Chapter 6
Regulation of Cortical Circuit Formation

Fernanda M. Rodríguez-Tornos, Beatriz Cubelos, and Marta Nieto

Abstract The complex functions of the mammalian neocortex depend on the formation of precise networks and subnetworks among its many neuron types during development. These networks are formed in a stereotyped manner that creates a reproducible human cortex and facilitates common human behavior. The accuracy and complexity of cortical circuitry predicts that the developmental mechanisms that direct each of these neurons to connect with its siblings must be precise. In recent years, remarkable advances have been made in our understanding of the several developmental mechanisms that direct cortical connectivity, but we still know only a fraction of the coordinated events and molecular elements involved. An additional difficulty is that the intricate connectivity and physiology of these circuits is far from being definitively untangled. Much of the knowledge comes from relatively simple animal models, such as rodents, ferrets, and cats. Relevant information is also derived from the study of human genetic conditions that affect intellectual capabilities. This chapter briefly describes the connectivity of excitatory neurons of the cerebral cortex, which integrate and transmit information among neocortex regions and to other regions of the brain. We will try to give an extended overview of the mechanisms that shape this connectivity during development, with special emphasis on implications in humans.

F.M. Rodríguez-Tornos • M. Nieto (✉)
Centro Nacional de Biotecnología, CSIC, Darwin 3, Campus de Cantoblanco, Madrid, Spain
e-mail: mnlopez@cnb.csic.es

B. Cubelos
Centro de Biología Molecular 'Severo Ochoa', Universidad Autónoma de Madrid, Madrid 28049, Spain

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6.1 Regulation of Cortical Circuit Formation

The mammalian neocortex is a complex, highly organized structure that contains hundreds of different neuronal cell types and diverse types of glial cells (Guillemot 2007; Molyneaux et al. 2007). It is the most anterior part of the telencephalon and is responsible for sensory perception, high cognitive functions, and consciousness; as such, it has undergone pronounced expansion during evolution, with maximal representation in the human cortex (Selzer 1990). The complex cortical functions rely on the formation of precise networks and subnetworks among the many neuron types during development. These networks form in a stereotyped manner able to create a reproducible human cortex and to facilitate common human behavior. Certain cortical circuits are also preserved among species throughout evolution, while new circuits and functions have been added to the more primitive existing structures (Innocenti 2011; Molnar 2011).

The accuracy and complexity of cortical circuitry predicts that the developmental mechanisms that direct each of these neurons to connect with its siblings must necessarily be precise. Several processes are conserved during evolution, and certain mechanisms are added or modified to create new networks that expand the cognitive capabilities of the cortex. Remarkable advances have been made in recent years in our understanding of these mechanisms and their spatial and temporal coordination, but we still know only a fraction of them. An additional difficulty is that the intricate connectivity and physiology of these circuits is far from being definitively untangled. Much of the knowledge comes from relatively simple animal models, including mice, which have a lissencephalic (smooth) cortical surface, whereas the close resemblance and evolutionary distance of the gyrencephalic brain of ferrets and cats provide excellent tools for deciphering processes exclusive to higher mammals. Relevant information is also derived from the study of human genetic conditions that affect cognitive capabilities, such as schizophrenia, autism, micro- and macroencephaly, and other syndromic and non-syndromic forms of mental retardation (Clowry et al. 2010; Manzini and Walsh 2011). In the near future, the field will benefit from the use of these approaches combined with new technologies and computer modeling to make a decisive step forward.

Neurons of the cerebral cortex can be classified into two broad classes, excitatory and inhibitory neurons. Inhibitory GABAergic, locally connecting neurons are born in the basal telencephalon and have modulatory functions. Excitatory neurons are of dorsal origin and are pyramidal neurons (most abundant) and spiny stellate excitatory interneurons of layer IV. Pyramidal neurons are projecting neurons; some extend their axons to distant subcortical and subcerebral targets, and others project to local and distant intracortical targets (Selzer 1990). This chapter will focus mainly on the connectivity of excitatory neurons, which integrate and transmit information between different neocortex regions and to other regions of the brain (subcortical targets).

6.2 General Structure of Cortical Connectivity

The cerebral cortex is a laminated structure, and each lamina or layer contains neurons with similar morphologies, connectivity patterns (Selzer 1990), and molecular identities (Molyneaux et al. 2007) that originate sequentially during development from radial precursors (Caviness et al. 1996; Takahashi et al. 1996; Heins et al. 2002; Malatesta et al. 2003; Hansen et al. 2010). The number of layers, their thickness, cell composition, and architecture varies throughout the tangential surface of the cortex and among the different functionally specialized areas. The neocortex, and by extension most of the cortex, is composed of six layers, numbered I to VI, which show further expansion and subdivisions in human. Most sensory information is routed to the cerebral cortex from the thalamus (Selzer 1990) and is conveyed to extracortical targets via corticofugal projections (Fig. 6.1). Nevertheless, the vast majority of cortical neuron connections are from one cortex region to another (intracortical) rather than to subcortical targets, allowing complex processing and integration (Fig. 6.2).

Cortical connectivity can be visualized in a simple scheme that reflects its hierarchical organization and the mechanism of origin during development. Radial interlaminar connectivity establishes the most essential intracortical circuit, the so-called cortical columns (Fig. 6.1). These columns, composed of neurons from different

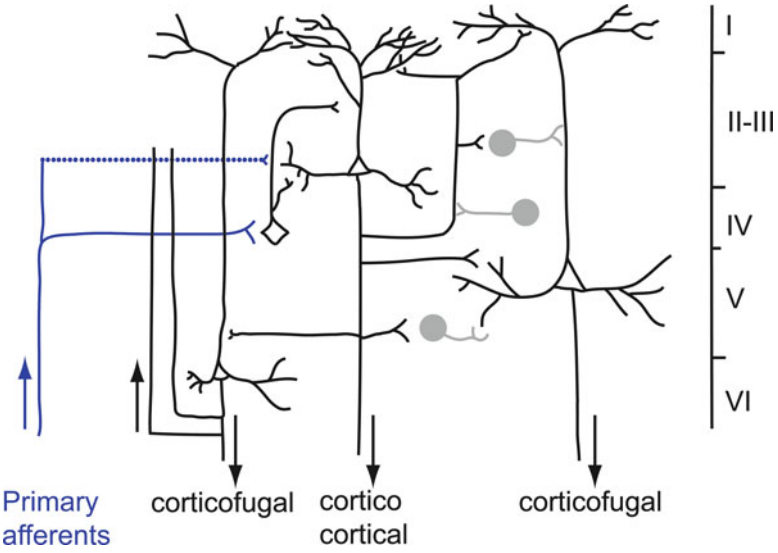

Fig. 6.1 The cortical column. Scheme showing the connectivity of a column in the somatosensory cortex. The precise connectivity of columns shows some variations on this general pattern among functional areas. *Circular grey cells* represent inhibitory interneurons; *diamonds* indicate excitatory interneurons in layer IV

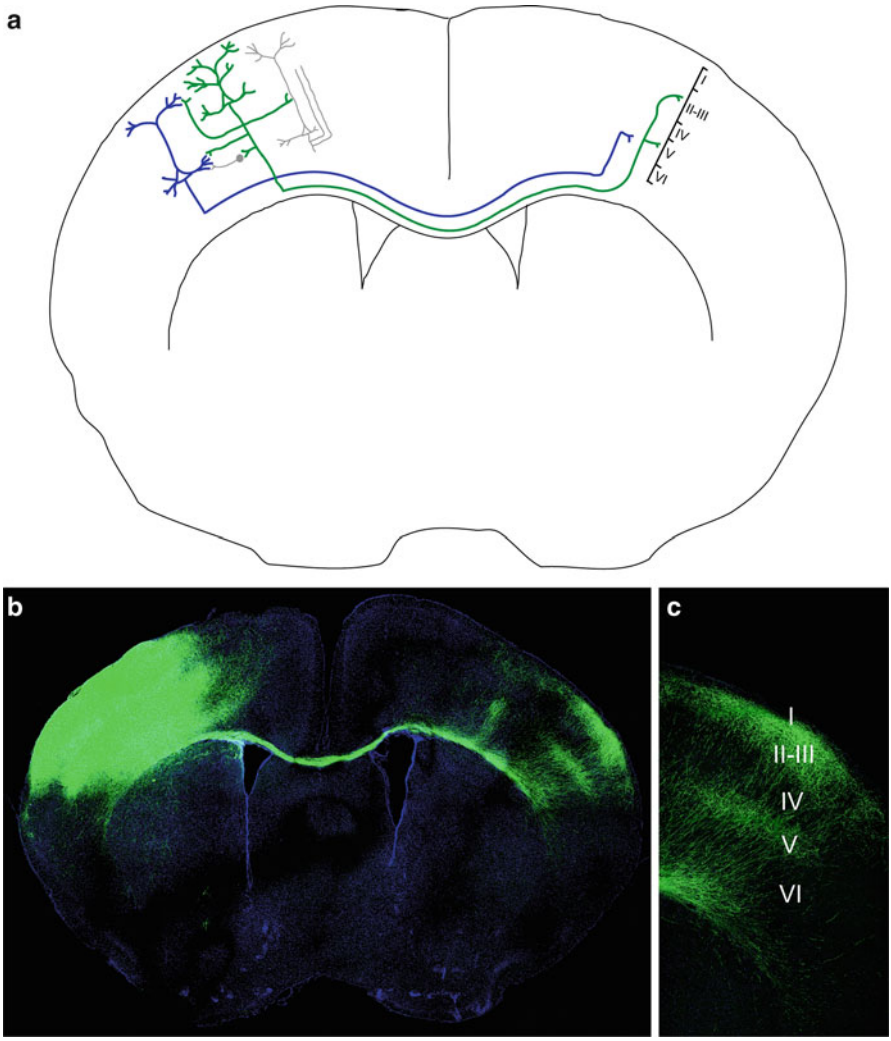

Fig. 6.2 The corpus callosum. (**a**) Myelinated axons of the CC project from neurons in layers II and III (~80 % in mouse) and in layer V (~20 %), and a very small population from cells in layer VI (not shown). (**b**) Confocal micrographs showing somas and CC axons of GFP-expressing layer II–III neurons in the P21 cortex. Neuronal morphology was analyzed at P21 after in utero electroporation at E15.5. Axons of CC neurons projecting from layers II–III invade the cortical plate at homotypic areas (six layered cortex), where they branch and profusely innervate layers II–III and V (**a** and **b**). (**c**) Layer specific pattern of innervation in the contralateral site. Magnification from **b**

