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Development of the Cerebral Cortex: Implications for Neurodevelopmental Disorders

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Abstract

The cerebral cortex has a central role in cognitive and emotional processing. As such, understanding the mechanisms that govern its development and function will be central to understanding the bases of severe neuropsychiatric disorders, particularly those that first appear in childhood. In this review, I highlight recent progress in elucidating genetic, molecular and cellular mechanisms that control cortical development. I discuss basic aspects of cortical developmental anatomy, and mechanisms that regulate cortical size and area formation, with an emphasis on the roles of fibroblast growth factor (Fgf) signaling and specific transcription factors. I then examine how specific types of cortical excitatory projection neurons are generated, and how their axons grow along stereotyped pathways to their targets. Next, I address how cortical inhibitory (GABAergic) neurons are generated, and point out the role of these cells in controlling cortical plasticity and critical periods. The paper concludes with an examination of four possible developmental mechanisms that could contribute to some forms of neurodevelopmental disorders, such as autism.

Keywords

Cortex; Development; Autism; Brain development; fibroblast growth factor; GABA

Introduction

The cerebral cortex has a central role in cognitive and emotional processing. As such, understanding the mechanisms that govern its development and function will be central to understanding the bases of severe neuropsychiatric disorders, particularly those that first appear in childhood. In this review, I highlight recent progress in elucidating genetic, molecular and cellular mechanisms that control cortical development. Selected definitions of specialized words can be found in Table 1.

The cerebral cortex is a bilateral structure forming the roof of the cerebral hemispheres (telencephalon), and consists of neurons, astrocytes, oligodendrocytes, blood vessels and ependyma. The ependymal layer faces the cerebral spinal fluid of the lateral ventricle; the exterior surface of the cortex is covered by the meninges. The largest region of the cerebral cortex is the neocortex, whose neurons are organized in roughly six layers. Each layer has distinct connections within the cortex and with subcortical structures. There are two major types of cortical neurons. Roughly 80% are projection neurons; these are excitatory cells that use glutamate as their neurotransmitter. Their axon extends long distances to its synaptic targets. The remainder (~20%) are local circuit neurons (interneurons), these are

inhibitory cells that use GABA as their neurotransmitter. Generally, their axon synapses on nearby neurons.

During evolution of the mammalian brain, perhaps the most salient morphological change has been the increased surface area of the neocortex, with a concomitant increase in its laminar complexity (Hill and Walsh; 2005; Bystron et al., 2008; Hansen et al., 2010). The human brain is roughly three times larger than the chimpanzee brain. The prefrontal cortex, which is the center of cognition and decision-making, also shows evolutionary changes. The prefrontal cortex of humans and greater apes is disproportionately large compared to the rest of the neocortex of lesser apes, monkeys and less complex mammals (Ongür and Price, 2000; Semendeferi et al., 2002).

Evolutionarily advantageous innovations can come with liabilities, particularly if selective pressures have not reduced the frequency of design flaws. Thus, perhaps the benefits of cortical enlargement in humans have come with imperfections that underlie some of the etiologies of neuropsychiatric disorders. Therefore, insights into some forms of neuropsychiatric disorders may come from understanding the mechanisms that underlie cortex development, including mechanisms that control the size, nature and connections of its subdivisions. For instance, the brain of autism spectrum disorder (ASD) patients shows a trend for increased brain volume (and head circumference) apparently due to increased growth during early postnatal life, followed by a deceleration at later stages (Piven et al., 1995; Carper et al., 2006; Amaral et al., 2008). Enlargements are greatest in the frontal lobes, although increases are also found in the temporal and parietal lobes. Within the frontal lobe, increases are seen in the dorsolateral and medial regions, but not the orbitofrontal cortex. Defects in other brain regions are also apparent (e.g. cerebellum, hippocampus, and amygdala) showing that one must also consider the importance of non-neocortical regions in the etiology of ASD. While brain enlargement is often associated with ASD children, reduced brain size (microcephaly) and cerebral malformations are also associated with learning disabilities, and often have a genetic basis that affects neuronal migration and/or signaling such as through the primary cilium (Mochida and Walsh, 2004; Lancaster and Gleeson, 2009).

The mechanisms for ASD brain enlargement are poorly understood; while it is unknown whether brain enlargement is a cause or consequence of ASD, insights into these disorders may come from understanding the basic mechanisms that regulate cortical growth and patterning that have been deciphered from work in rodents and other non-human organisms. In this review, I will discuss some developmental and molecular mechanisms that undoubtedly underlie cortical development in the prenatal human embryo and fetus.

Cortical Areas

The cerebral cortex develops from a morphologically uniform neuroepithelium located in the “dorsal” part of the telencephalic vesicles, or pallium. The pallium is further subdivided into the hippocampal formation (archicortex), neocortex, olfactory/piriform cortex (paleocortex), and claustrum/pallial amygdala (Puelles et al., 2000). Each of these large domains is further subdivided. For example, within the neocortex, rostral regions regulate motor and executive functions (i.e. prefrontal cortex), whereas caudal regions regulate sensory processing (i.e. somatosensation, audition and vision).

The prefrontal cortex (PFC) is a neocortical region that has critical roles in higher cognitive functions and has been implicated in neuropsychiatric disorders. The PFC can be divided into dorsal, medial and orbital regions (Ongür and Price, 2000; Price, 2006). The dorsomedial PFC is subdivided into dorsal (frontal association, anterior cingulate and

prelimbic) and ventral PFC (infralimbic and medial orbital) areas, while the orbital PFC is subdivided into ventral, lateral, dorsolateral and ventrolateral orbital areas.

Along its longitudinal dimension, the cortical sheet is organized into histologically discrete areas that emerge during embryonic and postnatal development as described in the protomap hypothesis of Rakic (1988, 1995). Molecular and cellular mechanisms have been identified that regulate cortical arealization (reviewed by: Grove and Fukuchi-Shimogori, 2003; Sur and Rubenstein, 2005; Mallamaci and Stoykova, 2006; Rash and Grove, 2006; O'Leary, Chou and Sahara, 2007; O'Leary and Sahara, 2008; Hoch et al., 2009). Prior to and after the arrival of thalamocortical afferents (the main source of input to the cortex), a subset of genes is expressed in graded and coarse areal patterns within the cortical progenitor zone and emergent cortical plate (Nakagawa et al., 1999; Rubenstein et al., 1999). Genetic ablation of thalamocortical input does not disrupt the establishment or maintenance of areal gene expression, providing evidence that early regionalization occurs independently of thalamic input (Miyashita-Lin et al., 1999; Nakagawa et al., 1999). Therefore, research over the last several years has focused on identifying genes that specify regional identity of neural progenitors and thereby contribute to cortical arealization.

