramidal, GABAergic neurons in neocortex are generated in the ganglionic eminences and arrive at the cortex via tangential migration[47]. One of their migration routes is within the intermediate zone and the subplate[47] (FIG. 3). The subplate, therefore, also contains radially and tangentially migrating neurons en route to their final destinations.

A recent study showed that the rostro-medial telencephalic wall (RMTW) in E11 mouse embryos can also give rise to future subplate neurons[11]. Thus, this study identified a further source of tangentially migrating subplate neurons (FIG. 3), although these are non-GABAergic. These cells migrate dorsally to reach the cortex and then disperse by tangential migration throughout the entire rostro-caudal and medio-lateral extent of the nascent subplate[11]. In addition, the RMTW region gives rise to Cajal–Retzius cells and deep-layer interneurons[11]. Despite the diverse origins of subplate neurons, and numerous clonal analyses using various methods[45,48–52], no studies to date have investigated the clonal origin of subplate neurons and how far related

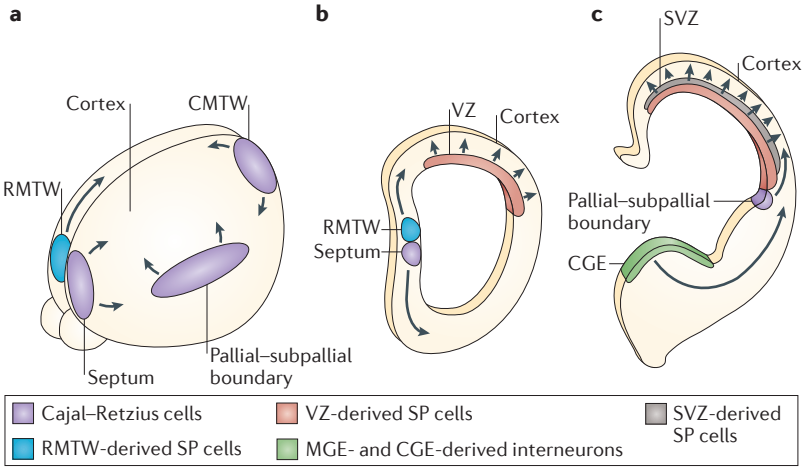

Figure 3 | **Origin and migratory routes of mouse subplate and other extracortical neurons.** **a** | Schematic of the whole mouse forebrain at embryonic day 11 (the anterior section is on the left and the dorsal section is at the top). Cajal–Retzius cells are generated at multiple locations adjacent to the cortex and migrate tangentially to populate the entire cortical sheet[148–151]. Recent evidence suggests that the same sources might contribute to the subplate (SP). Indeed, SP neurons can be generated in the rostro-medial telencephalic wall (RMTW) and migrate dorsally[11]. **b** | Illustration of a rostral coronal mouse brain section, showing the SP cell migration route and the generation of Cajal–Retzius cells in the RMTW and adjacent septum[150,152]. SP cells are additionally generated in the ventricular zone (VZ) of the cerebral cortex and migrate radially. **c** | Schematic illustration of a caudal coronal mouse brain section showing that SP neurons are generated not only in the cortical VZ but also the subventricular zone (SVZ), and that these cells also migrate radially. Cajal–Retzius cells are generated at the pallial–subpallial boundary and migrate tangentially. Interneurons are generated in the medial ganglionic eminence (MGE; not shown) and caudal ganglionic eminence (CGE) and migrate tangentially both within the marginal zone and the subplate. CMTW, caudo-medial telencephalic wall.

clones distribute tangentially. The recently developed CLoNe (clonal labelling of neural progenies) method could help to elucidate the source (or sources) of subplate neurons. By electroporating a cocktail of fluorescent probes that can stably integrate into the genome with the help of *piggyBac* transposase, it is possible to label clonally related cells with a multitude of fluorescent colours that enable subsequent identification of related cells even if they have dispersed across the cortical surface[45,53]. Better genetic definition of embryonic telencephalic regions may in the future enable us to exploit such clonal tracing experiments from various sectors of the developing telencephalon with modern lineage analysis[53].

Despite the evidence of late-born neuron migration through the subplate zone, there is little evidence for the later addition of (permanently resident) neurons to this region. Detailed radioactive birth-dating studies in primates did not identify any late neurogenetic events that contribute cells to the subplate layer or underlying white matter[2], with the last white matter neurons being generated well before the end of the neurogenesis of layer 6a cells[54]. Recent work with shorter post-injection survival times suggests that neurons born later in gestation (on E64 or E71) can be identified in the subplate 1 or 2 weeks after neurogenesis[55], but it is unclear whether they settle there or are in the process of migrating.

Bromodeoxyuridine
(BrdU). A synthetic analogue of the nucleic acid thymidine that incorporates into DNA during replication and repair. It can subsequently be detected by immunohistochemistry and is used to label cells during the DNA replication stage (S phase) of cell division.

In mice, subplate neurons are also mostly generated in a short time window from E10.5 to E12.5 (REFS 3,5). Curiously, interneuron genesis in the mouse medial ganglionic eminence starts at E9.5 and continues at least until E15.5 (REF. 56). It is surprising that birth-dating studies do not find earlier-born (<E10) or later-born (>E13) cell additions to the mouse subplate[3,5]. This means that subplate interneurons are either generated entirely at the same time as the presumed excitatory subplate neurons or there are very few interneurons in the mature subplate in rodents.

Molecular heterogeneity of subplate neurons. Histological, cytoarchitectonic and imaging characterization of the subplate zone was primarily driven by analysis of large brains, whereas the molecular characterization of subplate neurons has been primarily studied in transgenic mouse models (Supplementary information S1 (box)).

The preplate is predominantly of pallial origin, and its constituent cells express pallial markers[57]. Various gene expression profiling experiments were carried out in mice to identify subplate-specific gene expression patterns at different developmental ages[9,10,58–61]. Little overlap was found in the genes identified to be expressed in the subplates at embryonic and postnatal ages. This may indicate the changing roles of the subplate during development and emphasizes the need to study development and developmental gene expression patterns to understand the pathophysiology of subplate-associated diseases.

A gene ontology analysis of two subplate microarrays at young embryonic stages in mouse (E12.5 and E15) identified enrichment for developmental processes, including axonogenesis, but also for cell adhesion and exocytosis[10,58,59]. Gene expression profiling of the subplate was also conducted on mid-gestation fetal human brains, and this study found that the human subplate is functionally enriched for synaptic plasticity[62] and generally shows signs of more advanced maturity compared with the overlying cortical plate. Thus, some of the molecular hallmarks of the subplate zone during early development may primarily relate to cell maturity: subplate cells form and extend axons earlier than cortical plate cells and receive synaptic inputs earlier than surrounding cells.

