

# TOWARD A DEVELOPMENTAL NEUROBIOLOGY OF AUTISM

Franck Polleux<sup>1\*</sup> and Jean M. Lauder<sup>2</sup>

<sup>1</sup>Department of Pharmacology–Neuroscience Center, School of Medicine, University of North Carolina–Chapel Hill, Chapel Hill, North Carolina

<sup>2</sup>Department of Cell and Developmental Biology, School of Medicine, University of North Carolina–Chapel Hill, Chapel Hill, North Carolina

Autism is a complex, behaviorally defined, developmental brain disorder with an estimated prevalence of 1 in 1,000. It is now clear that autism is not a disease, but a syndrome with a strong genetic component. The etiology of autism is poorly defined both at the cellular and the molecular levels. Based on the fact that seizure activity is frequently associated with autism and that abnormal evoked potentials have been observed in autistic individuals in response to tasks that require attention, several investigators have recently proposed that autism might be caused by an imbalance between excitation and inhibition in key neural systems including the cortex. Despite considerable ongoing effort toward the identification of chromosome regions affected in autism and the characterization of many potential gene candidates, only a few genes have been reproducibly shown to display specific mutations that segregate with autism, likely because of the complex polygenic nature of this syndrome. Among those, several candidate genes have been shown to control the early patterning and/or the late synaptic maturation of specific neuronal subpopulations controlling the balance between excitation and inhibition in the developing cortex and cerebellum. In the present article, we review our current understanding of the developmental mechanisms patterning the balance between excitation and inhibition in the context of the neurobiology of autism. © 2004 Wiley-Liss, Inc. MRDD Research Reviews 2004;10:303–317.

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Autism is a developmental brain disorder characterized by a general inability to form reciprocal social interactions, severe impairment in verbal and nonverbal communication, and a markedly restricted repertoire of activities and interests. The incidence of autism is currently estimated at 1 in 1,000 children [Folstein and Rosen-Sheidley, 2001]. Familial recurrence of the disorder is 100-fold higher than in the general population, and the concordance rate among monozygotic twins is estimated at between 70 and 90%, but close to 0% in dizygotic twins, thus indicating a strong genetic component to the disease [reviewed in Veenstra-Vanderweele et al., 2003; Veenstra-Vanderweele and Cook, 2004; see also Wassink et al., this issue]. Furthermore, the prevalence of autism and Asperger syndrome are, respectively, 4 and 8 times higher in males than females, strongly suggesting an X-linked genetic component.

The etiology of autism is poorly understood [Piven, 1997]. However, the amount and quality of research performed in several fields, including epidemiology, early brain imaging, and genetic identification of candidate chromosomal regions, has led to new hypotheses regarding possible etiologies [Stokstad, 2001; Rubenstein and Merzenich, 2003; Belmonte et al., 2004]. Interestingly, upon more detailed clinical examination, approximately 10% of autistic cases reveal association with other genetic neuropathologies, such as fragile X; tuberous sclerosis, and Rett syndrome. Several other clinical features are frequently associated with autism, such as mental retardation [roughly 70% of cases have an intelligence quotient (IQ) < 70], epileptic seizures (30%), and macrocephaly (larger head circumference) and megencephaly (larger brain volume) [Tuchman, 2003]. Approximately one-third of autistic individuals develop clinically apparent seizures and, of those, more than 50% develop “sharp-spike” activity during sleep when recorded by EEG or magnetoencephalography [Lewine et al., 1999; Ballaban-Gil and Tuchman, 2000]. Taken together, these observations have recently led several investigators to hypothesize that the cortex of autistic individuals is characterized by an imbalance between excitation and inhibition, leading to hyperexcitability and an unstable activity of cortical networks following normal sensory stimulation [Husman, 2001; Rubenstein and Merzenich, 2003; Belmonte et al., 2004].