Cortical Patterning Centers

A current model of forebrain patterning suggests that Fgf signaling from a rostral source (the rostral patterning center, or RPC) imparts positional information to the adjacent neuroepithelium by regulating the expression of transcription factors and other regulatory molecules (Fig. 1; reviewed in Grove and Fukuchi-Shimogori, 2003; Garel and Rubenstein, 2004; Sur and Rubenstein, 2005; Rash and Grove, 2006; Mason, 2007; O'Leary, Chou and Sahara, 2007; O'Leary and Sahara, 2008; Hoch et al., 2009). There is increasing evidence that Fgf signaling from the RPC confers positional identity along the rostral-caudal axis in a dose-dependent manner, with high doses near the RPC defining the rostral-most cortical identity.

Early forebrain patterning is regulated by interactions between Fgfs secreted from the rostral patterning center and signaling proteins secreted from two other centers, a dorsal center which expresses bone morphogenic proteins (BMPs) and Wnts, and a ventral center which expresses Shh (Shimamura and Rubenstein, 1997; Crossley et al., 2001; Shimogori et al., 2004; Sur and Rubenstein, 2005; Storm et al., 2006).

The midline of the dorsal patterning center is called the roof plate, which has a central role in telencephalic development (Cheng et al., 2006). The roof plate, and adjacent structures, secrete molecules of the BMP and Wnt families that control patterning of the medial and dorsal pallium (Fig. 1; i.e. the choroid plexus, hippocampus and neocortex). Targeted inactivation of Wnt3a or the Wnt-activated transcription factor Lef-1 severely disrupts formation of the hippocampus (Galceran et al., 2000; Lee et al., 2000). Ectopic expression and conditional inactivation of BMP signaling alter development of the dorsal midline and paramedial structures (i.e. choroid plexus) and also affect patterning of the dorsal pallium (Panchision et al., 2001; Hebert et al., 2002; 2003; Ohkubo et al., 2002; Fernandes et al., 2007). BMP signaling represses Fgf8 expression, and Fgf signaling represses Bmp4 and Wnt8b expression, thus linking the functions of the dorsal and rostral patterning centers (Ohkubo et al., 2002; Shimogori et al., 2004; Storm et al., 2006).

Shh signaling, presumably from the ventral patterning center (Fig. 1), is required to maintain Fgf8 expression (Ohkubo et al., 2002; Aoto et al., 2002; Kuschel et al., 2003). As such, Shh mutants have rostral midline cortical defects (Rash and Grove, 2007) similar to Fgf8 mutants, but do form a dorsal telencephalic midline, a cortical hem, and two cortical hemispheres. Reducing dosage of the Gli3 transcriptional repressor (Gli3R) has the opposite

phenotype and can partially rescue rostromedial phenotypes in *Shh*^{-/-} mice (Rash and Grove, 2007). Thus, *Gli3* integrates *Fgf8*, *Shh* and probably BMP/Wnt signaling in patterning the cortex, tectum and cerebellum (Kuschel et al., 2003; Rash and Grove, 2007; Blaess et al., 2008).

Fgf Signaling and Cortical Patterning

The RPC expresses at least five *Fgf* genes: *Fgf3*, *Fgf8*, *Fgf15*, *Fgf17* and *Fgf18* (Crossley and Martin, 1995; Maruoka et al., 1998; Mason, 2007; Cholfin and Rubenstein, 2008; Borello et al., 2008). *Fgf8*, *Fgf17* & *Fgf18* are highly related, whereas *Fgf3* and *Fgf15* (the mouse ortholog of human *Fgf19*) are members of distinct *Fgf* subfamilies (Itoh and Ornitz, 2004). *Fgf8* and *Fgf18* are expressed in the core of the rostral patterning center (RPC), whereas *Fgf17* expression extends rostradorsally and *Fgf15* expression extends rostroventrally. *Fgf3* expression appears to be similar to *Fgf8* (Walshe and Mason, 2003; Theil et al., 2008). *Fgf2* and *Fgf10* are more broadly expressed in the cortical neuroepithelium, where they regulate progenitor maturation and neurogenesis (Vaccarino et al., 1999; Korada et al., 2002; Sahara and O'leary, 2009).

The nested expression of *Fgfs* in the rostral patterning center suggests that *Fgfs* may operate hierarchically. While *Fgf8* expression does not depend on *Fgf15* or *Fgf17*, the sizes of the *Fgf15*⁺ and *Fgf17*⁺ patterning center domains are reduced in *Fgf8* mutants (Cholfin and Rubenstein, 2008; Borello et al., 2008). *Fgf3* and *Fgf17* have subtle effects on cortical size (Cholfin and Rubenstein, 2007; Theil et al., 2008); the effect of the *Fgf18* mutation on cortical size and areal patterning has not been fully described, although its expression in the cortical plate complicates analysis of its earlier function in cortical regionalization (Hasegawa et al., 2004).

Currently it appears that among the *Fgfs* expressed in the RPC, *Fgf8* has the most pronounced role(s) in early forebrain patterning. *Fgf8* exhibits dosage-dependent functions in early forebrain patterning: this protein regulates neural specification, promotes rostral and ventral molecular properties, promotes progenitor proliferation and inhibits cell death (Shimamura & Rubenstein, 1997; Ye et al., 1998; Garel et al., 2003; Storm et al., 2003, 2006; Cholfin & Rubenstein, 2008; Borello et al., 2008; Okada et al., 2008; Theil et al., 2008). *Fgf8* severe hypomorphs (*Fgf8*^{null/neo}) and conditional null (*Fgf8*^{TelKO}) mutants have holoprosencephaly and exhibit severe regional patterning deficits (Storm et al., 2003, 2006).

Fgfs signal through four *Fgf* receptors (*Fgfrs*), which are receptor tyrosine kinases that can activate at least three pathways: 1) the RAS/mitogen-activated protein kinase (MAPK) pathway; 2) the phosphatidylinositol-3-kinase (PI3K)-AKT pathway; 3) the phospholipase-Cg/Calcium/PKCδ pathway (reviewed in Bottcher and Niehrs, 2005). *Fgfrs* are broadly expressed in the telencephalic neuroepithelium. Although *Fgf* gene (RNA) expression is highly localized, it is hypothesized that *Fgf* proteins diffuse from the RPC to activate receptors at a distance.