Gene expression comparisons have been also carried out on postnatal day 8 (P8) and adult mouse cortices[9,60]. Many of the postnatally expressed subplate-specific genes are still expressed in the subplate in adulthood. They are expressed in neurons generated at E11 or E12 (as assessed by bromodeoxyuridine (BrdU) birth dating at P8)[5], but the onset of expression is delayed until late embryonic or early postnatal ages[10]. At P8, expression of six subplate-specific or -enriched genes is excluded from GABAergic interneurons in the subplate but does not define molecularly distinct subclasses of glutamatergic neurons. By contrast, some of the embryonic subplate-enriched genes are exclusively expressed in GABAergic interneurons.

Some genes with subplate-enriched expression in the mouse are not expressed in rat or fetal human subplate, whereas others show common regional expression in all

Barrel hollows
The cell-sparse centre of each 'barrel' of cells in the barrel cortex. Thalamic afferents cluster inside this cylinder of cells.

Barrel septa
This is the region in between adjacent barrel walls.

three species[14,34,62]. There are additional genes that are expressed only in human and not mouse subplate[62], but so far species-specific expression patterns have not been used to identify species-specific functions of the subplate. The detection of conserved subplate-specific genes in the *Monodelphis domestica* cortex has provided support for the existence of subplate-equivalent cells in marsupials[14,15] (BOX 1). It remains to be seen whether gene expression analysis will lead to the identification of human-specific cell populations.

Variation in subplate cell death. In primates, interstitial white matter neurons are thought to be remnants of subplate neurons on the basis of morphological similarities with early-born neurons in the subplate[38]. The density of neurons labelled with the marker neuronal-nuclei (NeuN) within human white matter decreases sharply throughout the first year of life but remains stable thereafter[63]. A similar loss of early-born or NeuN-labelled subplate neurons has also been reported in mice[3,5] but not in rats[4,18].

In mice, in which molecular markers for subplate cells and serendipitous subplate-specific reporter gene-expressing transgenic lines are available, it is clear that some subplate cells are generated earlier than others[5]. In particular, the RMTW-derived cohort of subplate neurons is generated at early time points[5,11]. In addition, an analysis of subplate-specific gene expression combined with BrdU birth dating suggested that these genes are either selectively upregulated in surviving subplate neurons or, in fact, confer survival benefits on subplate neurons[5].

Diversity of subplate neuronal morphologies. Subplate neurons are not just diverse in terms of site of origin, birth date, survival and gene expression. They also have diverse morphologies and axonal projection patterns and participate in distinct functional networks at different times during development[64].

Subplate neurons in non-human primates, in cats and in humans fetuses are fusiform, multipolar, inverted pyramids or polymorphic. Interstitial white matter neurons in monkeys and humans are polymorphic neurons with large cell bodies and radially symmetric dendrites or fusiform neurons with a smaller soma and two main dendrites oriented parallel to the main fibre tracts[38]. In monkeys and humans, multipolar neurons are mainly found near the boundary with layer 6a and fusiform cells are located deep within white matter[38,65]. In cats, opposite distributions for these cell types are observed[36,66]. Around the time of birth, both types of interstitial white matter neurons are present in equal numbers[38] in monkeys and humans, but there are conflicting reports about which cell morphology is more common in adult brains[38,65]. It is not clear whether multipolar neurons alter their morphology as a result of spatial constraints (the increasing ingrowth of thalamic axons decreases the available space in a non-uniform manner) or whether they are clearly distinct cell types that have distinct functions. Is it also not clear whether cell shape or cell location is more likely to determine cell properties and functions.

In the postnatal rodent brain, subplate neurons have multipolar, horizontal bipolar or pyramidal cell shapes[18,67,68] and most subplate cells, as shown in Golgi preparations, are spiny[18], as they are in cats[66]. The length of subplate neuron primary dendrites in the radial direction reduces markedly throughout postnatal development in both rats and mice[4,68]. In fact, in mice, subplate neurites undergo several stages of pruning or remodelling. Shortly after birth, some subplate cell neurites extend to the marginal zone[68,69]. With further maturation, neurites from this group of cells coalesce into barrel hollows in mouse primary sensory cortex at P6 but then further retract until they end below layer 4 by P8. Some neurites subsequently reach into barrel septa while avoiding barrel hollows, whereas others remain restricted to below layer 4 at P10 (REF. 69). This process is experience dependent: removal of a row of whiskers at birth delayed the relocation of fibres from barrel hollow to barrel septum[69] (FIG. 4).

These changes in subplate cell morphology and distribution in some brains might be the consequence of gyrus formation. No published study has assessed the correlation between cell morphologies, connectivity patterns, physiological properties and recently described subplate molecular markers. Such a study would improve our understanding of abnormal circuit formation. This is particularly relevant for gyrencephalic species because the above data suggest that two morphologically distinct types

Box 1 | **Evolution of the subplate**

There are three hypotheses on the evolutionary origin of the subplate, namely the ancestral origin hypothesis, the derived hypothesis and the dual origin hypothesis.

The ancestral origin hypothesis emphasizes the conserved features between sauropsids and mammals, and states that subplate cells were already present in the common ancestors of mammals and sauropsids. According to this hypothesis, the primordial plexiform layer (preplate)-derived subplate and marginal zone are the 'reptilian framework' of the mammalian cerebral cortex[118], and a preplate is present during development of the dorsal cortex in reptiles[119]. Thus, the subplate originated as an embryonic structure before the appearance of the neocortex and the inversion of the cortical neurogenetic gradient observed in mammals.

The derived hypothesis states that subplate is a 'new invention' and is exclusive to mammals with increased complexity in larger brains. The subplate is an embryonic adaptation with more complexity in those areas of the brain that appear later in mammalian evolution. It evolved to support development of cortico-cortical connectivity[12,21,120].

The dual origin hypothesis suggests that there are both ancestral and new cell populations in the mammalian subplate. A subplate-like structure or its cellular elements were present in the common ancestor of reptiles and mammals, but the mammalian subplate has continued to evolve such that is cellular constituents are more complex, larger in size and perhaps more connected in species with larger cortices[15,121].