Most interestingly, recent advances in functional imaging in humans, but also in nonhuman primates, have revealed that rhythmic synchronization of neural discharges in the gamma frequency band (20–60 Hz) may provide the necessary spatial and temporal links that bind together the processing in different brain areas to build a coherent percept [Tallon-Baudry and

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\*Correspondence to: F. Polleux. E-mail: polleux@med.unc.edu or Jean Lauder E-mail: unclaud@med.unc.edu

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Bertrand, 1999]. These results suggest that one particular type of gamma oscillation resulting from synchronized neuronal activity at the cortical and subcortical levels not only plays an essential role in sensory perception, but also in attention-based cognitive tasks. Importantly, neuronal synchrony in hippocampal, cortical, and thalamic networks has been shown to be critically dependent on the integrity of the discharge of interneurons in relation to the activity of the pyramidal neuron. In the hippocampus, synchronous gamma oscillation is considered to occur through the GABA<sub>A</sub> receptor-mediated mutual inhibition among interneurons [Buhl et al., 1994; Cobb et al., 1995; Freund and Buzsaki, 1996]. In the neocortex; it is known that a class of pyramidal neurons termed fast rhythmic bursting cells or “chattering” neurons synchronize their activities at gamma frequencies and are thought to be necessary for selective attention or binding processing in object recognition [Aoyagi et al., 2003]. Therefore, a slight disruption of the balance between excitation and inhibition in the cortex could have dramatic consequences on the function of the neuronal networks underlying perception and attention.

In a very recent study, the brain activation of a group of high-functioning autistic individuals was measured using functional MRI during sentence comprehension and the results were compared with those of a verbal IQ-matched control group [Just et al., 2004]. Their results show that the functional connectivity; i.e., the degree of synchronization/correlation of brain activation was consistently lower for the autistic individual than for the control participants in the main cortical areas involved in language processing [Just et al., 2004]. These findings suggest that the neural basis of disordered language in autism entails a lower degree of information integration and synchronization across the large-scale cortical network for language processing [Brock et al., 2002; Just et al., 2004].

Taken together, these results suggest that the autistic brain might be characterized by a synchronization deficit during the activation of cortical networks involved in language processing (and maybe in other sensory modalities or attention) and that this synchronization deficit could be the result of an imbalance between excitation and inhibition [Rubenstein and Merzenich, 2003; Belmonte et al., 2004]. Although still speculative, this hypothesis is attractive because it is based on functional studies. The present review will discuss why this new hypothesis is especially at-

tractive to describe the pathophysiology of the autistic brain in light of recent progress made in understanding the generation, migration, and differentiation of glutamatergic and GABAergic neurons in the cortex. We will also discuss the development of neuromodulatory systems well known to control the global levels of neuronal excitability in the forebrain, including the serotonin inputs that have been suspected for a long time to be altered in the autistic brain. Finally, we review the classes of genes that have been linked to autism in recent genetic studies and discuss several candidate genes in the context of this neurodevelopmental hypothesis.

## NEUROANATOMICAL ABNORMALITIES IN AUTISM

A few studies using either neuroanatomical examination of autopsied brains of autistic patients or, more recently, functional and structural MRI studies, have revealed three main types of defects in (1) the brainstem and cerebellum, (2) the limbic system (amygdala and hippocampus), and (3) the cortex [Courchesne, 1997].

### Brain Stem and Cerebellum

An early anatomical study first revealed that several brainstem nuclei were either missing or displayed strong neuronal loss, such as the facial motor nuclei or the superior olive nuclei [Rodier et al., 1996]. A longitudinal study performed on a large set of cases using quantitative structural imaging also revealed hypoplasia of brainstem structures [Hashimoto et al., 1995]; however, this set of data probably needs to be replicated to evaluate its validity in light of the progress made in diagnosis of autism.