Fgf receptors (*Fgfr*) participate in telencephalon patterning and neurogenesis (the process of generating neurons from neuroepithelial stem and progenitor cells) (Hebert et al., 2003; Sansom et al., 2005; Gutin et al., 2006; Thomson et al., 2009). For example, while *Fgfr1* is necessary for hippocampal growth (Ohkubo et al., 2004), the attenuation of all *FGFR* signaling (via a dominant negative approach) results in loss of pyramidal neurons in frontal and temporal cortex (Shin et al., 2004). *Fgfr2*, by itself and in cooperation with *Fgf1* and *Fgfr3*, inhibits differentiation of progenitors into neurons, while promoting stem/progenitor cell self-renewal (Ever et al., 2008; Kang et al., 2009; Stevens et al., 2010). Loss of *Fgfr2* function impairs progenitor cell proliferation (Ever et al., 2008), while *Fgfr3* gain-of-function mutants show selective expansion of the occipitotemporal cortex (Thomson et al.,

2009). Mice lacking Fgfr1,2&3 have defects in the maturation of neural progenitors (Kang et al., 2009).

Fgf signaling has important effects on transcription factor expression. At the earliest stages of telencephalic morphogenesis, Fgf8 promotes the expression of Foxg1 (Bf1) (Shimamura and Rubenstein, 1997; Ye et al., 1998; Storm et al., 2006). Foxg1 has a fundamental role in promoting the progenitor cell state and repressing differentiation, perhaps by inhibiting Bmp expression and/or signaling (Dou et al., 1999; 2000; Seoane et al., 2004; Regad et al., 2007).

Rostral telencephalic patterning is transduced, at least in part, through Fgf8 and Fgf17 promoting the expression of several transcription factors, including the Six3 homeodomain protein, the Sp8 Zinc finger protein, and the ETS proteins Erm, Er81 and Pea3 (Fukuchi-Shimogori and Grove, 2003; Storm et al., 2006; Cholfin and Rubenstein, 2008; Sahara et al., 2007). Fgf8 and Fgf17 repress expression of COUP-TF1, and Emx2, which are transcription factors involved in caudal cortical patterning (Crossley et al., 2001; Fukuchi-Shimogori & Grove, 2003; Garel et al., 2003; Storm et al., 2006; Cholfin & Rubenstein, 2008).

Surprisingly, Fgf15 functions in an opposite manner to Fgf8 and Fgf17 (Borello et al., 2008). For instance, Fgf15^{-/-} mutants have reduced expression of COUP-TF1 and increased expression of Sp8. Currently, we do not know how Fgf8/17 and Fgf15 generate these opposite molecular changes, although the dynamics of pErk and pAKT phosphorylation induced by Fgf8 and Fgf15 are different (Borello et al., 2008), suggesting that these ligands differentially activate Fgfr signaling pathways.

Gain- and loss-of-function experiments point to a critical role for Fgf signaling in neocortical patterning and arealization (Fukuchi-Shimogori and Grove, 2001, 2003; Garel et al., 2003; Cholfin and Rubenstein, 2007). For example, Fgf8^{neo/neo} mild hypomorphic mutants exhibit rostral shifts in gradients of COUP-TF1 and Emx2 in the cortical neuroepithelium that correlate with reduced frontal cortex size and expanded caudal cortical regions (Garel et al., 2003). Ectopic expression of Fgf8 in the caudal cortical primordium results in partial duplication of the somatosensory cortex (Fukuchi-Shimogori and Grove, 2001). Although the initial pattern of thalamocortical connectivity is not affected in newborn Fgf8^{neo/neo} mutants (Garel et al., 2003), Fgf8 can regulate neocortical cues that guide area-specific thalamic innervation postnatally (Shimogori and Grove, 2005). Fgf8 appears to affect the early intracortical wiring pattern, another aspect of cortical arealization (Huffman et al., 2004). Finally, Fgf signaling is essential for differentiation of the dorsal telencephalic midline and for commissural crossing (Shanmugalingam et al., 2000; Huffman et al., 2004; Smith et al., 2006).

In Fgf17^{-/-} mutants, the gradients of COUP-TF1 and Emx2 are not altered as notably as in Fgf8 and Fgf15 mutants (Cholfin and Rubenstein, 2008), suggesting that Fgf17 may have a more specific role in controlling regional properties of the frontal cortex. Indeed, Fgf17^{-/-} mutants exhibit greatly reduced Erm expression in the frontal cortex anlage and specific defects in the dorsomedial frontal cortex of neonates and adults (Cholfin and Rubenstein, 2007, 2008).

Behavioral Defects of Fgf17^{-/-} mice

Fgf8 and Fgf17 mice have defects in the prefrontal cortex, inferior colliculus and the cerebellum; the latter defects arise from the role of Fgf-signaling in the midbrain-hindbrain patterning center. In this regard, it is note-worthy, that alterations in cerebellar vermis size are seen in ASD (Amaral et al., 2008; Bauman et al., 2006). While there is little understanding of the mechanism(s) for this, the En2 transcription factor, which lies

downstream of FGF-signaling, may contribute to some forms of ASD (Benayed et al., 2005).

The behavior of *Fgf17*^{-/-} mice may shed light on the phenotype of Humans with prefrontal cortex, tectal and cerebellar defects. They show deficiencies in assays of social recognition and interaction, as well as reductions in neonatal vocalizations (Searce-Levi et al., 2008). At this point, we can't directly ascribe these phenotypes to specific neuroanatomical lesions, although it seems very likely that the dorsomedial prefrontal hypoplasia contributes to the social deficits. However, given prefrontalthalamic-cerebellar connectivity (Dum and Strick, 2006), one can't dismiss the behavioral importance of the cerebellar deficit. Furthermore, the hypoplasia of the inferior colliculus could contribute to the vocalization deficits. In any case, a deficit in a single gene (*Fgf17*) disrupts functions of neural systems required for behaviors that are similar to those that are abnormal in ASD.

The analysis of mutations that reduce expression from *Fgf8* and *Fgf17* show that these genes promote growth of the cortex, and particularly of the dorsomedial frontal cortex – the region that shows the largest increase in size in ASD (Amaral et al., 2008). Thus, perhaps, there is over-activity of the FGF-signaling pathway in some forms of ASD. This could be due to several mechanisms, including increased expression of FGF ligands/receptors and/or increased signaling through the receptors and downstream transduction pathway. In this regard, mutation of several components that repress this pathway, including the PTEN phosphatase and TSC1/TSC2, are linked to ASD (see below). PTEN and TSC1/TSC2 probably regulate many steps in neural development and function, in addition to their known roles in neurite development and synaptic signaling.

Repressors of Fgf-Signaling

Given that Fgfs promote cortical growth, and evidence that the ASD brain is enlarged, it is important to investigate mechanisms that regulate Fgf signaling. In this regard, there are many negative regulators of this signaling pathway, including Sprouty1/2, TSC1/2 and PTEN; loss of function mutations in the latter genes are associated with ASD.