To test the three hypotheses, better knowledge of birth dates, origins, somatodendritic morphology, physiological properties and markers for the 'ancestral' versus newly developed subplate cell populations are required. Identifying respective markers may be difficult until the duration of subplate neurogenesis has been unequivocally identified in primates because it is possible that primate or 'large-brain' subplate neurons are generated throughout a longer period of gestation. Murine subplate-specific gene expression has been tested in various non-mammalian species, with the conclusion that some of these genes are consistently expressed in the dorsal cortex[14,15]. However, although similarities in gene expression may suggest similarities in cell populations, there are marked differences in subplate-specific gene expression patterns even between mouse and rat[14]. Ideally, gene expression, birth dating, cell morphology, projection pattern and neurophysiological characteristics should all be analysed together. Without this synthesis, each cell characteristic alone might lead to different conclusions because the links between these categories are elusive[122,123].

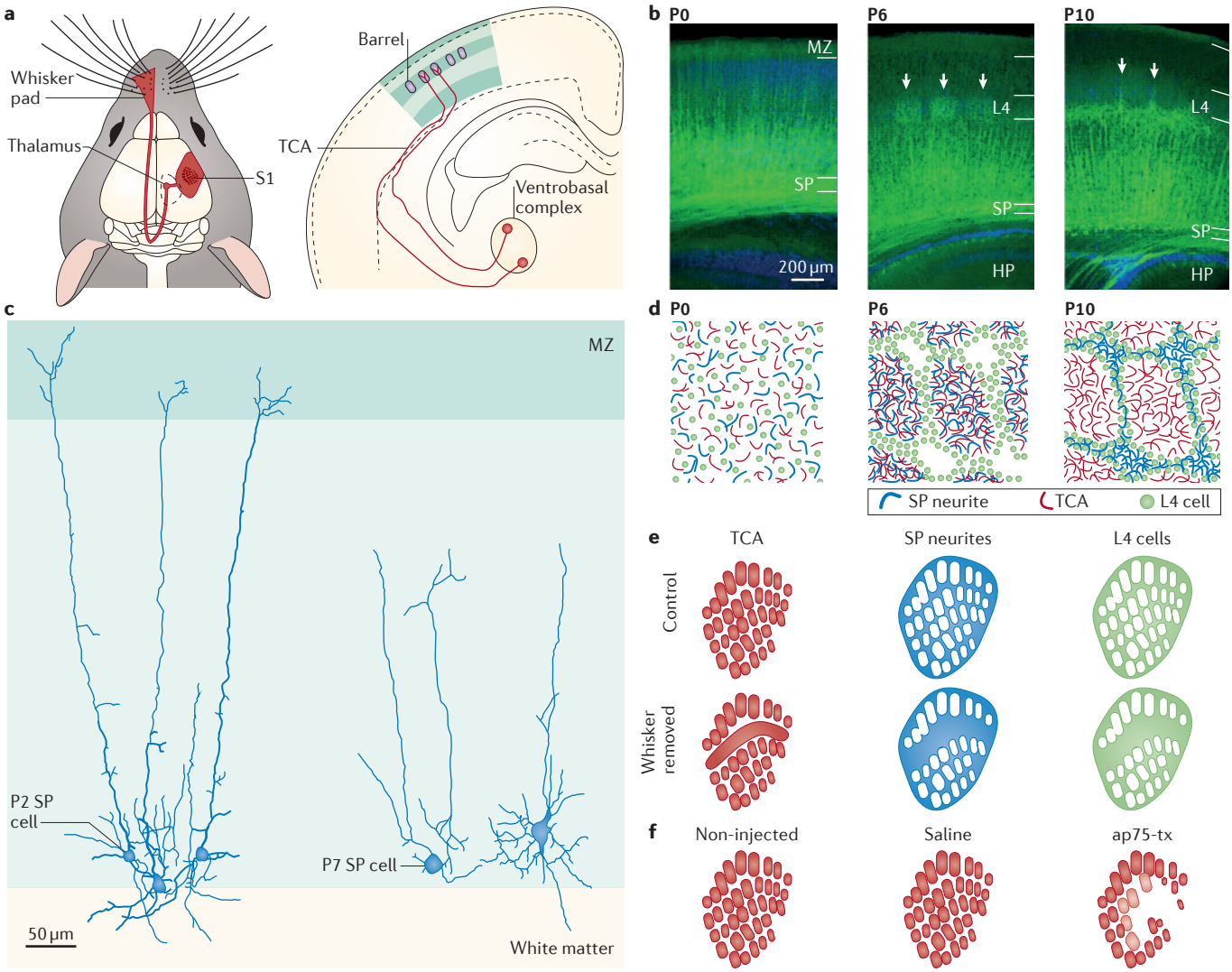

Figure 4 | **Subplate cell morphology and dendritic remodelling.** **a** | The diagram of the mouse somatosensory whisker system (left panel) shows the gross anatomical pathways (shown in red) from the whisker pad via the thalamus to the primary somatosensory cortex (S1). The schematic of a thalamocortical section through S1 (right panel) shows the layer-specific thalamic innervation (from the ventrobasal complex of the thalamus) of the barrel cortex (thalamocortical afferents (TCAs) are shown in red). **b** | Subplate (SP) cell neurites (shown in green) undergo time-dependent growth and pruning processes, as shown here in sections from the Golli-τ-eGFP (enhanced green fluorescent protein) mouse. In this transgenic model, both the axons and the dendrites of SP and some layer 6a (L6a) cells are labelled with eGFP. During early postnatal life (postnatal day 0 (P0)–P2), neurites of SP cells extend to the marginal zone (MZ). By P6, neurites end in L4 and are confined to the barrel hollow (arrows) in S1. By P10, most neurites end below L4 and are excluded from the barrel hollow, instead innervating the barrel septa (arrows)[69]. **c** | The gross anatomical changes of SP cells in barrel cortex are also mirrored at the level of single cell morphology, with P2 SP cells having much longer dendrites (that reach the marginal zone) than SP cells at P7. **d** | Schematic diagram of SP neurite reorganization in the tangential plane through mouse barrel cortex at P0, P6 and P10. Cells, SP neurites and thalamic afferents are unclustered at P0, but by P6 the cells have formed a barrel pattern and both SP and thalamic fibres cluster in the centre of each barrel. However, with further development at P10, TCAs remain clustered in the barrel hollow, whereas SP neurites have been remodelled to be located within the barrel septa[69]. **e** | Schematic representation of the distribution of the TCAs, the SP neurites and L4 cells across a larger area within the barrel cortex. The upper panel represents the normal development around the end of the first postnatal week, whereas the lower panel represents the same region in a mouse in which the second row of whiskers has been removed at birth. Ablation of a row of whiskers on the snout prevents withdrawal of SP neurites from L4. Instead, the neurites remain unclustered throughout the region. Neither the cells in L4 nor TCAs cluster in the region of whisker ablation. This experiment demonstrated that SP neurite reorganization during the second postnatal week is dependent on peripheral sensory input in mouse[69]. **f** | Schematic representation of the distribution of TCAs in the barrel cortex on tangential sections from a normal (non-injected), saline-injected and p75-immunotoxin (ap75-tx)-injected rat brain. ap75-tx targets and kills SP cells, but usually only in a relatively small area of the cortex. Clustering of thalamic afferents in the barrel cortex is dependent on an intact SP because ap75-tx-induced SP ablation prevents thalamic axon innervation in the SP ablated region in rats (pink regions denote low-density innervation)[91]. HP, hippocampus. Part **a** is modified, with permission, from REF. 69 Copyright © 2009 The Authors. Journal compilation © 2009 The Physiological Society. 1999-2015 John Wiley & Sons, Inc. All Rights Reserved; REF. 106, Nature Publishing Group; and REF. 153 Molnár, Z., López-Bendito, G., Blakey, D., Thompson, A. & Higashi, S. in *Development and Plasticity in Sensory Thalamus and Cortex* (Springer, 2006), with kind permission from Springer Science and Business Media. Parts **b** and **d** are modified, with permission, from REF. 69 Copyright © 1999-2015 John Wiley & Sons, Inc. All Rights Reserved.