layers, were described from early electrophysiological recordings in the sensory cortex demonstrating that neurons inside the column respond with similar activity to precise stimuli. The thalamic input is preferentially distributed vertically in columns to superficial and deep layers, rather than horizontally (Mountcastle et al. 1957). In the sensory cortex, neurons in a cortical column all process sensory

information from the same peripheral location and submodality (Feldmeyer et al. 2013). These studies were extended in the visual cortex by Hubel and Wiesel, who showed among other things that innervation of the visual cortex from the two eyes is also organized in columns (ocular dominance) and discovered orientation columns (Hubel and Wiesel 1962, 1963). Columnar organization is also the result of the common precursor foundation and the migration mode of cortical pyramidal neurons during development (Rakic 1988; Heins et al. 2002; Malatesta et al. 2003; Torii et al. 2009; Jones and Rakic 2010).

Columns communicate tangentially through laminar connectivity, essentially through layers II–III and V, to form the functionally specialized areas of the cortex generally classified as sensory, motor, and association areas (Rakic 1988). In the adult, the transition from one neocortical area to another can be defined by differences in cytoarchitecture, gene expression patterns, input projections, and by the specific mode of connections between neurons of the column. These properties determine the physiology and connectivity of specific circuits to allow the functional specializations that distinguish areas. For example, the somatosensory area in the mouse is defined by a thicker layer IV, expression of markers such as Rorβ, input from the whiskers and barrel formation. Finally, areas are interconnected, facilitating integration and complex behavior. Interhemispheric commissural axons permit information exchange between the cerebral hemispheres, whereas other axons that do not cross the midline, but run along the anterior posterior axis, connect areas from the same hemisphere.

In essence, the mechanisms that control cortical circuit formation during development select axon pathways and influence formation of dendritic structures and synapses, as will be discussed below. Studies in recent years have shown remarkable coordination between intrinsic molecular programs that specify neuronal cell identity and those regulating their connectivity. In the last two decades, numerous studies have reported examples of transcription factors (TF) expressed only by selected neuronal subtypes that regulate discrete aspects of connectivity (Hevner et al. 2001; Molnar et al. 2003; Jacobs et al. 2007). The pattern of overlapping functions of these TF creates cell diversity and acts as a genetic code that encrypts the rules that govern cortical networks. These intrinsic programs regulate fundamental aspects such as neurotransmitter expression, cell morphology, and the ability to respond selectively to external cues, including soluble factors and membrane-bound molecules. These mechanisms are discussed separately in this chapter. Finally, during postnatal stages, experience- and activity-mediated mechanisms involved in plasticity ultimately shape the circuits and give rise to the final stereotypical networks (Metin et al. 1997; Molnar and Cordery 1999).

6.3 Corticofugal Neurons

Projection neurons extend their axons to distant subcortical targets to transmit information to other brain regions. They are located mainly in the deeper layers of the cerebral cortex and are generally referred to as extracortical projection neurons or

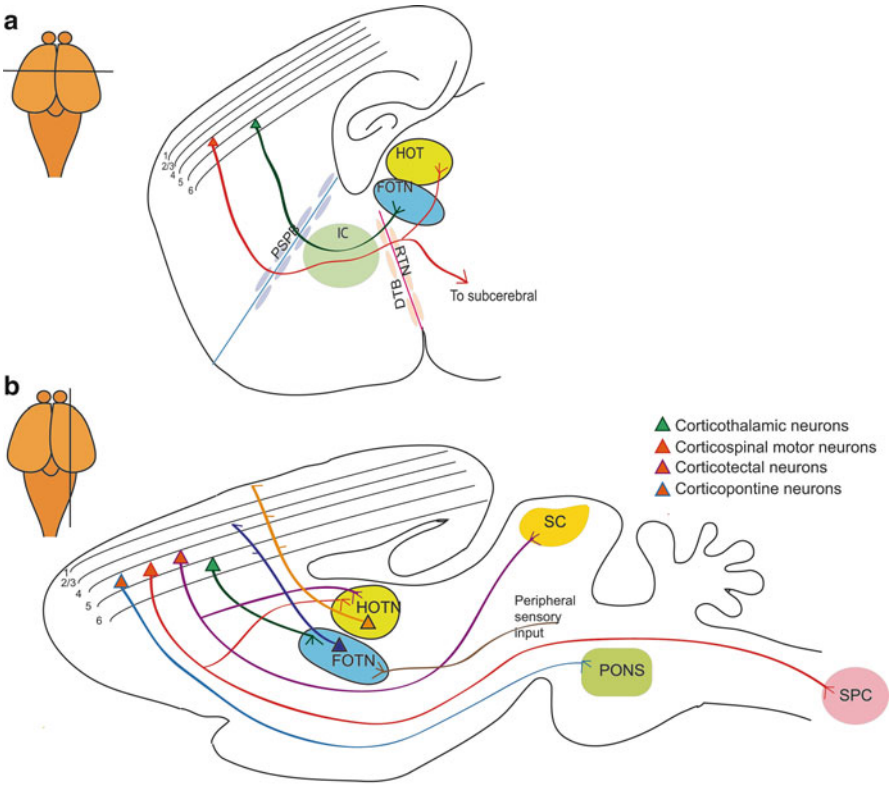

Fig. 6.3 The development of corticofugal axons. Scheme of the axonal pathway of corticofugal neurons of cortex layers V and VI. The different anatomic and genetic regions these axons encounter are depicted. Coronal (**a**) and sagittal views (**b**). *DTB* dorsal telencephalic boundary, *FOTN* first order thalamic nuclei, *HOTN* high-order thalamic nuclei, *PSPB* pallial-subpallial boundary, *RTN* reticular thalamic nuclei, *SC* superior colicullum, *SPC* spinal cord

corticofugal neurons, and are subdivided in *subcerebral* and *corticothalamic*. *Subcerebral* projection neurons reside mostly in layer V and innervate different parts of the brain stem and cerebellum, as well as the higher-order thalamic nuclei (HOTN) through secondary collaterals (Fig. 6.3). The neurons of the HOTN relay cortico-cortical information by projecting excitatory fibers to layers I, IV, and VI of a cortical area distinct from that from which they receive input. Subcerebral projecting layer V neurons can be subdivided into three major subpopulations, *corticotectal*, *corticospinal*, and *corticopontine. Corticotectal* neurons are located in the visual cortex; they send their primary axon to the superior colliculus and secondary collaterals to the rostral pons. *Corticospinal* motor neurons reside in the sensorimotor area of the cortex; they send primary projections to the spinal cord and secondary collaterals to the striatum red nucleus, caudal pons and medulla. Finally, *corticopontine* neurons are in charge of transmitting information to the pons (Molyneaux et al. 2007) (Fig. 6.3).

Corticothalamic neurons are located in layer VI and enable cortical processing of peripheral data. They project axons to and receive input from the first-order thalamic nuclei (FOTN) (Fig. 6.3). These nuclei receive peripheral sensory input and relay it to layer IV and VI neurons. Corticothalamic primary axons generate numerous small synapses with thalamic neurons, providing signals for peripheral information. Corticothalamic neurons projecting from layer VI primary visual cortex (V1) send axons to the dorsal lateral geniculate nucleus (dLGN); those in the auditory cortex (A1) project to the medial geniculate nucleus (MGN) and those in the primary somatosensory cortex (S1), to the ventrobasal nucleus (VB). The axon collaterals of these neurons innervate the reticular thalamic nucleus (RTN).

6.3.1 Development of Corticofugal Tracts

Development of corticofugal tracts follows a complex process by which distinct neuron subpopulations innervate specific extracortical regions in a temporal pattern with characteristic axon outgrowth kinetics. The subset of TF expressed by each neuron confers a unique identity, essential for its connectivity pattern and behavior. This identity would nonetheless be worthless in the absence of long- and short-range guidance cues that follow spatiotemporal dynamics. The development of corticofugal tracts is also closely associated with thalamocortical tract formation, since axons that form both tracts establish the physical association necessary to guide each other and to complete their development. Considerable controversy nonetheless remains regarding the relative importance of this interaction and of other intrinsic and extrinsic mechanisms. This will not be discussed here in detail, as the reader can find many complete reviews (Cang et al. 2005; Torii and Levitt 2005; Rash and Grove 2006; Rubenstein 2011).