Fgf8 induces the expression of Sprouty1 and 2, inhibitors of receptor tyrosine kinase signaling through the MAP Kinase pathway (Fukuchi-Shimogori and Grove, 2001, 2003; Storm et al., 2003; Mason, 2007). This suggests that negative feedback may regulate the level of Fgf signaling in the developing telencephalon. Sprouty mutants have increased Fgf-signaling in the frontal and ventrolateral cortex that results in subtle increases in cortical surface area (Faedo et al., 2010). The *Spred1* protein is also a repressor of FGF-signaling (Ras-Map Kinase-Erk pathway), which reduces proliferation while maintain neuroepithelial properties (Phoenix and Temple, 2010).

TSC1/TSC2 are negative regulators of signaling downstream of receptor tyrosine kinases such as EGFs, FGFs, IGFs, neurotrophins (Inoki et al., 2005). These signals activate a family of phosphatidylinositol lipid kinases (phosphatidylinositol-3 kinases, PI3K) that in turn activate the serine-threonine kinase AKT, which then represses TSC1/TSC2 (Inoki et al., 2005). TSC1/TSC2 are also regulated by intracellular amino acids and by the ATP/AMP ratio – the end product of this regulation is to promote appropriate levels of protein synthesis and cell size (Inoki et al., 2005).

TSC1 (hamartin, 9q34) & TSC2 (tuberin, 16p13) encode GTPase-activating proteins that inhibit the activity of the small G-protein Rheb. TSC1/TSC2 are tumor suppressors, because they repress growth responses through reducing activity of mTOR kinase (Inoki et al., 2005). mTOR promotes protein synthesis and other processes that increase cell growth.

Children with TSC (an autosomal dominant disorder) have greatly increased rates of autism (25-50%), epilepsy and mental retardation (Wiznitzer, 2004). While TSC patients develop focal CNS lesions (tubers), it is likely that the general function of TSC1&2 in most/all neurons underlies the ASD symptoms. For example, reduced TSC dosage in hippocampal pyramidal neurons results in increased size of the cell body and dendritic spines (Tavazoie et al., 2005) – this is intriguing given the increased size of the brain in some children with ASD. It is formally possible that reduced TSC dosage also leads to increased Fgf-signaling in the cortical neuroepithelium, and thus can lead to increased cortical size.

The PTEN phosphatase reduces activity of the PI3K pathway through dephosphorylation of phosphatidylinositol-tris-phosphate. Mice lacking CNS function of PTEN have increased signaling through the AKT, TSC and mTOR pathway (Kwon et al., 2006). These mutants have enlarged brains that are associated with increased dendritic and axonal arbors and increased dendritic spines and synapses. PTEN mutant mice also exhibit abnormal social behavior, further implicating this signaling pathway in ASD (Kwon et al., 2006). Some patients with mutations in the phosphatidylinositol phosphatase (PTEN; 10q23.31) have ASD with macrocephaly (Butler et al., 2005).

Transcription Factors that Regulate Cortical Patterning

Fgf8/17 and Fgf15 have opposite effects on the expression of transcription factors that are expressed in cortical progenitors. These transcription factors are expressed in spatial gradients along the neuroepithelium, and several of these genes, including COUP-TF1, Emx2 and Pax6, have major roles in regulating the size and nature of cortical regions (reviewed in O'Leary et al., 2007; see below).

COUP-TF1 (Nr2f1) is an orphan nuclear receptor protein that is expressed in bi-dimensional gradients: high caudoventral and low rostradorsal. We investigated the effects of changing these gradients (Faedo et al., 2008). In our study, which employed both gain-of-function and loss-of-function mutants, we provided evidence that COUP-TF1 coordinates cortical patterning, neurogenesis, and laminar fate through modulation of MAPK/ERK, AKT, and beta-catenin signaling (Faedo et al., 2008).

Using an enhancer to drive increased COUP-TF1 expression in rostral and dorsal cortical progenitors, we found that COUP-TF1 promotes caudal and ventral fates, respectively, in these regions. Loss-of-function results also support the model that COUP-TF1 has a major role in specifying caudal and ventral regional fate within the developing cortex (Armentano et al., 2007; Faedo et al., 2008). Indeed Armentano et al. (2007) demonstrated that COUP-TF1 promotes sensory and represses frontal/motor neocortical areas.

COUP-TF1 also promotes the differentiation of cortical progenitors into neurons (Faedo et al., 2008). Over-expression suppresses the number of progenitor cells (loss of function has the opposite phenotype), and increases the number of early-born neurons. Of note, the ventral-to-dorsal pattern of COUP-TF1 expression matches the gradient of neurogenesis; thus COUP-TF1, through promoting cell cycle exit, appears to coordinate cortical patterning with neurogenesis. COUP-TF1 also regulates laminar fate, promoting ER81⁺ and repressing Fezl⁺/p75⁺ layer 5 neurons (Faedo et al., 2008).

There is evidence that COUP-TF1 coordinates patterning, neurogenesis and neuronal fate by repressing the receptor tyrosine kinase (Fgf) and Wnt pathways (Faedo et al., 2008). Repression of Fgf and Wnt signaling could underlie COUP-TF1 repression of proliferation. Furthermore, repression of Fgf signaling could be a mechanism whereby COUP-TF1 represses rostral cortical identity; indeed over-expression of COUP-TF1 induces Sprouty (Faedo et al., 2010). Therefore, we postulate that Fgf8/17 and Fgf15 regulation of COUP-

TF1 expression are central processes in regulating cortical regional specification, proliferation and differentiation.

Emx2 is a homeodomain protein that is expressed in high-caudodorsal to low-rostroventral gradients in the cortical primordium (O'Leary et al., 2007). Emx2 regulates neocortical arealization in a direction opposite to Fgf8. Emx2^{-/-} mice have reduced caudal and expanded rostral cortical areas (Bishop et al., 2000; Mallamaci et al., 2000; Muzio et al., 2002a,b), defects that are rescued by reducing Fgf signaling (Fukuchi-Shimogori and Grove, 2003). By contrast, over-expression of Emx2 in neural progenitors results in expanded caudal and reduced rostral areas, despite normal Fgf8 expression (Hamasaki et al., 2004). Therefore, Emx2 may regulate cortical arealization both by repressing Fgf8 signaling and/or by direct specification of neural progenitors (Fukuchi-Shimogori and Grove, 2003; Hamasaki et al., 2004).

Pax6 is a paired-homeodomain protein that is expressed in a high-rostroventral to low caudodorsal gradient in the cortical primordium (Bishop et al., 2000; 2002; Yun et al., 2001). Pax6^{-/-} mice show cortical patterning changes: the ventral cortex takes on subpallial properties (Yun et al., 2001; Stenman et al., 2003) and the rostral cortex is caudalized (Bishop et al., 2000; 2002; Piñon et al., 2008). Surprisingly, mild overexpression of Pax6 does not seem to alter cortical patterning (Manuel et al., 2007). Both loss- and gain-of-function experiments show that Pax6 has a potent role in regulating the balance of proliferation and differentiation (Arai et al., 2005; Manuel et al., 2007; Berger et al., 2007; Sansom et al., 2009).