One of the most reproducible neuroanatomical findings in autistic brain is the paucity of Purkinje neurons in the cerebellum [from 35 to 90%; reviewed by Courchesne, 1997]. An early MRI study reported a significantly smaller area of the cerebellar vermis in lobules VIII–X [Courchesne et al., 1988]. Recent MRI studies have also revealed a near 40% increase in cerebellar white matter volume in autistic children compared to age-matched controls [Courchesne et al., 2001]. Subsequent independent reports, however, have failed to replicate the original observation of hypoplasia of the neocerebellar vermis [Piven et al., 1997] and additional studies of the volume of cerebellar cortical volume will be necessary before final conclusions can be drawn about the size of this structure in autism.

### Hippocampus and Amygdala

An isolated report has revealed a decreased level of dendritic branching in the CA1 and CA4 regions of the hippocampus of two autistic patients compared to two control cases [Raymond et al., 1996]. Other studies have reported an increased neuronal packing density in the hippocampus, subiculum, mammillary bodies, entorhinal cortex, medial septal nuclei, and several nuclei of the amygdala [reviewed by Courchesne, 1997]; the neuronal networks playing a critical role in the formation, maintenance, and retrieval of memory. However, other studies have failed to reproduce the observed differences in the packing density of CA1–CA4 pyramidal neurons [Bailey et al., 1998].

Interestingly, a recent analysis of brain morphometric features was performed in a large sample of carefully diagnosed 3- to 4-year-old children with autism spectrum disorder (ASD) compared with age-matched control groups of typically developing (TD) children and developmentally delayed (DD) children [Sparks et al., 2002]. This analysis revealed increased cortical and cerebellar volume and also an increased volume of the amygdala in autistic children that cannot be accounted for by a simple increased cortical volume. This and another recent analysis [Schumann et al., 2004] suggest abnormal brain developmental processes affecting the amygdala early in the clinical course of autism.

### Brain Enlargement in Autism

Although the defining behavioral features of autism are present from the earliest ages and change over time with age, perhaps the most compelling biological argument for autism being a disorder of abnormal brain development comes from studies showing increased brain volume in this disorder. Kanner in his first descriptions of autism [Kanner, 1943] noted that 5 of 11 children he described had no obvious dysmorphic features, but did show enlarged head size. Over the years others described this anecdotally in studies of dysmorphology [Steg and Rapoport, 1975]. In 1992, Piven et al. reported enlarged midsagittal brain area [Piven et al., 1992]. A systematic study of 35 autistic adults and adolescents versus 36 controls, to follow up on this initial report, similarly reported enlarged brain volume and brain tissue volume [Piven et al., 1995]. More recently Courchesne et al. [2001] examined head circumference in a sample of 60 boys compared to a nonmentally retarded group of controls and reported

enlargement of cortical gray and white matter limited to those in the 2- to 4-year-old age group [Courchesne et al., 2001], with an actual decrease in cortical white matter in the older 6- to 16-year-old group. Increased white matter in the cerebellum was also noted in this younger age group (2–4 years) with decreased gray matter in the older age group. Findings by Sparks et al. [2002] support the finding of increased brain volume in 45 3- to 4-year olds with either autism or pervasive developmental disorder—not otherwise specified (PDD-NOS) compared to both typically developing and mentally retarded controls, noting increased volume of both the cerebral cortex and cerebellum as well as increased volume of the amygdalae [Sparks et al., 2002]. Finally; increased brain volume was found in a large sample of high-functioning (nonmentally retarded) autistic individuals under 12 years of age; but not in those over 12 years of age [Aylward et al., 2002]. Others have reported evidence to suggest enlargement of the caudate nucleus [Sears et al., 1999], amygdalae [Schumann et al., 2004], and hippocampus [Schumann et al., 2004], with a decrease in cross-sectional area of the corpus callosum [Piven et al., 1997; Manes et al., 1999; Hardan et al., 2000]. The findings from brain MRI studies are also consistent with systematic large scale surveys showing that approximately 20% of autistic individuals have macrocephaly (greater than the 98<sup>th</sup> percentile for head circumference) that appears not to be present at birth [Lainhart et al., 1997; Stevenson, 1997]. More recent preliminary evidence suggests that the increase in head circumference may have its origins in the latter part of the first year of postnatal life [Courchesne et al., 2001].