of white matter cells exist in different subcompartments of subplate and white matter, and they might contribute differentially to circuit formation.

Interneurons in the subplate. The number of interneurons reportedly found in the subplate varies throughout development and between species, but it is unclear how much of that variation is due to underlying brain differences and how much of it is due to differences in methodology used.

Interstitial white matter neurons in primate brains are often identified using NADPH diaphorase (NADPHd) staining[70,71]. NADPHd staining indicates the presence of nitric oxide synthase (NOS), an enzyme that is commonly found in some GABAergic interneurons, but NADPHd in white matter neurons rarely colocalizes with GABA in primates[72]. In adult human brains, markers such as calretinin or parvalbumin have been used to detect interneurons in white matter, but quantifications exist only for superficial white matter cells. Calretinin and parvalbumin are rarely present in the sparsely distributed deep white matter neurons. One study found that within the superficial white matter, calretinin-positive cells comprise up to 15% of all neurons, whereas parvalbumin-positive cells comprise up to 6% of neurons, but the overlap between both populations was not assessed[65]. In cats, estimates of interneuron percentages range from 7% of cells in young postnatal subplate[66] to 25% of neurons in adult subplate[36]. In rodents, GABA-positive cells are abundant in embryonic and neonatal rat subplate (comprising up to 20% of neurons) but are much less abundant in postnatal subplate and white matter after the first postnatal week (comprising less than 5% of neurons)[73]. In addition, to date none of the identified postnatally expressed subplate-specific genes have been found to colocalize with GABA, and neither has serendipitous reporter-gene expression in the subplate[5,9]. Thus, compared with other cortical layers, postnatal subplate may have fewer GABAergic cells. Furthermore, GABAergic interneurons may not be providing inhibitory signals during the peak of subplate neuron activity. Although KCC2 expression distinguishes the upper from the lower subplate in humans from the halfway point of gestation onwards[22,34], it is unclear whether the level of KCC2 in subplate cells is sufficient to promote hyperpolarizing responses following GABA receptor activation.

Early projections from subplate neurons. Axonal projections from identifiable subplate neurons have been studied primarily in ferrets, cats and rodents[1,12]. Subplate cells have subcortical and cortical projections, the latter being both ipsi- and contralateral[1,68]. Subplate cells are the first cortical cells to send subcortical projections across the pallial–subpallial boundary in mice[74,75], but other cortical cell populations innervate the subplate-targeted thalamic nuclei first[76]. In the Golli-τ-eGFP (GTE) transgenic mouse, both subplate and layer 6a axons and dendrites are labelled with enhanced green fluorescent protein (eGFP)[77]. Ingrowth of eGFP-expressing axons into different first-order thalamic nuclei occurs in a strict temporal pattern[76–78] while avoiding higher-order thalamic nuclei. At birth, fibres extend into the ventrobasal thalamic nucleus but do not yet fill it. Over the next 4 days, this nucleus becomes filled with eGFP-expressing fibres, and they coalesce into a barreloid pattern. Despite close spatial proximity, the dorsal lateral geniculate nucleus does not become filled by eGFP-expressing fibres until P10–P14 in normally developing mice[76–78]. This temporal pattern of axon extension is dependent on spontaneous retinal activity[78] because the eyes are closed at this time. The expression of other subplate-marker genes correlates with target selection[76,79] but is insufficient to predict projection pattern on a cell-by-cell basis because of the high degree of overlap in gene expression and the potential for axonal collaterals to different target areas.

There are species-specific differences in the extent of subplate axonal projections. In all species studied, subplate projections are directed to the ipsilateral developing cortical plate, where they provide a substantial glutamatergic input into the maturing cortical plate, which has been associated with the establishment of functional cortical modules[1,8,67,69,74,80–84]. In contrast to those of ferrets and monkeys, rodent subplate cells may not establish many callosal projections to the contralateral cortex[75,85,86] or project to the superior colliculus[86].

In cats and ferrets, only glutamatergic subplate neurons project to contralateral and subcortical targets[79]. However, in monkeys and in the glutamate decarboxylase 67 kDa (GAD67)–GFP knock-in mouse, in which neurons normally expressing the GABA-synthesizing enzyme GAD67 (also known as GAD1) are labelled with GFP, GABAergic interneurons in subplate also form long-distance projections[87,88]. It is yet to be determined how many of these connections are temporary.

Functional insights from lesion studies

Subplate neurons are the first cortical cells that exhibit a mature morphology and membrane properties, and they participate in the maturation of early cortical circuits[12]. As the subplate has numerous developmental functions (FIG. 5), different phases of development can be disrupted depending on the timing of subplate ablation. Subplate neurons are necessary for thalamocortical axon pathfinding, synapse maturation and patterning of primary sensory areas in the cortex, as well as early oscillatory activity[12]. Genetic ablation of the subplate in mice prevents thalamic afferents from entering the telencephalon at the pallial–subpallial boundary[89]. The subplate is also essential for correct areal targeting of ingrowing thalamic afferents[90]. Excitotoxic or immunotoxic ablation of the subplate in the visual cortex of neonatal cats prevents strengthening of thalamocortical synapses onto layer 4 cells and the formation of ocular dominance columns, and inhibition in layer 4 does not mature[8,84]. Similarly, immunotoxic ablation of subplate neurons in newborn rats disrupts barrel field formation[91] (FIG. 4). Ablation of the subplate in rats also abolished endogenous and sensory-evoked spindle burst activity in the cortex[91,92]. This absence of oscillatory activity may underlie some maturational deficits observed following subplate ablation.