Sp8 is a zinc finger protein that is expressed in a dorsal domain that extends from the rostral to caudal cortex (Storm et al., 2006; Sahara et al., 2007; Zembrzycki et al., 2007). Sp8 conditional null mutants show reduced Pax6 expression and increased Emx2 expression, providing evidence that Sp8 promotes rostral properties. Of note, Fgf8 RPC expression at E10.5 appears normal in Sp8 mutants, whereas by E12.5 its expression in the septum is reduced (Zembrzycki et al., 2007); this consistent with the finding that Sp8 can positively regulate Fgf8 expression (Sahara et al., 2007). Lhx2 is a Lim homeodomain protein that has a potent role in specifying cortical identity. The cortex in Lhx2^{-/-} mutants takes on the fate of the dorsal patterning center (hem) and pallial-subpallial patterning center (antihem) (Monuki et al., 2001; Mangale et al., 2008). Furthermore, clones of Lhx2^{-/-} cells embedded in a wild type cortex become patterning centers that induce the adjacent cortex to have a hippocampal fate (Mangale et al., 2008). Early function of Lhx2 is required to specify the identity of the ventral-most cortical region (olfactory cortex) (Chou et al., 2009).

Development of Cortical Projection Neurons

Neocortical neurons are organized in roughly six layers; each layer has distinct connections within the cortex and subcortical structures (Molyneaux et al., 2007). There are two major types of cortical neurons; ~80% are excitatory projection neurons that use glutamate as their neurotransmitter; their axon extends long distances to its synaptic targets. ~20% are inhibitory local circuit neurons (interneurons), that use GABA as their neurotransmitter; generally their axon synapses on nearby neurons.

There are two general types of cortical projection neurons: corticocortical neurons and corticofugal neurons (Molyneaux et al., 2007). The corticocortical projection neurons send their axons ipsilaterally within the cortex, or contralaterally through the corpus callosum; these neurons are largely in layers 2 and 3. Corticofugal neurons also consist of two groups. Corticothalamic projection neurons reside in layer 6 and extend their axons into thalamus. Subcerebral projection neurons reside in layer 5, and extend their axons into the basal ganglia, diencephalon, midbrain, hindbrain, and spinal cord.

Cortical projection neurons are generated from the neocortical neuroepithelium (progenitor cells of the ventricular and subventricular zones; VZ and SVZ) (Rakic, 1998; Kriegstein et al., 2006; Hansen et al., 2010). There is evidence that retinoic acid (RA) has a key role in promoting cortical neuronogenesis (Siegenthaler et al., 2009). Asymmetric cell divisions from cortical primary progenitors (radial glia) in the VZ generate either an immature projection neuron, or a secondary progenitor (intermediate progenitor in the SVZ). Secondary progenitors also generate neurons. Once an immature neuron is generated, it migrates towards the pial surface along radial glial processes (Rakic, 1988; 2007), and then settles in specific cortical layers by sensing local molecular cues, such as the protein Reelin (Marin and Rubenstein, 2003).

The first cortical neurons to become postmitotic (to be born) migrate to the cortical preplate, which is split into the marginal zone (layer 1) and subplate by later arriving neurons of the cortical plate (Rakic 1988; 2007). Cortical plate neurons are generated in an inside-out pattern: layer 6 neurons are born first, followed by layer 5 neurons, and neurons of layers 4, 3 and 2 are born last.

Defects in migration can result in a dysmorphic brain (e.g. smooth cortex/lissencephaly or periventricular ectopias) that result in mental retardation and epilepsy. Several genetic defects cause these disorders, including mutations in *Lis1* and *doublecortin* (Mochida and Walsh, 2004). It is possible that subtle manifestations of these mutations could result in less severe neuropsychiatric disorders.

Molecular mechanisms that generate specific types of cortical projection neurons are beginning to be established. The *Sox5* and *Tbr1* transcription factors are required for the development of corticothalamic layer 6 neurons (Hevner et al., 2001; Kwan et al., 2008; Lai et al., 2008). *FEZF2* and *CTIP2*, two transcription factors expressed in deep-layer neurons, regulate the identity and differentiation of subcerebral projection neurons and the corticospinal tract (CST) (Arlotta et al., 2005; Chen et al., 2005a; Chen et al., 2008; Chen et al., 2005b; Molyneaux et al., 2005). Development of projection neurons in superficial layers require *SATB2*, an AT-rich DNA binding protein, that is regulates the identity of callosal projection neurons (Alcamo et al., 2008; Britanova et al., 2008). Defects in the generation, differentiation and wiring of cortical projection neurons have important impacts on cognition (Paul et al., 2007; Kumar et al., 2009).

Development of Cortical Local Circuit Neurons

Unlike neocortical projection neurons, most neocortical local circuit neurons are largely generated outside of the cortex, in the primordium of the basal ganglia called the ganglionic eminences. The immature interneurons then tangentially migrate into the immature cortical plate. In Humans, unlike in other species, there is evidence for the generation of local circuit neurons within the cortex (Letinic et al., 2002).

There are five ganglionic eminences: caudal, lateral, medial, preoptic and septal (CGE, LGE, MGE, PGE and SGE). The ganglionic eminences generate both GABAergic projection neurons (e.g. striatum and globus pallidus) and GABAergic local circuit neurons. Most of the GABAergic local circuit neurons tangentially migrate from the ganglionic eminences to the cortex, although some remain in the basal ganglia (e.g. striatal interneurons). The MGE also produces cholinergic neurons of the septum, nucleus basalis and striatum.

As in the cortex, the neuroepithelium consists of progenitor cells in the ventricular and subventricular zones (VZ and SVZ). CGE, LGE, MGE, PGE and SGE are each specified by distinct combinations of transcription factors which further subdivide these structures into

smaller domains that are implicated in generating distinct subtypes of neurons and glia (Flames et al., 2007). Below, I will introduce some of the key transcription factors that regulate the development of cells generated by the ganglionic eminences, which prominently include cortical interneurons.

Dlx Genes – Central Regulators of Forebrain Inhibitory Neurons

The Dlx homeobox transcription factors are broadly expressed prenatally in progenitors of forebrain GABAergic neurons, as well as postnatally in subsets of mature GABAergic neurons, such as in cortical interneurons (Cobos et al., 2005a, 2006). Mutations that simultaneously block the function of pairs of mouse Dlx genes (Dlx1&2) disrupt development (particularly migration and differentiation) of most forebrain GABAergic neurons (including cortical interneurons, projection neurons of the striatum, pallidum, central nucleus of the amygdala, and the reticular nucleus of the thalamus) (Anderson *et al.*, 1997a,b; Long et al., 2008). Such mutations have the potential to disrupt function within these regions in addition to the communication between the neocortex, basal ganglia and thalamus with obvious detrimental affects on cognitive and emotional functions (Fanselow and Poulos 2005; Yin and Knowlton, 2006; LeDoux, 2007).