In summary these reports provide firm support for the idea that the brain in autism is enlarged. There is evidence that the enlargement occurs in both gray and white matter and occurs in selected regions and structures in the brain (with a decrease in the size of some other structures). There is also evidence to suggest that this enlargement occurs predominantly during early postnatal brain development. More specific conclusions about the nature of this phenomenon, including the exact timing or window of brain enlargement, patterns over time in brain structures, regions, and tissues, and clinical correlates, are not yet known.

### **Cortical Columnar Architecture**

The finding of brain enlargement occurring in the early postnatal period suggests a range of possibilities about its

pathogenesis. Importantly, this early difference in brain size is not found in adult autistic patients compared to age-matched control brains [Courchesne et al., 2003], suggesting that the initially accelerated rate of cortical growth is followed by an abnormally slower period of growth [see Piven et al., this issue]. As we will discuss later, this abnormally rapid growth rate detected in cortical grey and white matter during the first years of life in the frontal, temporal, and parietal lobes could reflect (1) an increase in dendritic branching/outgrowth and a correspondingly increased rate of synaptogenesis (grey matter) accompanied by an accelerated rate of axon myelination (white matter) and/or (2) a reduced elimination of aberrant connections (reduced dendritic/synaptic pruning). Recent work has demonstrated that, during development, there is a causal relationship between the number of active pre-synaptic afferent inputs and the size and level of branching of the corresponding dendritic field harboring postsynaptic densities [reviewed in Cline, 2001; see below]. Therefore, one attractive hypothesis for the pathogenesis of autism, and potentially other developmental disabilities, could be a dysregulation of the developmental mechanisms patterning axonal outgrowth and/or dendritic arborizations/synaptic contacts between excitatory and inhibitory neurons in the cortex [Zoghbi, 2003]. The behavioral consequences of an abnormal balance between excitatory and inhibitory neurotransmission in the cortex are only starting to be explored experimentally [Powell et al., 2003] and are likely to be specific and complex, especially if a subpopulation of cortical interneurons is specifically reduced or absent as it has been proposed for schizophrenia [Lewis and Levitt, 2002].

A recent study by Casanova et al. [2002] has provided evidence for abnormal patterning of minicolumns in the frontal and temporal lobes of autistic patients. Interestingly, in humans, cortical minicolumns are the cellular manifestation of the basic columnar organization of excitatory and inhibitory neurons into canonical functional units (Fig. 1; [DeFelipe et al., 1990; Peters, 1994; Mountcastle, 1997; Jones, 2000]). Functional and anatomical investigations have enabled an accurate definition of the cellular composition of these minicolumns. Each column contains an array of pyramidal neurons in layers 5 and 2/3, characterized by bundled apical dendrites reaching layer 1 and pyramidal neurons in layer 6 with their bundled apical den-

drites reaching layer 4B (Fig. 1A). The second important component of minicolumns is GABAergic interneurons providing powerful and specific inhibitory control in cortical neuronal networks (Fig. 1B). Cortical interneurons can be classified based on morphological and functional criteria into four main classes, each characterized by a precise synaptic targeting of a specific part of the pyramidal neuron: (1) large and small basket cells, respectively, making their synaptic contacts onto the cell soma and proximal part of the apical dendrite of pyramidal neurons within the same layer; (2) double bouquet cells making their synaptic contacts onto more distal parts of the basal dendrites of pyramidal neurons and (3) Chandelier cells making synapses along the initial axonal segment of pyramidal neurons; and (4) Cajal-Retzius interneurons found in layer I making synapses onto the distal part of apical dendrites of cortical pyramidal neurons [reviewed in DeFelipe et al., 1990]. Based on this specialized synaptic targeting, each of these interneuronal subpopulations is thought to play a specific functional role in the control of synaptic integration, and therefore, on the propagation of information through cortical networks [Freund, 2003]. Disorganization of minicolumns could reflect distinct aspects of an abnormal cortical cytoarchitecture, including: (1) a disrupted laminar distribution of a specific subclass of interneurons and/or (2) a numerical imbalance between pyramidal neurons and interneurons and/or (3) a disrupted level of branching of the axons and dendrites of both pyramidal neurons and interneurons. Future investigations are required to characterize the number, laminar distribution, and morphology of specific classes of interneurons in the autistic cortex to assess whether this disorder could be due to the abnormal patterning of a subpopulation of GABAergic neurons.