The preplate contains the first subsets of cortical differentiated neurons and gives rise to Cajal-Retzius and to subplate cells. The latter are the first cortical neurons to extend their axons into the internal capsule, the natural path to extracortical territories. These initial projections act as a scaffold for subsequent corticofugal axons; the majority will disappear in the early postnatal period, correlating with a wave of cell death that eliminates their somas (Hevner et al. 2001; Jacobs et al. 2007). Neurons that form permanent connections between the cortex and extracortical regions will begin to extend their neurites at around embryonic day (E)10. Depending on their location and identity, their axons take a lateral, medial, rostral, or caudal trajectory, and grow until they reach the region adjacent to the lateral internal capsule. The distinct populations arrive at this zone at slightly different times between E13 and E15.5, depending on the position of their somas; the lateral fibers are the first to arrive and the dorsally derived fibers, the last (Fig. 6.3a). At this point, temporal synchronization requires axons to align in order to continue their journey together. The first incoming axons await the arrival of the others before continuing growth; this is termed the first waiting period. All the axons then cross the pallial-subpallial boundary (PSPB) and enter the internal capsule. The PSPB is a major boundary that

expresses a very specific subset of TF (high Pax6, null Emx1, Dlx1). This territory has modulatory potential, making early corticofugal projections turn sharply from their original ventrolateral to a medial trajectory, to cross the subpallium. The internal capsule is the site at which early corticofugal axons emitted from the subplate and thalamocortical projections first meet and establish a close interaction that will be maintained throughout the intermediate zone, PSPB, and the lateral sector of the internal capsule; this interaction is needed for guidance (Hevner et al. 2001, 2002; Lopez-Bendito et al. 2007; Chen et al. 2012; Grant et al. 2012) (23).

Once the axons exit the internal capsule, they arrive at the diencephalon-telencephalon boundary (DTB), where they enter the prethalamus and encounter the cells of the perireticular nuclei (PRN) and RTN at E16 (Fig. 6.3a). The extension will undergo a second pause that lasts until E17.5 (second waiting period). At this time, corticofugal projections continue through different pathways (Fig. 6.3a, b). Layer V primary axons continue to grow and cross the cerebral peduncle to the brainstem and spinal cord. Layer VI primary axons and layer V collaterals change direction to enter the thalamus, a process that takes several days and results in postnatal innervation of most thalamic nuclei. In higher mammals, this correlates with the functional establishment of behaviors associated with the relevant sensory systems; somatosensory and motor functions mature before visual and auditory functions. For example, in mice, somatosensory ventrobasal and motor ventrolateral nuclei are innervated earlier (E18.5 and P0.5) than auditory MGN and visual dLGN, which are not fully innervated until postnatal day (P)8 (O'Leary and Koester 1993; Metin et al. 1997; Molnar and Cordery 1999; Molnar et al. 2003; Jacobs et al. 2007; Grant et al. 2012; Lickiss et al. 2012).

One of the most fascinating characteristics of layer V and VI axons is therefore that they must navigate through several distinct territories until they reach their target. This requires dynamic recognition of territory-specific signals and modulation of axon responses. It has become apparent that several neuron populations and their axons, such as the thalamic afferents discussed above, provide structural support essential for crossing these anatomic regions and their boundaries. Pioneer axons are those of neurons (in this case, subplate neurons) that, thanks to their intrinsic electrical activity, can navigate without the help of preexisting axons and pave the way for follower axons. Voltage-gated ion channels, which in subplate neurons are voltage-gated K+3.4 (Kv3.4), are responsible for the intrinsic electrical activity patterns of neurons, and are thus necessary for corticofugal development (Huang et al. 2012). The corridor cells, a population derived from the lateral ganglionic eminence, also illustrate these cooperative interactions. These cells are needed to generate a permissive substrate for cortical axon growth across the medial ganglionic eminence (MGE). The axon guidance functions of corridor cells overlap with the guidance and sorting functions of PRN neurons, thought to have a role in directional change in the internal capsule (Lopez-Bendito et al. 2006; Grant et al. 2012).

Following spatiotemporal dynamics, axons respond differently to distinct sets of cues in the environment they traverse. These specific behaviors enable correct navigation and innervation of their targets. These guiding factors include intrinsic factors at the neuron that emits the axon (e.g., cell surface receptors or molecules that

influence intracellular signaling) as well as extrinsic factors (membrane-bound and soluble factors presented or secreted by intermediate or final targets); the latter act at short and long range, and affect growth cone extension as well as orientation by generating repulsive or attractive responses. Soluble molecules often establish concentration gradients critical for precise axon guidance of corticofugal neurons.

6.3.2 Guidance Factors and Receptors that Direct Corticofugal Axons

Although gaps remain in our knowledge, several families of guidance molecules are known to determine the trajectory of corticofugal axons. We summarize a series of illustrative examples. The semaphorin family provides early context-dependent cues. Pioneer explant experiments showed that Sema3A expression in the most superficial cortical plate, the marginal zone (MZ), is responsible both for repelling axons toward the VZ (Polleux et al. 1998) and for attracting apical dendrites (Polleux et al. 2000). Further complementary studies demonstrated that combinations of Sema3 molecules have a specific effect on the corticofugal axon pathway. For example, in addition to the superficial cortical plate, Sema3A is expressed throughout the ventricular zone and lower subventricular zone, and Sema3C is expressed in the intermediate and the subventricular zones. Although cortical axons are exposed to Sema3A and Sema3C concurrently, Sema3A has a repulsive effect that overrides Sema3C attraction, even at very low concentrations. As a result, corticofugal axons grow over the corridor generated at the intermediate zone and the upper SVZ, where Sema3C is expressed alone (Ruediger et al. 2012). Likewise, Sema5B is expressed in many regions of the corticofugal pathway, including the ventricular zone and the ventrolateral cortices, and inhibits axon entry into these territories (Bagnard et al. 2001; Lett et al. 2009). Sema molecules bind to neuropilins, whose expression and differential association with plexins also critically modulate cortifugal axon responses and dynamics (Pasterkamp 2012). Several pathways involving Sema signaling alone can thus explain many of the corticofugal axon turns and trajectories.

Netrin-1 is expressed in the internal capsule and mediates long-range attraction of corticothalamic axons at E12.5–13.5. The attractive effects of netrin-1 can induce axon turning and thus appears to be responsible for corticofugal growth cone reorientation toward the ventral telencephalon. Slit1 and 2 have a major role in corticothalamic and thalamocortical axon guidance within the ventral telencephalon and diencephalon, mainly through binding to Robo1 and Robo2 receptors, which appear to have partially redundant functions. In Robo mutant mice, and more markedly in Robo1 and Robo2 double mutants, corticothalamic axons do not grow through the internal capsule but are aberrantly directed to cross the midline. In addition, Robo1 (but not Slit) appears to act as a slowing signal, since both corticothalamic and thalamocortical axons grow faster in Robo1 knockouts (ko) than in WT mice. This deceleration might be relevant in the developmental control of the temporal

dynamics of these tracts, specifically in the regulation of the two waiting periods (Andrews et al. 2006; Lopez-Bendito et al. 2007; Grant et al. 2012).

Finally, the EphA family of tyrosine kinase receptors and their ligands are essential for the initial establishment of corticothalamic targeting. Neocortical neurons express an EphA7 gradient that controls the topography of corticothalamic projections, through local interactions within individual thalamic nuclei. Other EphA proteins, such as EphA5, also have a role in the correct patterning of corticothalamic and thalamocortical wiring (Sestan et al. 2001; Cang et al. 2005; Torii and Levitt 2005; Torii et al. 2013).

Further studies are needed to better delineate the elements that determine corticofugal connectivity. As these neurons are characterized by their long-distance journeys, the challenge is not only to understand what these signals are and how they are transduced, but also the nature of the spatiotemporal mechanisms that regulate them.

6.4 The Formation of Intracortical Circuits

6.4.1 The Development of Callosal Projecting Neurons

Interhemispheric connections are essential components of intracortical circuits and contribute to the integration ability and high associative function of the mammalian brain. The corpus callosum (CC) and the anterior commissure formed by axons of layer V are the main commissures that connect the hemispheres. The CC is the major commissural track of the mammalian brain. Partial or total CC agenesis is associated with many human developmental syndromes that affect the brain (Fame et al. 2011). Most myelinated axons of the CC project from neurons in layers II and III (~80 % in the mouse) and in layer V (~20 %), and a very minor population from cells in layer VI. A number of callosal neurons also send axonal collaterals to the same hemisphere (ipsilateral) and communicate cortical areas. There are also dual connections to the contra- and ipsilateral striatum. Axon guidance cues and synaptic maturation mechanisms that target callosal neurons and their projections are critical in the development of this important cortical circuitry.