While mice lacking pairs of Dlx genes die neonatally, mice lacking Dlx1 are viable. However, after ~1 month, there is a selective degeneration of a subset of cortical interneurons, that results in epilepsy (Cobos et al., 2005a). Thus, Dlx1^{-/-} mutants have an age-dependent onset of seizures analogous to a subset of ASD patients who have late onset of epilepsy (Levisohn, 2007). Furthermore, Dlx genes regulate craniofacial morphogenesis, including the ossicles (Qiu et al., 1995; Jeong et al., 2008); as a result, Dlx1 mutants have reduced hearing acuity (Polley et al., 2006), which has obvious implications for auditory comprehension. This result also brings up an important point. Many transcription factors that control brain development also have important roles in regulating craniofacial development, suggesting that analysis of craniofacial morphology may provide insights into the etiologies of ASD.

While mutations in the Dlx2 and Dlx5 genes have been detected in some autistic individuals, it is unknown whether these contribute to the development of ASD (Hamilton *et al.*, 2005), or co-morbid symptoms such as epilepsy. Despite the lack of firm evidence implicating Dlx mutations genes in ASD susceptibility, examining the function of the Dlx genes is illustrative of a genetic pathway, whose dysfunction, could predispose to ASD, through defects in forebrain inhibitory neurons (Rubenstein and Merzenich, 2003). This could be through Dlx function in the neocortex and/or basal ganglia (including the amygdala). For instance, in the cortex, reduced Dlx dosage (function) would weaken inhibitory tone in the cortex, thereby increasing the ratio of excitation/inhibition, which would decrease the signal/noise ratio, altering neural processing and predisposing to epilepsy. In the basal ganglia, Dlx mutations could alter development of the striatum and pallidum – key components of the cortico-basal ganglia-thalamic circuit that is important in controlling Pavlovian (appetitive) learning, habit learning, and goal directed behaviors (Yin and Knowlton, 2006). Thus, understanding the genetic circuits downstream of the Dlx genes will identify genes required for forebrain inhibitory neuronal function – perhaps many of these, alone or in combination with other genes – are susceptibility factors for ASD.

Currently, the Dlx genes are known to (directly or indirectly) regulate the expression of large numbers of genes that are implicated in GABAergic neuronal development. For example, the Arx transcription factor is downstream of Dlx (Cobos et al., 2005b; Colasante et al., 2008; Long et al., 2009a,b). Mutation of Human Arx can cause epilepsy and autism, and in mice results in defects in cortical interneuron development (Colombo et al., 2007).

Dlx genes also promote the expression of glutamic acid decarboxylase and vesicular GABA transporter (Stuhmer et al., 2002; Long et al., 2007, 2009a,b), and thereby can regulate inhibitory tone. Furthermore, reduced Dlx dosage is associated with reduced synapse formation and reduced expression of neuroligin3, a neuroligin ligand (Cobos, Long and Rubenstein, unpublished). Dlx repression of the Pak3 kinase expression is implicated in regulating neurite growth (Cobos et al., 2007); Human Pak3 mutants have mental retardation (van Galen and Ramakers, 2005). Finally, Dlx genes promote the balance of neuronal vs oligodendrocyte production; reduced Dlx function can reduce the number of GABAergic neurons while perhaps altering the extent of myelination (Petryniak et al., 2007).

In sum, the Dlx genes can regulate the development and function of a single generic class of neurons: forebrain inhibitory neurons (e.g. interneurons in the cortex, and projection neurons in the basal ganglia). As such, alterations in the function of the Dlx genes, or of genes downstream of them, can weaken forebrain inhibitory tone, and thereby impact neural systems that underlie cognition.

Transcriptional control of development of MGE derivatives, including cortical interneurons

While the Dlx genes are core regulators of basal ganglia (CGE, LGE, MGE, PGE and SGE) development, other transcription factors have more specific functions. For instance MGE development is dependent on a gene expression program controlled by Nkx2.1, Lhx6, Lhx7(8) and Sox6. The MGE is the progenitor domain that generates projection neurons of the globus pallidus and interneurons of the striatum and pallidum (Wonders and Anderson, 2006; Batista-Brito R et al., 2009; Flandin et al., 2010). MGE-derived interneurons include the parvalbumin⁺ neurons that innervate the cell body of cortical projection neurons. These interneurons control progression through the critical period of ocular dominance plasticity (Hensch, 2005), and their numbers appear reduced in schizophrenia (Lewis and Hashimoto, 2007). The MGE also generates somatostatin⁺ interneurons that innervate the dendrites of cortical projection neurons.

Initial patterning of the MGE depends on the Nkx2.1 (Sussel et al., 1999), which leads to the expression of a cascade of transcription factors [e.g. Lhx6, Lhx(8) and Sox6] and secreted proteins (e.g. Shh) that promotes cell type specification and differentiation (Sussel et al., 1999; Azim et al., 2009; Batista-Brito et al., 2009; Flandin et al., 2010; Xu et al., 2010). Shh expression during gastrulation is required for Nkx2.1 expression in the forebrain, which in turn is required to induce Shh expression in the telencephalon (Sussel et al., 1999). Later, Shh expression in the telencephalon helps maintain Nkx2.1 expression and interneuron development (Xu et al., 2010; Flandin and Rubenstein, unpublished).

The Lhx6 and Lhx7(8) LIM-Homeodomain transcription factors have very similar expression patterns from the earliest stages of MGE development and both continue to be expressed in subsets of neurons such as in the globus pallidus (GP). However, only Lhx6 expression is detectable in tangentially migrating cells to the cortex, where it continues in parvalbumin⁺ and somatostatin⁺ interneurons (Cobos et al., 2007). Mice with reduced Lhx6 expression have a reduced rate of interneuron tangential migration (Alifragis et al., 2004; Liodis et al., 2007; Zhao et al., 2008). Lhx6 null mutants have reduced numbers of cortical interneurons, and almost none expressing somatostatin and parvalbumin (Liodis et al., 2007; Zhao et al., 2008). Lhx7(8) mutants have a more restricted phenotype, which is largely associated with reduced numbers of cholinergic neurons (Zhao et al., 2003; Fragkouli et al., 2009). Lhx6/Lhx7(8) double mutants fail to express Shh in MGE early born neurons, which in turn leads to defects in interneuron production (Flandin, Zhao and Rubenstein, unpublished). Sox6 also directs the development of MGE-derived interneurons; Sox6

mutants have reduced numbers of parvalbumin⁺ and somatostatin⁺ and increased numbers of neuropeptide Y interneurons; they also have epilepsy (Azim et al., 2009; Batista-Brito et al., 2009).