First-order thalamic nuclei
The thalamic nuclei that receive direct innervation from the sensory periphery.

Higher-order thalamic nuclei
The thalamic nuclei with a cortico-cortical relay function that receive the majority of extrinsic excitatory input from the cerebral cortex.

Barrel field
The mouse and rat barrel field is the somatosensory region of the cerebral cortex that receives input from the whiskers. The name derives from the 'barrel-like' cylindrical cell arrangement in layer 4.

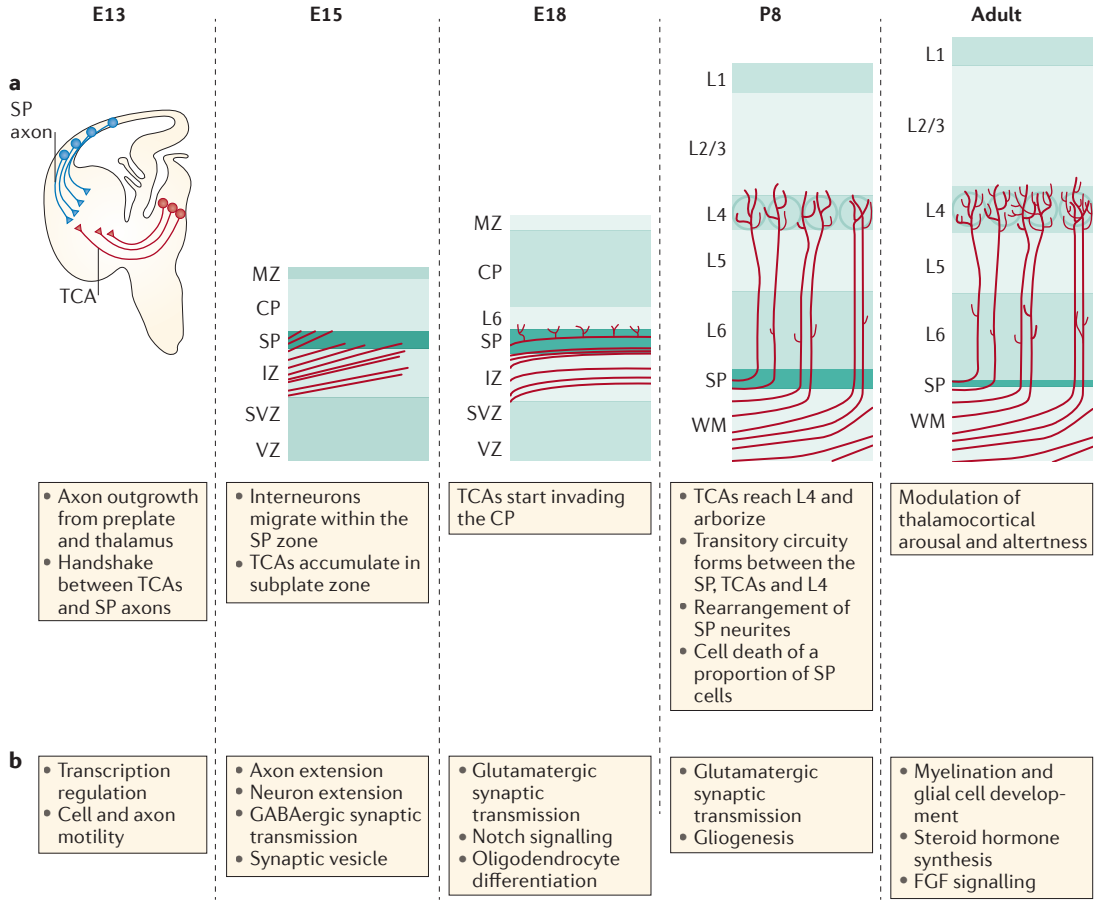

Figure 5 | **Transient subplate developmental stages.** **a** | Schematic depicting the stages of thalamocortical interactions in mice. At embryonic day 13 (E13), subplate (SP) axons and thalamocortical afferents (TCAs) are starting to extend but are not yet in contact with one another. By E15, the TCAs have reached the intermediate zone (IZ) and are in contact with SP cells, and by E18, the TCAs have formed synapses onto SP cells and are beginning to extend processes into the overlying cortical plate (CP). At postnatal day 8 (P8), the TCAs have reached their final target layer (layer 4 (L4)) and have branched within this layer. L4 is organized in a periphery-related barrel pattern (denoted by circles). Although synapses may still be refined, the gross axonal projection pattern remains similar into adulthood[154]. **b** | Summary of the different gene ontology terms associated with SP-specific or SP-enriched gene expression at the various developmental stages from E13 to adulthood (based on data from REFS 10,58,59). FGF, fibroblast growth factor; MZ, marginal zone; SVZ, subventricular zone; VZ, ventricular zone; WM, white matter. For an overview of mouse subplate neuron gene expression at these developmental stages to adult see Supplementary information S2 (figure). Part **a** is modified, with permission, from REF. 69 Copyright © 2009 The Authors. Journal compilation © 2009 The Physiological Society. 1999-2015 John Wiley & Sons, Inc. All Rights Reserved; REF. 106 Nature Publishing Group; and REF. 155 © Graham Publishing.

Much focus has been placed on ablation of the subplate and the ensuing consequences for cortical plate organization and maturation. However, various cortical and thalamic inputs to the subplate exist as well[93,94], and more effort should be directed to identifying the functional consequences of subtle alterations in cell numbers or synaptic strength within this entire transient circuit to gain a better understanding of how perinatal injuries affect cognitive development.

Impact of altered subplate on cortical development. Several mouse mutants have been described in which subplate cells are displaced within the cortex, incorrectly specified or altogether undetectable (FIG. 6). Some descriptions of mutants make use of subplate-specific markers, such as connective tissue growth factor (CTGF)[9,10,95], or transgene expression, such as GTE (or Golli-LacZ)[77,96], which in normal cortices are exclusively expressed in the subplate layer, thereby making it easier to identify mislocalized subplate cells in malformed brains.