In the several steps of axon routing involved in CC formation, different glial and neuronal cells act as intermediate guideposts and present secreted and membrane-bound navigation signals. Defects in hemisphere fusion cause partial or total CC agenesis; fusion occurs simultaneously as callosal neurons are born, just before they extend their axons, and is necessary for axons to cross the midline. Early studies showed that CC axons are guided across the cerebral midline by a glial population, then termed sling-like glial and now known as the glial sling. These astroglial populations form a bridge-like structure at the midline between the two lateral ventricles (Hankin et al. 1988; Silver et al. 1993). It was shown early on, that in acallosal mice midline crossing could be restored postnatally when this glial scaffold was

presented artificially (Silver and Ogawa 1983). More recent observations in mice and humans nonetheless show that many neurons are also present within the glial slings (Shu et al. 2003a; Ren et al. 2006). Semaphorin 3C expression in one of these transient neuronal populations helps to attract callosal axons to and through the midline (Niquille et al. 2009). Additional glial structures in the CC are considered relevant for axon navigation, including radial glial cells in the glial wedge (GW) and astrocytes in the indusium griseum (IG) (Shu and Richards 2001; Shu et al. 2003b). In the developing CC, GW-expressed Slit2 guides callosal axons to the corticoseptal boundary (Bagri et al. 2002; Shu et al. 2003c). Robo receptors bind to Slit proteins; callosal axons express Robo1, and mice deficient in this protein (*Robo1*$^{-/-}$) have defects in CC formation (Shu and Richards 2001; Andrews et al. 2006; Lopez-Bendito et al. 2007). Once axons cross the midline, the same signal repels them from this boundary (Bagri et al. 2002; Shu et al. 2003c). Other long-range molecules such as Wnt are necessary for the guidance of callosal axons. Wnt5a is expressed by the GW and the IG cells, and stimulates both outgrowth and repulsion of developing callosal axons via Ryk receptors (Keeble et al. 2006; Li et al. 2010). Other signals such as ephrins and their receptors (EphA5, EphB1 and EphrinB3) act at a shorter range and are essential not only for callosal formation, but also have a broader effect on other commissures (Mendes et al. 2006; Lindwall et al. 2007).

CC formation is also highly dependent on the earlier extensions emitted by a population of pioneer callosal neurons. This is the earliest neuron population to extend axons across the midline, at around E17 in the mouse. The cell bodies of these neurons are located in the most medial part of the cortical plate and the cingulate cortex, and their axons appear to guide the neocortical callosal projections (Koester and O'Leary 1994; Rash and Richards 2001; Fame et al. 2011). Short-range signals such as neuropilin 1 (Nrp1) regulate crossing of these early axons (Hatanaka et al. 2009; Piper et al. 2009).

Callosal axons initiate their journey guided by this plethora of signals. After midline crossing, they travel along the CC; they make a sudden turn in their trajectory and invade the contralateral cortical plate at homotypic areas. Little is known about the mechanisms that trigger this turn, but it might imply changes in axon capacity to respond to cortical cues, similar to those that occur when they cross the midline. Recognition of the correct contralateral territories might also imply recognition of lateral gradients at the cortical plate, although these mechanisms remain unclear.

Axons are able to branch and extend many synapses along their length, which allows neurons to send information to various cells simultaneously. Callosal axons branch at several points during their trajectory; most branches profusely innervate layers II–III and V in the ipsilateral and contralateral columns (Fig. 6.2), although some neurons (termed dual projecting) also send collaterals to other areas and regions. Despite their probable importance in human cognition, the patterns of these branched connections are not fully resolved, although they are likely to be responsible for certain associative properties of the cortex. For example, an undetermined number of callosal projecting neurons from the sensory cortex simultaneously extend exuberant projections to the contralateral homotypic cortex and to both

contralateral and ipsilateral areas of the motor cortex. Laterally located superficial neurons can also extend dual axons toward the midline and the internal capsule, although in the latter case, they apparently retract at P11 (Garcez et al. 2007). Similar schemes of dual projections are found in certain callosal neurons of the motor cortex, which send dual axonal projections to sensory areas (Mitchell and Macklis 2005). In mice, these dual projections show maximum numbers at P8; they are refined until approximately P21, probably through activity-dependent mechanisms, but many persist into adulthood (Innocenti and Price 2005; Mitchell and Macklis 2005). Little is currently known of the molecular control of these double connections.

6.4.2 Factors that Regulate Selectivity of the Synapse: From Intra-Columnar and Intra-Laminar Connectivity to Microcircuits

Based on the work discussed above, it is clear that scientists have successfully identified several crucial regulatory mechanisms responsible for delivering axons to the vicinity of their targets. After this arduous journey, however, only half the job is done. Axons do not establish synapses without a pattern. The nervous system shows considerable specificity at this level, and connections are made only with certain neurons; there is even selection of specific cell compartments. This is extreme in the case of cortical circuits, which implicate hierarchical organization in layers: axons selectively establish connections with certain layers, certain cells within the layers, and even choose between apical or basal dendrites. The cellular and genetic mechanisms responsible for the assembly of specific connections in the nervous system are the subject of intense study. These mechanisms involve coordinated expression of homophilic adhesion molecules by both pre- and postsynaptic partners, including the diverse cadherins and immunoglobulin superfamily (IgSF) proteins. Repulsive signals also prevent abnormal innervation (Shen and Scheiffele 2010; de Wit et al. 2011).

Few of the mechanisms known to select synaptic targets in other parts of the nervous system have been reported or tested in the cortex; there is an intriguing relative lack of knowledge about the elements that implement the beautiful patterns of cortical laminar connectivity. Barrels, which are prominent sensory units in the rodent somatosensory cortex, have been examined in detail. Data suggest that the initial gross formation of the barrel map relies on molecular cues, while refinement of its topography depends on neuronal activity. Temporal and cell-specific expression of cadherins contributes to the barrel-like distribution of thalamic axonal inputs into layer IV (Huntley and Benson 1999; Inan and Crair 2007). The development of excitatory synapses between axons emitted from layer II–III neurons with dendrites in layers II–III and V, but not those in layers IV and VI, is another perfect paradigm of layer-specific synaptic organization. Activity has a role in determining the relative innervation of layers II–III and V by contralateral CC afferent connections. Reduced firing results in increased innervation of superficial layers at the expense

of layer V innervation (Mizuno et al. 2007). Recent work identified an unexpected molecular regulators of innervation of layers II–III and V in Shh, a secreted molecule known mainly for its patterning and axon guidance effects, and in its high-affinity receptor Brother of CDO (Boc) (Okada et al. 2006). The restricted Shh expression in layer V promotes synaptic formation with Boc-bearing axons; these axons are precisely those of neurons in layers II–III. Genetic manipulation of mice showed that conditional Shh deletion in the dorsal telencephalon mimics Boc ko phenotypes of layer V neurons. Boc-depleted layer V neurons show reduced dendritic complexity, spine density and synaptic strength as a result of decreased innervation from layer II–III callosal projecting neurons (Harwell et al. 2012). Although alteration of activity or the Shh-Boc pathway did not result in layers being ectopically innervated, these studies open the path to understand layer-specific connections and the possible implications of other patterning molecules in cortical wiring, perhaps in conjunction with activity.

These studies of synaptic specificity mechanisms are also extremely important when considering the existence and formation of microcircuits and subnetworks embedded within cortical circuits. There is cellular and molecular heterogeneity not only between layers and cortical areas, but also within the neurons of the same layer (Fame et al. 2011); this results in the expression of different membrane and secreted proteins that might contribute to generating networks in the cortex. In layers II–III, microcircuits have been described functionally by the characterization of neuron firing patterns (Burgalossi et al. 2011). They have also been identified genetically, through visualization of GFP-labeled neurons that express high c-fos levels, and are highly interconnected, as shown by electrophysiology studies (Yassin et al. 2010). Common neuronal birth origin might be implicated in the formation of these microcircuits and in columnar formation. A common progenitor increases the probability of synapse between neurons, the probability to form strong electrical coupling with each other rather than with adjacent non-sister excitatory neurons, and the likelihood of producing similar excitatory responses (Yu et al. 2009, 2012; Li et al. 2012).

6.4.3 The Regulation of Dendritic Structures

Another facet of the regulation of cortical circuitry is the modulation of postsynaptic structures: dendrites, spines, and synapses. Dendritic branching specifies connectivity with selected axonal input and determines neuron morphology (Shen and Scheiffele 2010). Morphology in turn influences the way information is processed, amplifies or silences presynaptic input depolarization signals (Mainen and Sejnowski 1996), and even affects plasticity (Feldman 2012). Spine density and spine morphology determine the number, strength, and stability of synaptic contacts (Tada and Sheng 2006; Edbauer et al. 2010; Shen and Scheiffele 2010).

Developmental mechanisms that target regulation of postsynaptic structures and compartments have considerable importance in cortical function and circuit modulation, and are critical for the acquisition of higher intellectual abilities. Alterations

in dendritic morphology and in spine number and structure are defects that often correlate with cognitive disorders and mental retardation (Tada and Sheng 2006; Bourgeron 2009; Jan and Jan 2010; Kulkarni and Firestein 2012). Many of the mechanisms involved in the control of dendritic structures and synapses were thus identified during the study of human intellectual disabilities, including autism and fragile X syndrome, the most frequent cause of mental retardation. Analysis of human mutations linked to autism often shows alterations in genes that regulate the cytoskeleton and synaptic scaffold (Segal 2001); this is the case of Shank proteins (Bourgeron 2009), kalirin (Penzes and Remmers 2012), and mutations that affect the Ras/Epac2 pathway (Srivastava et al. 2012). Autism-related genes also appear to target postnatal mechanisms of plasticity and synaptic refinement. Human mutations linked to fragile X syndrome (Zhang et al. 2001) affect *FMR1*, a gene that encodes the RNA-binding protein FMRP (fragile mental retardation protein), which regulates transport and local translation to axons and dendrites (Tada and Sheng 2006; Napoli et al. 2008; Boda et al. 2010; Darnell et al. 2011; Penzes et al. 2011; van Bokhoven 2011; De Rubeis et al. 2012).