Interestingly, an early study by Bailey et al. [1998] carefully assessing the clinicopathology of six carefully diagnosed autistic individuals revealed several abnormalities that suggest abnormal migration of pyramidal neurons and/or patterning of their dendrite outgrowth. In four of six cases, postmortem examination of the frontal cortex revealed (1) the presence of ectopic neurons in the white matter and in layer 1 as well as (2) mis-oriented pyramidal neurons in layer 5 with their apical dendrite not oriented toward layer 1 as in normal cortex and (3) a disorganized cellular organization in supragranular layers of areas located in the superior temporal gyrus [Bailey et al.,

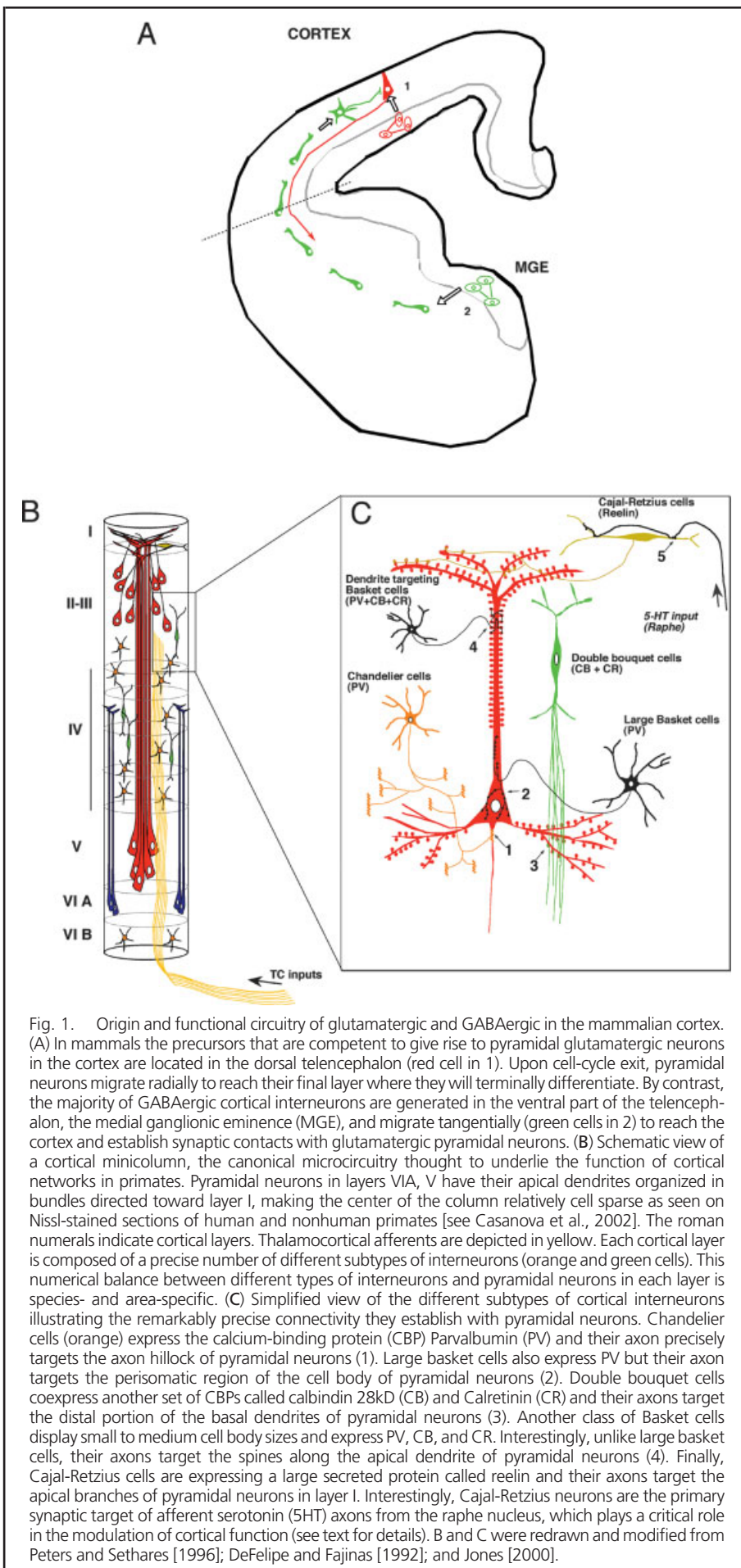


Fig. 1. Origin and functional circuitry of glutamatergic and GABAergic in the mammalian cortex. (A) In mammals the precursors that are competent to give rise to pyramidal glutamatergic neurons in the cortex are located in the dorsal telencephalon (red cell in 1). Upon cell-cycle exit, pyramidal neurons migrate radially to reach their final layer where they will terminally differentiate. By contrast, the majority of GABAergic cortical interneurons are generated in the ventral part of the telencephalon, the medial ganglionic eminence (MGE), and migrate tangentially (green cells in 2) to reach the cortex and establish synaptic contacts with glutamatergic pyramidal neurons. (B) Schematic view of a cortical minicolumn, the canonical microcircuitry thought to underlie the function of cortical networks in primates. Pyramidal neurons in layers VIA, V have their apical dendrites organized in bundles directed toward layer I, making the center of the column relatively cell sparse as seen on Nissl-stained sections of human and nonhuman primates [see Casanova et al., 2002]. The roman numerals indicate cortical layers. Thalamocortical afferents are depicted in yellow. Each cortical layer is composed of a precise number of different subtypes of interneurons (orange and green cells). This numerical balance between different types of interneurons