The Reeler mutant mouse is characterized by the superficial position of subplate neurons and superficial ingrowth of early thalamocortical afferents (FIG. 6). Mutations in reelin (*Reln*) or disabled 1 (*Dab1*), or mutations in both very low density lipoprotein receptor (*Vldlr*) and apolipoprotein E receptor type 2 (*Apoer2*; also known as *Lrp8*) all cause a similar cortical malformation phenotype in mice (reviewed in REF. 97). Initially, the preplate forms normally, but subsequently generated cortical plate cells fail to split the preplate

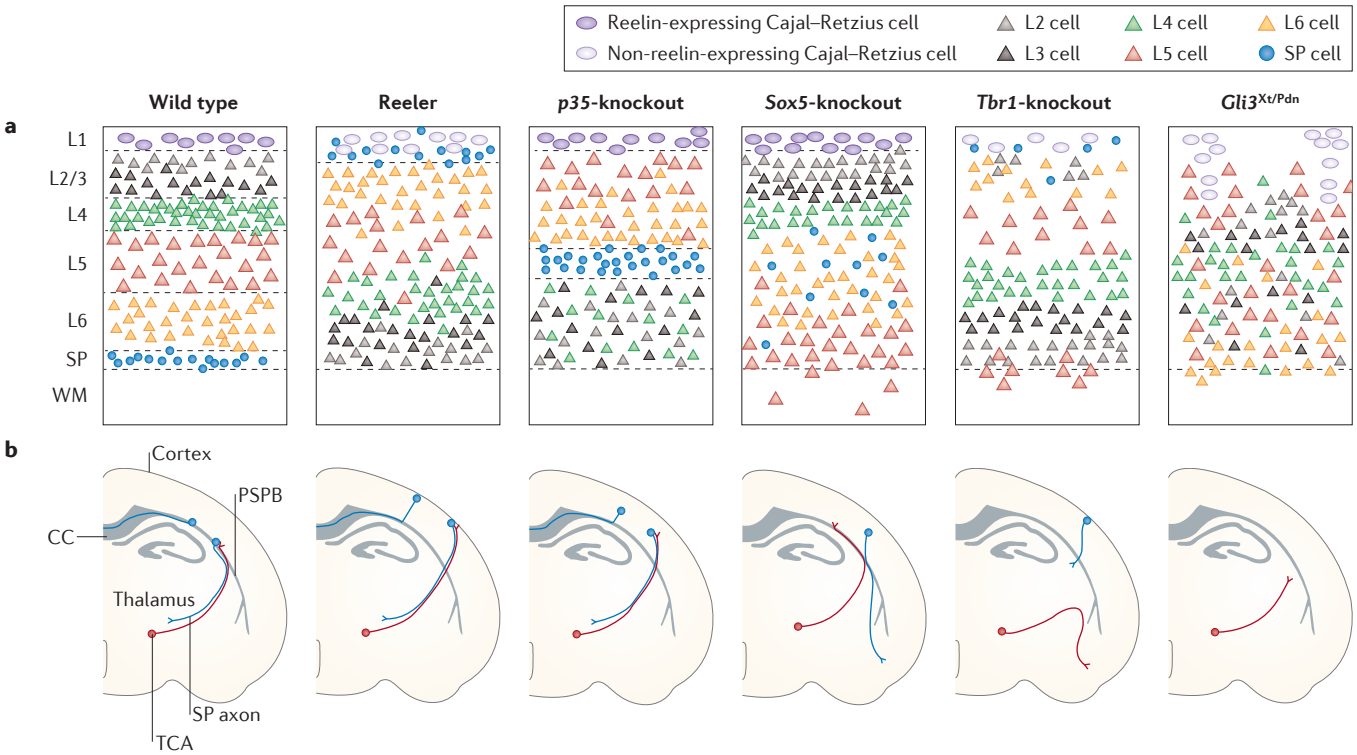

Figure 6 | **Mouse mutants with abnormal subplate position.** **a** | In the wild-type mouse cortex, subplate (SP) cells form the deepest layer and reelin-expressing Cajal–Retzius cells are located in the marginal zone. The cortical plate is formed in an inside-out gradient, with earlier born cells located more deeply, whereas the later born cells of layer 4 (L4) and L2/3 are progressively more superficial. In the Reeler mouse cortex, Cajal–Retzius cells do not express reelin, and the preplate fails to split into marginal zone and SP. The cortical plate forms underneath this 'superplate' in an outside-first gradient. In the cortex of *p35*-knockout mice, the SP is located in the middle of the cortical plate, with normally deep-layer neurons being correctly positioned above the SP, whereas normally upper-layer neurons are mixed together underneath the SP. In the sex-determining region Y-box 5 (*Sox5*)-knockout mouse cortex, SP cells are also located in the middle of the cortex, although they are more scattered than those in *p35*-knockout mice. In addition, some early-born neurons fail to differentiate and do not express connective tissue growth factor, which normally labels most SP neurons. The upper-layer neurons are positioned roughly correctly, but the deep-layer neurons are mixed in the lower half of the cortical plate. In the cortices of both T-box brain gene 1 (*Tbr1*)-knockout mice and *Gli3*$^{Xt/Pdn}$ (extra toes/polydactyly Nagoya) mutant mice, the SP is almost completely absent. The SP cells are missing altogether or express undetectable levels of commonly used molecular markers of SP cells. In both mutants, reelin-expressing Cajal–Retzius cells are also severely affected in their protein expression and cellular localization. **b** | The location of the SP has an impact on the targeting of thalamocortical projections, and the outgrowth of cortical projections to the internal capsule and the corpus callosum (CC). Thalamocortical afferents (TCAs) target the SP, irrespective of its location within the cortex. In wild-type brains, TCAs cross the pallial–subpallial boundary (PSPB) and grow within the developing white matter and SP, forming synapses within the latter. In Reeler, *p35*-knockout and *Sox5*-knockout brains, the SP cells are displaced outwards within the developing cortex but still form projections towards subcortical targets that cross the PSPB. TCAs cross the PSPB and invade the deep cortical plate until they reach SP neurons in Reeler and *p35*-knockout brains. Similarly, TCAs in the *Sox5*-knockout brain reach and cross the PSPB, grow within the white matter and extend side branches into the cortical plate. However, if SP cells are absent or their fibres do not reach internal capsule, such as in *Tbr1*-knockout and *Gli3*$^{Xt/Pdn}$ mutant mice, TCAs extend from the thalamus towards the PSPB but do not cross it.

and, instead, accumulate underneath it in an outside-to-inside gradient of cell age[97,98]. Subplate neurons are therefore located above the cortical plate instead of being adjacent to the white matter. Consequently, thalamic afferents initially obliquely grow through the cortical plate to target the marginal zone or superplate before turning sharply and invading the cortical plate to innervate their eventual target cells, the stellate cells of layer 4 (REF. 99). Molecular markers of subplate, including CTGF and complexin 3, are expressed in the superplate in the brains of Reeler mice[9].