Human and mouse genes that encode TF also control dendrite and synapse development. In mice, *Mef2a* controls activity-dependent dendritogenesis (Fiore et al. 2009) as well as activity-dependent spine deletion (Flavell et al. 2006), which involves downstream use of FMRP (Pfeiffer et al. 2010). *Neurog2* regulates early neuritogenesis and alters neuron migration via phosporylation of the small GTPase Rnd2 (Hand et al. 2005), and by forming a DNA-binding complex with the LIM-only protein LMO4 (Asprer et al. 2011). Calcium signals and calcium-binding TF such as CREB are also involved in migration and dendritogenesis in the cortex (Redmond et al. 2002; Redmond and Ghosh 2005). Of the several cortical layer-specific TF described so far, the expression in mice of *Fezf2/Zfp312* in layer V neurons (Chen et al. 2005) and of *Cux1* and *Cux2* in layers II-IV regulate dendrite formation, and also synaptogenesis in the case of Cux proteins (Chen et al. 2005; Cubelos et al. 2010). *Cux* TF functions might be linked to evolution; the number of superficial layers in mammals expands together with brain volume and is maximal in humans (Hill and Walsh 2005). This correlates with the fact that upper layer neurons participate in highly associative circuits and tasks, and show an extreme degree of interconnectivity. It is thus possible that *Cux* optimize these neurons to increase their connectivity and their capacity to integrate information.

In a similar conceptual line, the two human-specific duplications of *SRGAP2* are proposed to be a delay mechanism for synaptic maturation which expands the temporal window of neonatal plasticity in humans. Mice bear one copy of the SRGAP2 gene, while humans have three alleles (A, B, and C). In the mouse neocortex, *SRGAP2* promotes spine maturation and limits spine density. The human *SRGAP2B* and *SRGAP2C* duplications are partial and encode truncated forms that dimerize with the ancestral SRGAP2 (SRGAP2A) protein. Surprisingly, this dimerization inhibits normal SRGAP2 function. Thus, experiments in mice show that ectopic expression of hSRGAP2C phenocopies SRGAP2 deficiency; in both cases, mice have abundant, immature long spines. These findings suggest that inhibition of SRGAP2 function by its human-specific paralogs has contributed to evolution of the

human neocortex (Charrier et al. 2012). In sum, these studies suggest that specific mechanisms that target dendritic structures and synapses contribute to the evolution of cerebral cortical circuits and the definition of human intellectual capacity.

6.5 Molecular Identity of Cortical Neurons: Layer and Area Identity as Determinants of Connectivity

Molecular identity is broadly defined by the subset of genes expressed by each neuron. Subtype-specific TF ultimately determine the molecular identity of neurons by initiating and maintaining specific genetic programs. Expression of these TF is often interconnected through gene expression cascades (Molyneaux et al. 2007; Leone et al. 2008; Fame et al. 2011). Neuron identity programs are initiated early in dividing cells by progenitor-specific TF and passed on to neuronal progeny through expression of the same or other subtype-specific TF (Molyneaux et al. 2007; Leone et al. 2008; Fame et al. 2011). Because laminar organization of the cortex coincides with the segregation of neuron subpopulations, many of the TF that specify neuronal identity have been identified as layer specific (Fig. 6.4). In the last two decades, genetic studies in mice have shown how several of these layer-specific TF modulate

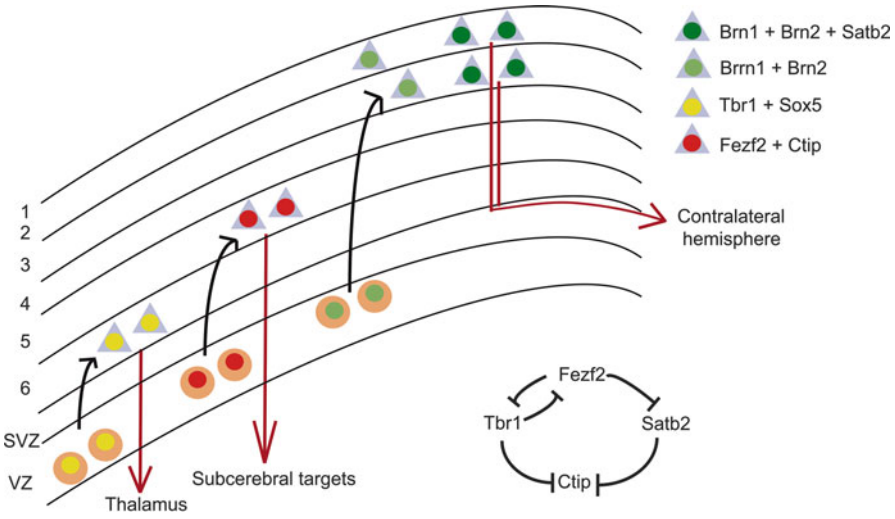

Fig. 6.4 The molecular identity of cortical neurons. Molecular identity is defined by the subset of TF expressed by each neuron. Many of the TF that specify neuronal identity have been identified as layer-specific factors. Neuron identity programs are initiated early in dividing cells by progenitor-specific TF and passed on to neuronal progeny through expression of the same or other subtype-specific TF. This identity determines the connectivity pattern of these neurons. Schematic representation of reported molecular and genetic interactions that inter-regulate the expression of subclass-specific TF

different aspects of connectivity during development and indicate that they are related to almost every process the neurons undergo. This is a fascinating and dynamic field, as indicated by the ongoing identification of genes essential for determination of each neuron's fate and behavior and, thus, its connectivity. The most recent studies clearly established that there is even further molecular diversity within the layers, which could explain the instructive signals that direct formation of cortical circuits and microcircuits.

It is increasingly apparent that there are many TF genes common to all projection neurons, which would explain the common pattern of initial development. A smaller group of TF defines closely related subtypes of projection neurons and an even smaller group is characteristic of each neuron population (Arlotta et al. 2005; Molyneaux et al. 2007; Leone et al. 2008). Most studies analyze the phenotypes of neurons with loss and gain of function of specific genes. More research is needed to fully understand the specification of all these neuron subtypes and the molecular mechanisms underlying their integration into selected circuitries. We can nonetheless begin to define some mechanisms that are quite illustrative of the extreme importance of the TF selective mode of control.

6.5.1 Transcription Factors in Lower Layers

Sox5, *Ctip2* (*COUP-TF*-interacting protein 2), and *Tbr1* expression patterns selectively mark distinct subtypes of corticofugal populations (Fig. 6.4). Subplate neurons express an intermediate level of *Sox5*, high *Tbr1*, and low *Ctip2* levels; corticothalamic neurons in layer VI express *Sox5* and *Tbr1* strongly and little *Ctip2*, and subcerebral projection neurons in layer V show high *Ctip2* levels, intermediate *Sox5*, and little *Tbr1*. These expression patterns prompt the hypothesis that these proteins form a coregulatory network that governs the adoption of neuronal fates (Fig. 6.4) (Arlotta et al. 2005; Molyneaux et al. 2007).

Tbr1, a T-box family TF gene, is expressed soon after cortical progenitors begin to differentiate (Fig. 6.4). It is found at high levels in early-born neurons of the preplate and layer VI and is necessary for their correct differentiation, as it is for cortical laminar organization and guidance of cortical afferent and efferent axons (Bulfone et al. 1995; Hevner et al. 2001). Several studies suggest that its functions overlap partially with those of *Sox5*, although defects in *Tbr1* ko mouse cortex are more severe. In the absence of *Tbr1*, the corticothalamic tract disappears and there is greater upregulation of neuronal markers than in *Sox5* ko mice. Chromatin immunoprecipitation and luciferase assays demonstrated that Tbr1 binds to and inhibits *Fezf2* promoter (McKenna et al. 2011).

Studies of *Sox5* ko mice and of its overexpression demonstrate that *Sox5* is critical for generation of diversity in extra-cortical projecting neurons, as it regulates and coordinates timing of sequential emergence of the different corticofugal neuron types (subplate, corticothalamic, and subcerebral) during early corticogenesis.

Sox5 expression is essential for correct differentiation of corticothalamic and subplate neurons, and blocks premature emergence of subcerebral neurons. When *Sox5* is absent, subplate and corticothalamic neurons locate to more superficial areas, while subcerebral neurons accumulate within layer VI and the white matter. This is interpreted as an anomalous overlap in the generation of the three principal corticofugal neuron subtypes. In addition, in the *Sox5* ko mouse cortex, subplate neurons aberrantly express molecular hallmarks and connectivity patterns of subcerebral projection neurons, resulting in the appearance of additional subcerebral projection tracts. Differentiation of corticothalamic neurons is imprecise, and that of subcerebral projection neurons is accelerated. In contrast, *Sox5* gain of function at later stages of corticogenesis causes reemergence of neurons with corticofugal features (Lai et al. 2008).

Ctip2 is one of the molecular targets of *Sox5* that is upregulated in the subplate of Sox5 ko mice (Lai et al. 2008). *Ctip2* is also a major downstream effector of *Fezf2*; it is expressed at high levels in layer V corticospinal and corticotectal neurons, and at much lower levels in layer VI corticothalamic neurons. *Ctip2* expression begins once neurons reach the cortical plate and is not implicated in early specification of cortical precursors (Arlotta et al. 2005). *Ctip2* participates in directing the extension, fasciculation, and refinement of subcerebral axonal projections, particularly the ability of corticospinal neurons to extend projections to the spinal cord during formation of the corticospinal tract. Thus, *Ctip2* ko axons fail to extend past the pons to reach the spinal cord (Arlotta et al. 2005; Lickiss et al. 2012).