Current efforts are aimed at elucidating developmental programs that regulate later stages of interneuron development, particularly as they relate to their integration into cortical circuits and modulation of cortical plasticity. In this regard, it is clear that the activity of interneurons, particularly parvalbumin⁺ basket cells, have a central role in regulating the maturation of cortical circuits, including the opening and closing of critical periods (e.g. ocular dominance) (Hensch, 2005). Furthermore, there is evidence that transplantation of immature interneurons into the postnatal rodent cortex can have therapeutic effects on seizures (Baraban et al., 2009), further supporting the role of this cell type in regulating brain plasticity, and providing evidence that cell replacement therapies may someday have practical uses in Humans.

Four Neurodevelopmental Models of Childhood Neuropsychiatric Disorders

Neuropsychiatric disorders such as ASD can arise through many mechanisms. Below I highlight four possible models that are based on cortical neurodevelopmental processes described in this article.

1. Detection of Salient Synaptic Information - Signal/Noise Processing

Molecular lesions that alter the development and/or function of excitatory, inhibitory and neuromodulatory synapses can disrupt neural systems that process cognition and social behaviors. For instance, mutations that alter the balance of excitatory and inhibitory synaptic function may impede the ability to detect salient sensory signals above ambient noise, affect maturation of cortical regions (Hensch, 2005), and have been postulated to contribute to some forms of ASD (Rubenstein & Merzenich, 2003; Rubenstein, 2010). For instance, defects that alter the ratio and/or function of cortical excitatory and inhibitory neurons could result in excitatory/inhibitory imbalance. An example of this is selective interneuron apoptosis (death) in *Dlx1*^{-/-} mutant mice, that leads to reduced cortical/hippocampal inhibition and to epilepsy (Cobos et al., 2005a).

The high prevalence of epilepsy in ASD suggests an increase in the excitatory/inhibitory balance. However, current evidence from some mouse models of ASD provides evidence for decreased excitatory/inhibitory balance. For instance, female mice lacking one copy of *MeCP2* (the gene responsible for Rett Syndrome) show reduced synaptic excitation (Dani et al., 2005). Likewise, mice expressing a human allele of *neuroligin3* found in an autistic individual, showed increased synaptic inhibition (Tabuchi et al., 2007). None-the-less, one would expect decreased signal-to-noise (reduced signal salience) in the cortical/hippocampal circuits of these mutants. Therefore, there is good reason to continue exploring the model that disruption of excitatory/inhibitory balance, through multiple molecular/cellular/developmental mechanisms, contributes to ASD. Furthermore, many ASD susceptibility/causative genes regulate synapse formation, signaling and homeostasis (Walsh et al., 2008), and therefore have the potential to impact E:I balance. Furthermore, analyses of several mutant mice lines, which have mutations in genes that cause ASD (e.g. *Neuroligin3*, *Fmr1*, *MeCP2*), have cortical interneuron defects (Gogolla et al., 2009).

2. Cerebral Hyperplasia Secondary to Increased Fgf-Signaling

Neuropsychiatric disorders of childhood, such as ASD, have no simple or single neuroanatomical phenotype that points to obvious or consistent neurodevelopment mechanisms. However, structural neuroimaging and histological studies provide evidence for anatomical defects, at least in some individuals that include: 1) increased growth of the

cerebral cortex (~10%) during early childhood (years 1-3), with the largest effect in the frontal lobes; the growth rate then decreases; and 2) increased cerebellar size (~7%) in children under age 5, but decreased in older patients. One possible mechanism underlying these increases in cortical and cerebellar size in young children could be an increase in Fgf (and related) signaling, as this pathway is known to regulate the size of the cerebral and cerebellar hemispheres in vertebrates (see earlier sections of this article). For instance, the analysis of mutations that reduce expression from Fgf8 and Fgf17 show that these genes promote growth of the cortex, and particularly of the dorsomedial frontal cortex – the region that shows the largest increase in size in ASD (Amaral et al., 2008). Thus, perhaps, there is over-activity of the FGF-signaling pathway in some forms of ASD. This could be due to several mechanisms, including increased expression of FGF ligands and/or increased signaling through the receptors and downstream transduction pathway. In this regard, mutation of several components that repress this pathway, including the PTEN phosphatase and TSC1/TSC2, are linked to ASD. Thus, these genes could increase Fgf-signaling in neural progenitors, which in principle could lead to an increase in the frontal cortex/sensory cortex size ratio. (Fukuchi-Shimogori and Grove, 2001; Garel et al., 2003; Cholfen et al., 2007; Vaccarino et al. 2009, Faedo et al., 2010; Rubenstein, 2010). The behavioral ramifications of increasing the frontal/sensory surface area ratio are not known. In addition, increasing Fgf-signaling through mutations in PTEN and TSC1/TSC2 can regulate many other steps in neural development and function, including synaptic signaling, which would impact cognition.

3. Cortical-Basal Ganglia Circuits

Cortical basal ganglia circuits have central functions in learning and executing behaviors. These circuits play central roles in how animals and Humans learn complex cognitive and motor process (Yin and Knowlton, 2006), such as how birds learn to sing (Doupe et al., 2005).

Inroads into the genetic regulation of this pathway, and their roles in complex Human behaviors, have been made through analysis of FoxP2 function, a transcription factor that is expressed in the cortex and basal ganglia (developing and mature). Mutation of the FoxP2 transcription factor is implicated in human language disorders (Fisher and Scharff, 2009). It is likely that developmental lesions in cortical-basal ganglia circuits can disrupt language acquisition and generation in children. For this reason it is important to understand the anatomy and the development of components of this circuit,

The basal ganglia consist primarily of neurons in the striatum, accumbens, globus pallidus, ventral pallidum, and related parts of the extended amygdala. A simplified summary of this circuit is that glutamatergic inputs from the cortex excite GABAergic striatal projection neurons. There are two types of striatal projection neurons: those that express dopamine receptor 2 (DR2), and project to and inhibit the external globus pallidus (GPe; GABAergic) (indirect pathway); and those that express dopamine receptor 1 (DR1), project to and inhibit the internal globus pallidus (GPi; GABAergic) and the substantia nigra reticulata (GABAergic) (direct pathway). The GPe projects to and inhibits the subthalamic nucleus (glutamatergic), which in turn projects to and excites the GPi and substantia nigra reticulata. The GPi and substantia nigra reticulata project to and inhibit the thalamus (largely the MD, VL and VM nuclei)(glutamatergic), which project to the prefrontal and motor cortex.