Mutations in cyclin-dependent kinase 5 (*Cdk5*) or the gene encoding the neuron-specific regulator p35 (also known as CDK5R1) also lead to abnormal positioning of the subplate in mice[100,101]. In such animals, the preplate initially forms normally, and the earliest-born cortical plate neurons migrate past the subplate and cause the preplate to split. However, later-born cortical plate neurons then fail to pass the subplate and accumulate underneath it in an outside-in gradient of neuronal age. Consequently, the subplate is located in the upper third of cortex but is distinctly separated from Cajal–Retzius

cells in the marginal zone[100]. The thalamic afferents also develop abnormally: they enter the cortex obliquely and grow towards the marginal zone[101].

The zinc-finger transcription factor GLI3 is required for formation of the preplate[102]. There are several naturally occurring *Gli3* mouse mutants with phenotypes of varying severity. In the *Gli3*$^{Xt/Xt}$ (extra toes) mutant, there is a reduction in and abnormal clustering of reelin-producing Cajal–Retzius cells, which greatly affect cortical lamination. Subplate neurons fail to be generated or do not differentiate sufficiently to express subplate-specific markers. Consequently, corticothalamic projections fail to emerge from the cortex, and thalamocortical fibres cannot cross the pallial–subpallial boundary[89,103]. In *Gli3* mutants, the septal region does not display normal gene expression patterns and Cajal–Retzius neurons fail to be generated in this region. It is therefore possible that the effect of *Gli3* ablation extends dorsally to the RMTW region and affects subplate neuron formation in this region.

The transcription factor TBR1 is expressed in postmitotic cells of the cerebral wall, with particularly high protein levels detected in preplate cells[104]. The cerebral cortex is severely malformed in *Tbr1*$^{-/-}$ mutants. The preplate initially appears histologically normal but fails to split into the marginal zone and subplate by E14.5. Expression of the subplate- and layer 6a-specific transgene Golli-LacZ revealed an almost complete absence of subplate cells during embryonic development and at birth[104]. In the *Tbr1*$^{-/-}$ mutant, all cortico-thalamic fibres end in growth cones within the internal capsule, and thalamocortical afferents only extend as far as the internal capsule and then become derailed and extend into the external capsule and amygdala[104]. Normally, subplate neurons are the first cortical cells to send axons into the internal capsule, but the perinatal and postnatal innervation of sensory thalamic nuclei is dominated by layer 5 and 6a projections[76]. Thus, in the absence of subplate neurons in the *Tbr1*$^{-/-}$ mutant, the later-developing (follower) layer 6a projection neurons may be unable to progress through the internal capsule. In the absence of subplate axons in the internal capsule, the 'handshake' between subplate and thalamocortical axons proposed by Molnár and Blakemore[105,106] cannot occur and the thalamic axons fail to cross the pallial–subpallial boundary.

The laminar positioning and differentiation of subcerebral projection neurons are severely affected in sex-determining region Y-box 5 (*Sox5*)$^{-/-}$ mice[107]. *Ctgf* mRNA-expressing subplate neurons are located throughout the cortical plate in these animals, but cortico-thalamic projections still form relatively normally, although they are probably mostly the projections from layer 6a cells. Moreover, the thalamic afferents reach the cortical plate normally[107]. This is in stark contrast to the situation in *Tbr1*$^{-/-}$ mice and *Gli3* mutants, in which subplate cells and their subcortical projections seem to be completely absent and thalamocortical afferents fail to reach the cortical plate. On the basis of the results described above, it seems that the presence of even a few subplate cells that extend a process through the pallial–subpallial boundary is sufficient for thalamic afferents to be able to invade the cortex.

These mouse mutations are severe cortical malformations, but the subplate might be affected in more subtle developmental abnormalities in humans. Heterotopias are common in the white matter and subplate, and they might contribute to the development of epilepsy. In addition, an abnormally developed subplate compartment has been reported in the brains of individuals with schizophrenia or autism (BOX 2).

Function from transcriptomics

The multiple functions of subplate neurons in axon guidance and early circuit maturation derive from different attributes of these cells, such as expression of distinctive surface molecules, and the secretion of extracellular matrix proteins, growth factors or proteases. Gene expression analysis has helped to clarify these functions. The transcriptome of mouse subplate neurons has been analysed at various developmental stages from E12.5 to adult[9,10,58–60] (FIG. 5; Supplementary information S2 (figure)). Similarly, transcriptomes of human embryonic, fetal and adult cortex have been published[62,108,109], as have transcriptomes of layers of the adult monkey cortex[110,111]. The adult monkey data unfortunately do not extend to white matter, where residual subplate neurons would reside, and most of the adult human brain data are at a regional, not layer-specific, level of detail. However, the human transcriptomic analyses highlight that gene expression in different brain regions varies considerably during development, and that there are human-specific gene transcripts or variants expressed during development. Thus, caution should be applied when extrapolating from animal models to humans in this context.

Analysis of subplate-specific gene expression for functional annotation in mice and humans has primarily highlighted the relative maturity of the subplate compared with other (postmitotic) cortical layers[10,62], but it has also identified known functions such as axon extension and pathfinding, synaptic maturation and myelination at relevant ages of mouse development[10] (FIG. 5). Unexpectedly, gene expression in the mouse subplate also suggests a role in the regulation of cell division, cellular secretion[10] or oestrogen-regulated signalling in early development[58]. Gene expression in the subplate is also functionally enriched for the gene ontology term "behaviour of fear or defensive responses", although it is unclear how this relates to cellular function in the subplate[10].

Transient secretory function of subplate. The preplate and its derivatives, the subplate and the marginal zone, contain special pericellular and extracellular matrix. The subplate is specifically rich in chondroitin sulphate proteoglycans (CSPGs), whereas the adjacent intermediate zone is not[112]. CSPGs are generally secreted from cells, and the subplate transcriptome is enriched for genes involved in the production of extracellular matrix and proteoglycans[10,60]. CSPGs are known to interact with laminin, fibronectin, tenascin and collagen.

The differential distribution of CSPGs suggests that they have a role in axon pathfinding and possibly cell migration. The intracortical trajectory of afferent axons from the thalamus is centred on the CSPG-rich

Heterotopias
Clusters of cells or neurons in abnormal locations.

Box 2 | **Subplate involvement in cognitive abnormalities**

Subplate neurons have been investigated for their contribution to various psychiatric disorders and brain abnormalities, including epilepsy, autism, bipolar disorder and schizophrenia.