and pyramidal neurons in each layer is species- and area-specific. (C) Simplified view of the different subtypes of cortical interneurons illustrating the remarkably precise connectivity they establish with pyramidal neurons. Chandelier cells (orange) express the calcium-binding protein (CBP) Parvalbumin (PV) and their axon precisely targets the axon hillock of pyramidal neurons (1). Large basket cells also express PV but their axon targets the perisomatic region of the cell body of pyramidal neurons (2). Double bouquet cells coexpress another set of CBPs called calbindin 28kD (CB) and Calretinin (CR) and their axons target the distal portion of the basal dendrites of pyramidal neurons (3). Another class of Basket cells display small to medium cell body sizes and express PV, CB, and CR. Interestingly, unlike large basket cells, their axons target the spines along the apical dendrite of pyramidal neurons (4). Finally, Cajal-Retzius cells are expressing a large secreted protein called reelin and their axons target the apical branches of pyramidal neurons in layer I. Interestingly, Cajal-Retzius neurons are the primary synaptic target of afferent serotonin (5HT) axons from the raphe nucleus, which plays a critical role in the modulation of cortical function (see text for details). B and C were redrawn and modified from Peters and Sethares [1996]; DeFelipe and Fajinas [1992]; and Jones [2000].

1998]. To our knowledge, this finding has never been reexamined or replicated but suggests the interesting possibility of a neuronal migration defect and/or dendrite patterning defect in the cortex of some autistic patients. This finding could also explain partially the reduced regularity of minicolumn spacing because, as explained above (Fig. 1), the spacing between the cell-body column is formed by tight fascicles of apical dendrites ascending to layer 1. Therefore, a defect in the orientation of apical dendrites might result in a disorganized columnar organization. Future investigations should be directed at testing how reproducible these findings are.