The direct pathway activates the thalamus, whereas activation of the indirect pathway inhibits the thalamus. This part of the thalamus activates the prefrontal and motor cortex. Dopaminergic inputs from the substantia nigra pars compacta onto striatal neurons activate DR1⁺ and inhibit DR2⁺ neurons. In this way, dopamine signaling has a key role in

regulating the salience of the direct pathway and thereby activation of the thalamus and frontal cortex.

Several lines of evidence support the existence of three parallel cortical-basal ganglia networks that progressively control higher-order types of learning: Limbic, Associative and Sensorimotor (Yin and Knowlton, 2006). The limbic network regulates appetitive Pavlovian learning (stimulus outcome, S-O) that is fundamental to addictive behaviors underlying drug abuse. It includes projections from the ventral and orbital prefrontal cortex to the limbic striatum (accumbens) and pallidum (ventral pallidum). This system exerts influence on associative and sensorimotor networks.

The associative network controls the acquisition and performance of goal-directed actions and flexible choice behavior [action-outcome (A-O) learning] involved in working memory. These behaviors are modulated by reward expectancy and operate in conjunction with the dorsal hippocampus. In the course of habit formation, this network gives up control over behavior to the sensorimotor network. The core of this network includes projections from the dorsomedial prefrontal cortex to the associative striatum (caudate/dorsomedial) and associative pallidum.

The sensorimotor network controls inflexible response learning [stimulus response (S-R)]. These behaviors are related to movements and discriminative stimuli, and are not modulated by reward expectancy. The core of this network includes projections from the sensorimotor cortex to the putamen (dorsolateral striatum in rodents) and motor pallidum.

These three cortico-basal ganglia networks participate in controlling Pavlovian (appetitive) learning, habit learning, and goal directed behaviors. Whereas habit and goal directed behaviors are encoded in pathways that utilize the dorsal striatum and pallidum, Pavlovian (appetitive) behaviors engage the ventral striatum (nucleus accumbens) and ventral pallidum (substantia innominata) (Schoenbaum et al., 2006). The cortico-basal ganglia networks are integrated with other brain systems. For instance, the orbital cortex-accumbens network is regulated by the efferents from the hippocampus (Lisman and Grace, 2005).

Molecular and/or anatomical defects in these pathways may underlie susceptibility to several neuropsychiatric disorders. For instance, circuits involving the dorsal striatum (caudate/putamen) are implicated in Tourette's Disease and Schizophrenia (Seeman and Kapur, 2000; Saka and Graybiel, 2003; Kellendonk et al., 2006), whereas circuits involving the ventral striatum (accumbens) are implicated in drug addiction (Hyman et al., 2006; Schoenbaum et al., 2006). As noted above, these pathways are involved in the acquisition and use of language.

4. Male/female ratio

One of the great mysteries of ASD, and other neuropsychiatric disorders of childhood, such as Attention Deficit Disorder, is the roughly 4:1 ratio of affected boys to girls. This could arise because maleness may bias cognitive style, emotional response and instinctual behavior (Baron-Cohen et al., 2005; Rubenstein, 2010). It is possible that some males are more likely to have a highly focused cognitive style that is less subject to emotional influences, and are less interested in verbal communication, an idea akin to the extreme male brain model Autism (Baron-Cohen et al., 2005). This bias may sensitize the male brain to the effects of certain alleles or environmental factors, and thereby lead to ASDs in males more frequently than in females.

If this conception were correct, how would it arise? While there are several ASD-susceptibility genes on the X chromosome (e.g. Arx, Fmr1, MeCP2, Neuroligin3 and 4),

numerically this is not thought to account for the increased male prevalence of ASD. Thus, other models should be considered. Perhaps the simplest model is that male:female hormonal differences, such as brain concentrations of androgens and estrogens, account for the bias (androgens are converted to estrogens in the brain via aromatase; males have higher estrogen concentrations). These hormones are potent regulators of behavior. Furthermore, exposure to them during various stages of brain development have multiple effects, including regulating cell survival (which modulates the number of neurons in particular nuclei: sexually dimorphic nucleus of the preoptic area, and the anteroventral periventricular nucleus), and regulate neuronal connectivity and function (McCarthy, 2008). For instance, estrogens can regulate synapse numbers through controlling the numbers of dendritic spines in some hypothalamic nuclei (ventromedial nucleus and the arcuate nucleus). In addition estrogens appear to regulate the expression of glutamic acid decarboxylase in the arcuate nucleus of the hypothalamus, and thus can modulate GABA signaling (McCarthy, 2008). Estrogens also can modulate whether GABA is excitatory or inhibitory, via expression of the potassium-chloride co-transporter KCC2 (Galanopoulou, 2005). Finally androgens can predispose males to GABAA-mediated excitotoxicity (Nunez and McCarthy (2008). Therefore, sex steroids could modulate excitatory/inhibitory balance, which could sensitive the male brain to ASD. Perhaps one should consider attempting to devise a therapy for ASD that modulates sex-steroid signaling in males.

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Key Points

- Development of the cerebral cortex involves an intricate sequence of genetically regulated steps that is orchestrated by transcription factors.
- The nature and size of cortical regions is regulated by secreted molecules, such as Bmp, Fgf, RA, Shh and Wnt, often produced by patterning centers.
- Cortical excitatory projection neurons are produced by cortical neuroepithelial progenitors, whereas cortical inhibitory local circuit neurons are produced by subcortical neuroepithelial progenitors.
- Insights into childhood learning disorders, such as ASD, are gleaned through understanding the mechanisms of cortical development.

Fig. 1.

Forebrain Patterning Centers Legend: Schema showing the morphology of the embryonic mouse forebrain and patterning centers that express BMPs and WNTs (dorsal patterning center), FGFs (rostral patterning center, also known as the commissural plate, CP), and SHH (ventral patterning center). Other abbreviations: Cx: cortex, Hyp: hypothalamus, LGE: lateral ganglionic eminence; M: mesencephalon, MGE: medial ganglionic eminence.

Table 1

Definitions of Selected Terms

Name	Definition
Cortical Plate	Transient structure during development consisting of immature cortical neurons that accumulate following their migration from their origins in cortical (excitatory neurons) and subcortical (inhibitory neurons) progenitor zones. The cortical plate becomes the cortical grey matter.
Morphogen	Secreted molecule, such as Bmp, Fgf, Shh and Wnt, that regulates growth and identity of developing structures in a concentration dependent manner.
Patterning Center	Transient structure during development that controls growth and identity of adjacent regions by secreting morphogens.
Preplate	Transient structure during development consisting of the earliest produced immature cortical neurons that later split into the layer 1 (marginal zone) and subplate layers.
Receptor Tyrosine Kinase (RTK)	Plasma membrane protein that binds to protein ligands, and that transduces its signal into the cytoplasm through tyrosine phosphorylation. RTKs include Fgf and neurotrophin receptors.
Transcription factors	Proteins that regulate whether or not a gene is expressed (transcribed into mRNA).