Fezf2 represses callosal neuron identity, is sufficient for specification of layer V subcortical projection neurons, and is needed for layer VI neuron maturation (Rouaux and Arlotta 2010). *Ctip2*- and *Fezf2*-null mice have very similar phenotypes. In *Fezf2* ko mice, the corticospinal tract disappears; corticotectal and pontine projections are also greatly reduced; inappropriate new projections appear instead (Chen et al. 2005; Molyneaux et al. 2005). In *Fezf2* ko mice, *Ctip2* expression is absent, whereas forced expression of *Fezf2* by in utero electroporation induces upregulation of *Ctip2* in neurons that would not normally express it (Chen et al. 2005, 2008). This suggests that these two genes might act in a common pathway and that *Fezf2* is a key upstream regulator of corticospinal projection neuron differentiation.

Although the genetic regulatory pathways of the TF described above are relatively well characterized, there are many other TF that define lower layer identities or are involved in axon extension and pathfinding. *Otx1* is expressed in 40–50 % of subcerebral neurons, primarily those of the visual cortex, as well as by a number of cells in layer VI; it is essential for development of the corticotectal projection neurons and controls the refinement and pruning of their axon collaterals (Weimann et al. 1999). *Opn3* is a marker of layer V and *Foxp2* of layer VI. *Er81* is expressed in layer V cortico-cortical and subcerebral projection neurons; *Nfh* and *Pou3f1* are expressed primarily in layer V subcerebral projection neurons (Frantz et al. 1994; Ferland et al. 2003; Hevner et al. 2003; Voelker et al. 2004; Arlotta et al. 2005).

6.5.2 Transcription Factors in Superficial Layers

Several TF define the molecular identity of the superficial layers. We will mention some that exemplify distinct roles in neuron differentiation. *Brn1* and *Brn2* are two POU domain transcriptional regulators expressed in superficial cortical neurons and are necessary for correct migration and cortical lamination (McEvilly et al. 2002; Sugitani et al. 2002). Genetic loss of *Brn1* and *Brn2* in mice thus abrogates the appearance of late-born superficial neurons (Sugitani et al. 2002). Other TF directly implement programs that regulate connectivity. *Satb2* (AT-rich sequence-binding protein 2) is a chromatin-remodeling TF expressed in a broad subset of layer II–III neurons and in a smaller subpopulation of layer V neurons. Loss of *Satb2* expression in mice results in agenesis of the corpus callosum and reorientation of axons toward subcortical targets through the internal capsule. This abnormal wiring scenario is explained by the observation that *Satb2* represses expression of *Ctip2*, which regulates corticofugal identities; *Satb2*-deficient neurons also have other molecular features of corticofugal projecting neurons (Alcamo et al. 2008; Britanova et al. 2008). An epigenetic regulator, the proto-oncogene *Ski*, cooperates with *Satb2* for callosal axon guidance (Baranek et al. 2012).

Cut-like homeobox proteins *Cux1* and *Cux2* also mark layers II–III and IV specifically (Nieto et al. 2004; Zimmer et al. 2004). As mentioned above, in cortical layers II–III, both genes regulate dendritogenesis, spine formation, and synaptogenesis in a non-redundant manner and act in combination to specify the final dendritic tree and the synapses of these neurons (Cubelos et al. 2010). *CUX2* also defines the upper layers of the human cerebral cortex (Arion et al. 2007), and a possible association of *CUX1* polymorphisms with failure of antidepressant response is reported (Sasayama et al. 2012). Additional TF, including Id2, act as markers of the molecular identity of superficial layers. The functions of *Bhlhb5*, which marks superficial layers but is also found in layer V, are described below.

6.5.3 Area-Specific TF

Neocortical areas are characterized by unique molecular profiles and cyto-architecture, which ultimately reflect specific modes of axonal and dendritic connectivity. A strong deterministic function of TF expressed in the progenitor pools was demonstrated in relation to cortical area formation. Four murine TF, *Coup-TFI* (Armentano et al. 2007; Faedo et al. 2008), *Emx2, Pax6* (Bishop et al. 2000; Mallamaci et al. 2000), and *Sp8* (Sahara et al. 2007), all of which are expressed in gradients across the embryonic cortical axis, determine cortical area sizes and positions by specifying or repressing area identities within cortical progenitors. Early expression of area-specific progenitor TF is modulated by morphogens and signaling molecules secreted by patterning centers that are positioned at the perimeter of the dorsal telencephalon. These centers generate graded TF expression in cortical progenitors. Two major patterning centers are the commissural plate, which expresses *Fgf8* and

Fgf17, and the cortical hem, which expresses *Bmps* and *Wnts* (O'Leary and Nakagawa 2002). Progenitor area-specific TF also interact genetically, thus modifying the expression of one another; for example, *Pax6* and *Emx2* are mutually exclusive (Bishop et al. 2000; Mallamaci et al. 2000). There is interplay between intrinsic genetic mechanisms and extrinsic information conveyed by thalamocortical input to the cortex, especially to layer IV. The relative contribution of each of these early mechanisms to area formation is still debated, and has been reviewed extensively (O'Leary et al. 2007; O'Leary and Sahara 2008).

Expression of progenitor area-specific TF can be downmodulated (*Emx2*, *Pax6*) or maintained in postmitotic neurons (*Coup-TFI*). Area-specific TF generally inhibit or promote expression of other area-specific genes including *Cadherin8, Eph* receptors and other layer-specific TF such as *Satb2, Rorβ*, and *Id2* (O'Leary et al. 2007; O'Leary and Sahara 2008). *Coup-TFI* is expressed as a gradient and, during corticogenesis, is needed to maintain the balance between frontal/motor and sensory areas (Armentano et al. 2007). This factor temporally inhibits generation of corticospinal motor neurons, which in large numbers characterize motor areas (Tomassy et al. 2010), and regulates axon outgrowth as well as the formation of the CC and other brain commissures (Armentano et al. 2006), and governs neuronal migration (Alfano et al. 2011).

Arealization is closely linked to the identity of the postmitotic neurons. Moreover, certain layer-specific TF have a role in this process. *Bhlhb5* is selectively expressed in layers II–IV and V and regulates area identity; during embryonic development, it shows a transient high caudomedial to low rostrolateral gradient. It is gradually downmodulated in the postnatal brain to produce a sharp boundary between sensory and caudal motor cortices around P4, and practically disappears at P14. *Bhlhb5*-null mice show aberrant expression of layer-specific markers and disorganization of vibrissal barrels, and those layer V corticospinal motor neurons of the motor cortex that normally express this TF also show aberrant development (Joshi et al. 2008).

Our picture of arealization mechanisms is still incomplete. Fortunately, considerable research is ongoing to further our understanding of this process. These studies include the contribution of other TF expressed in postmitotic neurons to area specification and how they might coordinate with the action of thalamocortical input, as well as with activity and experience. Unraveling circuit formation in the cerebral cortex will help us to comprehend the precise modes of connections in the cortex and that are altered in many human conditions that affect cognition, from mental retardation to neurodegeneration.

References

Alcamo EA, Chirivella L et al (2008) Satb2 regulates callosal projection neuron identity in the developing cerebral cortex. Neuron 57(3):364–377

Alfano C, Viola L et al (2011) COUP-TFI promotes radial migration and proper morphology of callosal projection neurons by repressing Rnd2 expression. Development 138(21):4685–4697

Andrews W, Liapi A et al (2006) Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain. Development 133(11):2243–2252
Arion D, Unger T et al (2007) Molecular markers distinguishing supragranular and infragranular layers in the human prefrontal cortex. Eur J Neurosci 25(6):1843–1854
Arlotta P, Molyneaux BJ et al (2005) Neuronal subtype-specific genes that control corticospinal motor neuron development in vivo. Neuron 45(2):207–221
Armentano M, Filosa A et al (2006) COUP-TFI is required for the formation of commissural projections in the forebrain by regulating axonal growth. Development 133(21):4151–4162
Armentano M, Chou SJ et al (2007) COUP-TFI regulates the balance of cortical patterning between frontal/motor and sensory areas. Nat Neurosci 10(10):1277–1286
Asprer JS, Lee B et al (2011) LMO4 functions as a co-activator of neurogenin 2 in the developing cortex. Development 138(13):2823–2832
Bagnard D, Chounlamountri N et al (2001) Axonal surface molecules act in combination with semaphorin 3a during the establishment of corticothalamic projections. Cereb Cortex 11(3):278–285
Bagri A, Marin O et al (2002) Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain. Neuron 33(2):233–248
Baranek C, Dittrich M et al (2012) Protooncogene Ski cooperates with the chromatin-remodeling factor Satb2 in specifying callosal neurons. Proc Natl Acad Sci U S A 109(9):3546–3551
Bishop KM, Goudreau G et al (2000) Regulation of area identity in the mammalian neocortex by Emx2 and Pax6. Science 288(5464):344–349
Boda B, Dubos A et al (2010) Signaling mechanisms regulating synapse formation and function in mental retardation. Curr Opin Neurobiol 20(4):519–527
Bourgeron T (2009) A synaptic trek to autism. Curr Opin Neurobiol 19(2):231–234
Britanova O, de Juan Romero C et al (2008) Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. Neuron 57(3):378–392
Bulfone A, Smiga SM et al (1995) T-brain-1: a homolog of Brachyury whose expression defines molecularly distinct domains within the cerebral cortex. Neuron 15(1):63–78
Burgalossi A, Herfst L et al (2011) Microcircuits of functionally identified neurons in the rat medial entorhinal cortex. Neuron 70(4):773–786
Cang J, Kaneko M et al (2005) Ephrin-as guide the formation of functional maps in the visual cortex. Neuron 48(4):577–589
Caviness VS Jr, Takahashi T et al (1996) Regulation of normal proliferation in the developing cerebrum potential actions of trophic factors. Exp Neurol 137(2):357–366
Charrier C, Joshi K et al (2012) Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation. Cell 149(4):923–935
Chen B, Schaevitz LR et al (2005) Fezl regulates the differentiation and axon targeting of layer 5 subcortical projection neurons in cerebral cortex. Proc Natl Acad Sci U S A 102(47):17184–17189
Chen B, Wang SS et al (2008) The Fezf2-Ctip2 genetic pathway regulates the fate choice of subcortical projection neurons in the developing cerebral cortex. Proc Natl Acad Sci U S A 105(32):11382–11387
Chen Y, Magnani D et al (2012) Evidence that descending cortical axons are essential for thalamocortical axons to cross the pallial-subpallial boundary in the embryonic forebrain. PLoS One 7(3):e33105
Clowry G, Molnar Z et al (2010) Renewed focus on the developing human neocortex. J Anat 217(4):276–288
Cubelos B, Sebastian-Serrano A et al (2010) Cux1 and Cux2 regulate dendritic branching, spine morphology, and synapses of the upper layer neurons of the cortex. Neuron 66(4):523–535
Darnell JC, Van Driesche SJ et al (2011) FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146(2):247–261
De Rubeis S, Fernandez E et al (2012) Molecular and cellular aspects of mental retardation in the Fragile X syndrome: from gene mutation/s to spine dysmorphogenesis. Adv Exp Med Biol 970:517–551