Epilepsy

Drug-resistant epilepsy is often accompanied by severe cortical dysplasias, in which large groups of cells may be abnormally located within white matter[124]. Such an excess of neurons could be the result of a failure of programmed cell death in subplate cells[125], but without further molecular characterization, which has only become feasible recently, it is not possible to say whether these neurons are extra subplate neurons or cortical plate neurons that failed to migrate to their final destinations. Type II focal cortical dysplasia (FCD) is associated with blurring of the white–grey matter boundary, as seen by MRI[126], which might also suggest an abnormality of subplate or interstitial white matter cells. However, analysis of type II FCD cases for layer-specific gene expression did not indicate any involvement of layer 6a or subplate cells[127].

Schizophrenia

Excess numbers of neurons within the superficial white matter have also been documented in post-mortem samples from patients with schizophrenia[124,128,129]. Moreover, studies found that interstitial white matter neurons (identified by NADPH diaphorase (NADPHd) staining) were abnormally distributed in the dorsolateral prefrontal cortex and lateral lobe of brains from patients with schizophrenia, with a preferential redistribution of such neurons to deep white matter, compared with control cases[130,131]. However, NADPHd-stained cells make up a small minority of all neurons in adult human white matter, and studies examining presumed glutamatergic neuron or pan-neuronal markers found a widespread increase in the number of white matter neurons in brains from individuals with schizophrenia[63,132,133]. Furthermore, not all brains examined showed alterations in white matter neuron density compared with controls[128]. In addition, an increase in the number of subplate neurons and an unusual persistence of expression of the subplate 'marker' nuclear receptor related 1 (NURR1; also known as NR4A2) was documented in a rodent model of developmental hypothyroidism[134], a model for pre-term birth. The link between a rodent model of premature birth (a known risk factor for schizophrenia[135]) and an increase in the number of NURR1-expressing subplate neurons found shortly after birth is intriguing.

Autism

Post-mortem histopathological analysis of autistic brains revealed various cellular and structural abnormalities in the cerebral cortex, white matter tracts, cerebellum and brainstem. Supernumerary mature-looking neurons in white matter (presumed to be interstitial neurons) were identified in a number of adult brains[136] of patients with autism. Heterotopias, including some in the white matter, were found in child and adult cases[137]. MRI of brains of patients with autism spectrum disorders revealed an indistinct boundary between grey and white matter, possibly also indicating supernumerary subplate neurons[136,138,139]. However, it should be noted that epilepsy is a common co-morbidity of autism, and some overlap between the underlying brain malformations may be expected.

Vulnerability to hypoxic–ischaemic injury

The neurons of the subplate mature earlier than most of the surrounding neurons, and it was proposed that this makes them particularly vulnerable to hypoxic injury during late gestation and in the perinatal period[140,141]. Pre-term birth or perinatal hypoxic–ischaemic injuries are risk factors for both epilepsy and schizophrenia[142].

Recent evidence suggests that subplate cells do not die in excess compared to other deep-layer neurons in the Rice–Vannucci model of hypoxia–ischaemia in young postnatal rats[143,144]. Similar cell death across all cortical layers was also reported following oxygen–glucose deprivation in an *in vitro* assay[145]. Using molecular markers of subplate neurons on human post-mortem tissue with hypoxic injury may help to resolve this.

Transcriptomic evidence for disease association

Genes expressed in a subplate-enriched pattern at some point in mouse cortical development between embryonic day 15 and adult are markedly enriched for genes that have an association with either autism or schizophrenia[10]. Human genetic studies also show that autism- and schizophrenia-associated genes are selectively associated with distinct cortical layers. Co-expression network analysis identified deep-layer projection neurons — including subplate cells — as being implicated in the pathogenesis of autism[146]. Of the autism-associated genes with subplate-enriched expression, most are expressed during development in mouse brains[10]. They encode proteins that are functionally and molecularly diverse, although most are transmembrane proteins. Their common link is likely to be their role in early subplate formation, axon extension and subsequent network maturation.

subplate, whereas early efferents cross the subplate and follow a deeper pathway that contains fewer CSPGs[113]. The CSPG-rich subplate is also a major corridor for migrating GABAergic neurons, and radially migrating neurons change their migratory mode in the subplate region. Many cortical lamination defects are associated with cells that are abnormally located below the subplate, suggesting a failure of cell migration specifically through the subplate or a failure to transition between modes of cell migration at the subplate.

There are additional genes with subplate-restricted expression in the cortex that encode secreted proteins, including *Serpini1* (which encodes neuroserpin), neuronal pentraxin 1 (*Nptx1*) and insulin-like growth factor binding protein 5 (*Igfbp5*)[10]. Neuroserpin and NPTX1 are both nervous system-specific secreted proteins that have proposed roles in synaptic function or maturation[114–117], and in subplate cells they associate with the endoplasmic reticulum (S. Kondo, H. Al-Hasani, A.H.-S., W. Z. Wang and Z.M., unpublished observations). Thus, the subplate might additionally influence cortical circuit formation through a transient secretory function.

Conclusions and perspectives

Transient populations of cortical neurons generate such great interest because they are the link between developing and mature cortical circuits. One such transient population is located in the subplate compartment. The subplate itself changes during development with regard to cellular composition, the circuits that cellular elements participate in and the genes that they express. The subplate compartment, its cellular constituents and gene expression are so dynamic that definition of subplate cellular elements is problematic.

Normal development involves dynamic interactions between the subplate and the cortical plate and extracortical targets, and each interaction is the beginning of a new phase of development that eventually culminates in the formation of a mature circuit and dissolution of the subplate. Thus, subplate dissolution is dependent on normal cortical development. Although it is well documented that abnormalities in subplate alter thalamocortical afferent development, we propose that abnormalities in thalamocortical or intracortical circuits may also lead to altered numbers and/or distributions

Rice–Vannucci model
A rodent model of perinatal hypoxia injury obtained by ligation of the common carotid artery on one side of the body followed by a period of low oxygen in the inspired air.

of subplate and interstitial white matter cells. Further insights into these bidirectional interactions are likely to hold the key to understanding how subtle alterations in functional circuit elements can lead to cognitive impairment.

There are still big gaps in our understanding of human subplate function and how cell groups identified in the mouse may be involved in human brain development. It is essential to identify the one-to-one correspondence between structures identified with imaging and histological and gene-expression data if we are to bridge this gap. Furthermore, we need clarification of which cell groups within the subplate are affected in perinatal hypoxic brain injury and whether there are molecular pathways that can be exploited to protect these cells. In addition, to elucidate the circuit function of the subplate, more refined experiments permitting silencing of groups of cells, rather than their ablation, are required.

Finally, the subplate is far from being the only transient structure of the developing brain, and further insights may be gained from devoting time to studying other dynamic, largely transient structures, such as the thalamic reticular nucleus or transient cells of the internal capsule or cerebral peduncle.

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Competing interests statement
The authors declare no competing interests.

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