de Wit J, Hong W et al (2011) Role of leucine-rich repeat proteins in the development and function of neural circuits. Annu Rev Cell Dev Biol 27:697–729
Edbauer D, Neilson JR et al (2010) Regulation of synaptic structure and function by FMRP-associated microRNAs miR-125b and miR-132. Neuron 65(3):373–384
Faedo A, Tomassy GS et al (2008) COUP-TFI coordinates cortical patterning, neurogenesis, and laminar fate and modulates MAPK/ERK, AKT, and beta-catenin signaling. Cereb Cortex 18(9):2117–2131
Fame RM, MacDonald JL et al (2011) Development, specification, and diversity of callosal projection neurons. Trends Neurosci 34(1):41–50
Feldman DE (2012) The spike-timing dependence of plasticity. Neuron 75(4):556–571
Feldmeyer D, Brecht M et al (2013) Barrel cortex function. Prog Neurobiol 103:3–27
Ferland RJ, Cherry TJ et al (2003) Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. J Comp Neurol 460(2):266–279
Fiore R, Khudayberdiev S et al (2009) Mef2-mediated transcription of the miR379-410 cluster regulates activity-dependent dendritogenesis by fine-tuning Pumilio2 protein levels. EMBO J 28(6):697–710
Flavell SW, Cowan CW et al (2006) Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number. Science 311(5763):1008–1012
Frantz GD, Bohner AP et al (1994) Regulation of the POU domain gene SCIP during cerebral cortical development. J Neurosci 14(2):472–485
Garcez PP, Henrique NP et al (2007) Axons of callosal neurons bifurcate transiently at the white matter before consolidating an interhemispheric projection. Eur J Neurosci 25(5):1384–1394
Grant E, Hoerder-Suabedissen A et al (2012) Development of the corticothalamic projections. Front Neurosci 6:53
Guillemot F (2007) Cell fate specification in the mammalian telencephalon. Prog Neurobiol 83(1): 37–52
Hand R, Bortone D et al (2005) Phosphorylation of Neurogenin2 specifies the migration properties and the dendritic morphology of pyramidal neurons in the neocortex. Neuron 48(1):45–62
Hankin MH, Schneider BF et al (1988) Death of the subcallosal glial sling is correlated with formation of the cavum septi pellucidi. J Comp Neurol 272(2):191–202
Hansen DV, Lui JH et al (2010) Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464(7288):554–561
Harwell CC, Parker PR et al (2012) Sonic hedgehog expression in corticofugal projection neurons directs cortical microcircuit formation. Neuron 73(6):1116–1126
Hatanaka Y, Matsumoto T et al (2009) Distinct roles of neuropilin 1 signaling for radial and tangential extension of callosal axons. J Comp Neurol 514(3):215–225
Heins N, Malatesta P et al (2002) Glial cells generate neurons: the role of the transcription factor Pax6. Nat Neurosci 5(4):308–315
Hevner RF, Shi L et al (2001) Tbr1 regulates differentiation of the preplate and layer 6. Neuron 29(2):353–366
Hevner RF, Miyashita-Lin E et al (2002) Cortical and thalamic axon pathfinding defects in Tbr1, Gbx2, and Pax6 mutant mice: evidence that cortical and thalamic axons interact and guide each other. J Comp Neurol 447(1):8–17
Hevner RF, Daza RA et al (2003) Beyond laminar fate: toward a molecular classification of cortical projection/pyramidal neurons. Dev Neurosci 25(2–4):139–151
Hill RS, Walsh CA (2005) Molecular insights into human brain evolution. Nature 437(7055): 64–67
Huang CY, Chu D et al (2012) Coexpression of high-voltage-activated ion channels Kv3.4 and Cav1.2 in pioneer axons during pathfinding in the developing rat forebrain. J Comp Neurol 520(16):3650–3672
Hubel DH, Wiesel TN (1962) Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J Physiol 160:106–154
Hubel DH, Wiesel TN (1963) Shape and arrangement of columns in cat's striate cortex. J Physiol 165:559–568

Huntley GW, Benson DL (1999) Neural (N)-cadherin at developing thalamocortical synapses provides an adhesion mechanism for the formation of somatopically organized connections. J Comp Neurol 407(4):453–471
Inan M, Crair MC (2007) Development of cortical maps: perspectives from the barrel cortex. Neuroscientist 13(1):49–61
Innocenti GM (2011) Development and evolution: two determinants of cortical connectivity. Prog Brain Res 189:65–75
Innocenti GM, Price DJ (2005) Exuberance in the development of cortical networks. Nat Rev Neurosci 6(12):955–965
Jacobs EC, Campagnoni C et al (2007) Visualization of corticofugal projections during early cortical development in a tau-GFP-transgenic mouse. Eur J Neurosci 25(1):17–30
Jan YN, Jan LY (2010) Branching out: mechanisms of dendritic arborization. Nat Rev Neurosci 11(5):316–328
Jones EG, Rakic P (2010) Radial columns in cortical architecture: it is the composition that counts. Cereb Cortex 20(10):2261–2264
Joshi PS, Molyneaux BJ et al (2008) Bhlhb5 regulates the postmitotic acquisition of area identities in layers II-V of the developing neocortex. Neuron 60(2):258–272
Keeble TR, Halford MM et al (2006) The Wnt receptor Ryk is required for Wnt5a-mediated axon guidance on the contralateral side of the corpus callosum. J Neurosci 26(21):5840–5848
Koester SE, O'Leary DD (1994) Axons of early generated neurons in cingulate cortex pioneer the corpus callosum. J Neurosci 14(11 Pt 1):6608–6620
Kulkarni VA, Firestein BL (2012) The dendritic tree and brain disorders. Mol Cell Neurosci 50(1):10–20
Lai T, Jabaudon D et al (2008) SOX5 controls the sequential generation of distinct corticofugal neuron subtypes. Neuron 57(2):232–247
Leone DP, Srinivasan K et al (2008) The determination of projection neuron identity in the developing cerebral cortex. Curr Opin Neurobiol 18(1):28–35
Lett RL, Wang W et al (2009) Semaphorin 5B is a novel inhibitory cue for corticofugal axons. Cereb Cortex 19(6):1408–1421
Li L, Hutchins BI et al (2010) Wnt5a induces simultaneous cortical axon outgrowth and repulsive turning through distinct signaling mechanisms. Sci Signal 3(147):pt2
Li Y, Lu H et al (2012) Clonally related visual cortical neurons show similar stimulus feature selectivity. Nature 486(7401):118–121
Lickiss T, Cheung AF et al (2012) Examining the relationship between early axon growth and transcription factor expression in the developing cerebral cortex. J Anat 220(3):201–211
Lindwall C, Fothergill T et al (2007) Commissure formation in the mammalian forebrain. Curr Opin Neurobiol 17(1):3–14
Lopez-Bendito G, Cautinat A et al (2006) Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell 125(1):127–142
Lopez-Bendito G, Flames N et al (2007) Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain. J Neurosci 27(13):3395–3407
Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. Nature 382(6589):363–366
Malatesta P, Hack MA et al (2003) Neuronal or glial progeny: regional differences in radial glia fate. Neuron 37(5):751–764
Mallamaci A, Muzio L et al (2000) Area identity shifts in the early cerebral cortex of Emx2-/- mutant mice. Nat Neurosci 3(7):679–686
Manzini MC, Walsh CA (2011) What disorders of cortical development tell us about the cortex: one plus one does not always make two. Curr Opin Genet Dev 21(3):333–339
McEvilly RJ, de Diaz MO et al (2002) Transcriptional regulation of cortical neuron migration by POU domain factors. Science 295(5559):1528–1532
McKenna WL, Betancourt J et al (2011) Tbr1 and Fezf2 regulate alternate corticofugal neuronal identities during neocortical development. J Neurosci 31(2):549–564

Mendes SW, Henkemeyer M et al (2006) Multiple Eph receptors and B-class ephrins regulate midline crossing of corpus callosum fibers in the developing mouse forebrain. J Neurosci 26(3):882–892
Metin C, Deleglise D et al (1997) A role for netrin-1 in the guidance of cortical efferents. Development 124(24):5063–5074
Mitchell BD, Macklis JD (2005) Large-scale maintenance of dual projections by callosal and frontal cortical projection neurons in adult mice. J Comp Neurol 482(1):17–32
Mizuno H, Hirano T 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(25):6760–6770
Molnar Z (2011) Evolution of cerebral cortical development. Brain Behav Evol 78(1):94–107
Molnar Z, Cordery P (1999) Connections between cells of the internal capsule, thalamus, and cerebral cortex in embryonic rat. J Comp Neurol 413(1):1–25
Molnar Z, Higashi S et al (2003) Choreography of early thalamocortical development. Cereb Cortex 13(6):661–669
Molyneaux BJ, Arlotta P et al (2005) Fezl is required for the birth and specification of corticospinal motor neurons. Neuron 47(6):817–831
Molyneaux BJ, Arlotta P et al (2007) Neuronal subtype specification in the cerebral cortex. Nat Rev Neurosci 8(6):427–437
Mountcastle VB, Davies PW et al (1957) Response properties of neurons of cat's somatic sensory cortex to peripheral stimuli. J Neurophysiol 20(4):374–407
Napoli I, Mercaldo V et al (2008) The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP. Cell 134(6):1042–1054
Nieto M, Monuki ES et al (2004) Expression of Cux-1 and Cux-2 in the subventricular zone and upper layers II-IV of the cerebral cortex. J Comp Neurol 479(2):168–180
Niquille M, Garel S et al (2009) Transient neuronal populations are required to guide callosal axons: a role for semaphorin 3C. PLoS Biol 7(10):e1000230
O'Leary DD, Koester SE (1993) Development of projection neuron types, axon pathways, and patterned connections of the mammalian cortex. Neuron 10(6):991–1006
O'Leary DD, Nakagawa Y (2002) Patterning centers, regulatory genes and extrinsic mechanisms controlling arealization of the neocortex. Curr Opin Neurobiol 12(1):14–25
O'Leary DD, Sahara S (2008) Genetic regulation of arealization of the neocortex. Curr Opin Neurobiol 18(1):90–100
O'Leary DD, Chou SJ et al (2007) Area patterning of the mammalian cortex. Neuron 56(2):252–269
Okada A, Charron F et al (2006) Boc is a receptor for sonic hedgehog in the guidance of commissural axons. Nature 444(7117):369–373
Pasterkamp RJ (2012) Getting neural circuits into shape with semaphorins. Nat Rev Neurosci 13(9):605–618
Penzes P, Remmers C (2012) Kalirin signaling: implications for synaptic pathology. Mol Neurobiol 45(1):109–118
Penzes P, Cahill ME et al (2011) Dendritic spine pathology in neuropsychiatric disorders. Nat Neurosci 14(3):285–293
Pfeiffer BE, Zang T et al (2010) Fragile X mental retardation protein is required for synapse elimination by the activity-dependent transcription factor MEF2. Neuron 66(2):191–197
Piper M, Plachez C et al (2009) Neuropilin 1-Sema signaling regulates crossing of cingulate pioneering axons during development of the corpus callosum. Cereb Cortex 19(Suppl 1):i11–i21
Polleux F, Giger RJ et al (1998) Patterning of cortical efferent projections by semaphorin-neuropilin interactions. Science 282(5395):1904–1906
Polleux F, Morrow T et al (2000) Semaphorin 3A is a chemoattractant for cortical apical dendrites. Nature 404(6778):567–573
Rakic P (1988) Specification of cerebral cortical areas. Science 241(4862):170–176
Rash BG, Grove EA (2006) Area and layer patterning in the developing cerebral cortex. Curr Opin Neurobiol 16(1):25–34

Rash BG, Richards LJ (2001) A role for cingulate pioneering axons in the development of the corpus callosum. J Comp Neurol 434(2):147–157
Redmond L, Ghosh A (2005) Regulation of dendritic development by calcium signaling. Cell Calcium 37(5):411–416
Redmond L, Kashani AH et al (2002) Calcium regulation of dendritic growth via CaM kinase IV and CREB-mediated transcription. Neuron 34(6):999–1010
Ren T, Anderson A 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 288(2):191–204
Rouaux C, Arlotta P (2010) Fezf2 directs the differentiation of corticofugal neurons from striatal progenitors in vivo. Nat Neurosci 13(11):1345–1347
Rubenstein JL (2011) Annual research review: development of the cerebral cortex: implications for neurodevelopmental disorders. J Child Psychol Psychiatry 52(4):339–355
Ruediger T, Zimmer G et al (2012) Integration of opposing semaphorin guidance cues in cortical axons. Cereb Cortex 23:604–614
Sahara S, Kawakami Y et al (2007) Sp8 exhibits reciprocal induction with Fgf8 but has an opposing effect on anterior-posterior cortical area patterning. Neural Dev 2:10
Sasayama D, Hiraishi A et al. (2012) Possible association of CUX1 gene polymorphisms with antidepressant response in major depressive disorder. Pharmacogenomics J. doi:10.1038/tpj.2012.18 (Epub ahead of print)
Segal M (2001) New building blocks for the dendritic spine. Neuron 31(2):169–171
Selzer ME (1990) Cajal on the cerebral cortex—an annotated translation of the complete writings, By Javier DeFelipe and Edward G. Jones New York, Oxford University Press, 1988 654 pp, illustrated. Ann Neurology 27(4):453–453
Sestan N, Rakic P et al (2001) Independent parcellation of the embryonic visual cortex and thalamus revealed by combinatorial Eph/ephrin gene expression. Curr Biol 11(1):39–43
Shen K, Scheiffele P (2010) Genetics and cell biology of building specific synaptic connectivity. Annu Rev Neurosci 33:473–507
Shu T, Richards LJ (2001) Cortical axon guidance by the glial wedge during the development of the corpus callosum. J Neurosci 21(8):2749–2758
Shu T, Li Y et al (2003a) The glial sling is a migratory population of developing neurons. Development 130(13):2929–2937
Shu T, Puche AC et al (2003b) Development of midline glial populations at the corticoseptal boundary. J Neurobiol 57(1):81–94
Shu T, Sundaresan V et al (2003c) Slit2 guides both precrossing and postcrossing callosal axons at the midline in vivo. J Neurosci 23(22):8176–8184
Silver J, Ogawa MY (1983) Postnatally induced formation of the corpus callosum in acallosal mice on glia-coated cellulose bridges. Science 220(4601):1067–1069
Silver J, Edwards MA et al (1993) Immunocytochemical demonstration of early appearing astroglial structures that form boundaries and pathways along axon tracts in the fetal brain. J Comp Neurol 328(3):415–436
Srivastava DP, Woolfrey KM et al (2012) An autism-associated variant of Epac2 reveals a role for Ras/Epac2 signaling in controlling basal dendrite maintenance in mice. PLoS Biol 10(6):e1001350
Sugitani Y, Nakai S et al (2002) Brn-1 and Brn-2 share crucial roles in the production and positioning of mouse neocortical neurons. Genes Dev 16(14):1760–1765
Tada T, Sheng M (2006) Molecular mechanisms of dendritic spine morphogenesis. Curr Opin Neurobiol 16(1):95–101
Takahashi T, Nowakowski RS et al (1996) The leaving or Q fraction of the murine cerebral proliferative epithelium: a general model of neocortical neuronogenesis. J Neurosci 16(19):6183–6196
Tomassy GS, De Leonibus E et al (2010) Area-specific temporal control of corticospinal motor neuron differentiation by COUP-TFI. Proc Natl Acad Sci U S A 107(8):3576–3581
Torii M, Levitt P (2005) Dissociation of corticothalamic and thalamocortical axon targeting by an EphA7-mediated mechanism. Neuron 48(4):563–575

Torii M, Hashimoto-Torii K et al (2009) Integration of neuronal clones in the radial cortical columns by EphA and ephrin-A signalling. Nature 461(7263):524–528
Torii M, Hackett TA et al (2013) EphA signaling impacts development of topographic connectivity in auditory corticofugal systems. Cereb Cortex 23:775–785
van Bokhoven H (2011) Genetic and epigenetic networks in intellectual disabilities. Annu Rev Genet 45:81–104
Voelker CC, Garin N et al (2004) Selective neurofilament (SMI-32, FNP-7 and N200) expression in subpopulations of layer V pyramidal neurons in vivo and in vitro. Cereb Cortex 14(11): 1276–1286
Weimann JM, Zhang YA et al (1999) Cortical neurons require Otx1 for the refinement of exuberant axonal projections to subcortical targets. Neuron 24(4):819–831
Yassin L, Benedetti BL et al (2010) An embedded subnetwork of highly active neurons in the neocortex. Neuron 68(6):1043–1050
Yu YC, Bultje RS et al (2009) Specific synapses develop preferentially among sister excitatory neurons in the neocortex. Nature 458(7237):501–504
Yu YC, He S et al (2012) Preferential electrical coupling regulates neocortical lineage-dependent microcircuit assembly. Nature 486(7401):113–117
Zhang YQ, Bailey AM et al (2001) Drosophila fragile X-related gene regulates the MAP1B homolog Futsch to control synaptic structure and function. Cell 107(5):591–603
Zimmer C, Tiveron MC et al (2004) Dynamics of Cux2 expression suggests that an early pool of SVZ precursors is fated to become upper cortical layer neurons. Cereb Cortex 14(12):1408–1420