### MULTIPLE NEUROTRANSMITTER SYSTEMS CONTROLLING EXCITATION/INHIBITION RATIO MAY BE ABNORMAL IN AUTISM

Neurochemical features of autism include evidence of disrupted development of multiple neurotransmitter systems from functional brain imaging, neurochemistry, and clinical pharmacological studies [reviewed by Trotter et al., 1999; Korvatska et al., 2002]. So far, the strongest evidence implicates the glutamatergic and GABAergic and serotonergic systems, although weaker evidence also exists for changes in catecholaminergic, peptidergic, and cholinergic systems.

#### Glutamate

Contrasting hypotheses have been proposed to underlie autism; one current hypothesis is that autism is a hypoglutamatergic disorder [Purcell et al., 2001; Jamain et al., 2002; Serajee et al., 2003], whereas the other suggested a decreased level of inhibition, i.e., [Rubenstein and Merzenich, 2003; Belmonte et al., 2004]. It is difficult to reconcile these two opposite models, but both have strengths and weaknesses with regard to the neurobiology of autism reflecting the need for more accurate and direct investigation of the cortical cytoarchitecture in autism using cellular and molecular techniques. As mentioned previously, the hypothesis of a decreased GABAergic inhibition in specific cortical regions is attractive to explain the hyperexcitability of cortical tissue frequently associated with autism. Importantly, these two hypotheses are not totally incompatible: specific cortical regions could be characterized by an increased excitatory/inhibitory ratio whereas some other regions could show the reverse trend. The behavioral consequence of such a regional

imbalance will be important to assess using new genetic animal models [Levitt et al., 2004].

The reduction in global levels of glutamate signaling might occur by overactivation of excitatory receptors on cortical GABA interneurons, such as those of the 5-HT<sub>2A</sub> receptor subtype, leading to pronounced depression of the excitatory glutamatergic circuitry [Carlsson, 1998]. This possibility is supported by the relative efficacy of combined treatment with partial glutamate agonists and 5-HT<sub>2A</sub> receptor antagonists (e.g., risperidone) in autism as well as in hypoglutamatergic animal models [Carlsson et al., 1998, 1999; Nilsson et al., 2001]. Recent evidence also suggests involvement of polymorphisms in genes encoding both metabotropic and ionotropic glutamate receptors in autism [Jamain et al., 2002; Serajee et al., 2003]. Recently, a postmortem study has reported elevated expression of the glutamate transporter in autistic brain [Purcell et al., 2001].

### Developmental Origin of Glutamatergic Cortical Neurons

In mammals, the vast majority of glutamatergic pyramidal neurons are generated in the dorsal telencephalon by precursors in the ventricular and subventricular zones during embryonic development. In rodents, these neurons are generated during the second week of gestation (E11 to E17 in mouse or E13 to E19 in rat). In nonhuman primates, cortical neurons are generated from E40 and E100 approximately [Rakic, 1972]. In humans, the generation of cortical neurons is thought to occur between the 6<sup>th</sup> and the 13<sup>th</sup> week of gestation based on histological appearance of VZ and SVZ mitotic figures [reviewed in Sidman and Rakic, 1973]. Upon their last mitosis in the germinal pseudoepithelium lining the ventricles, glutamatergic neurons migrate along the radial glial scaffold to accumulate in the cortical plate in an inside-first outside-last manner, so that neurons in infragranular layers 6 and 5 are generated first, before neurons populating more superficial layers 4, 3, and 2 [Rakic, 1972].

Recent progress in the analysis of the genetic mechanisms underlying the generation of cortical neurons has revealed that cortical glutamatergic neurons are generated by dorsal precursors under the control of bHLH transcription factors, *Neurogenin1* and 2 (*Ngn1*, *Ngn2*) and as well as *Pax6* and *Emx2* [reviewed in Schuurmans and Guillemot, 2002]. Interestingly, *Ngn1* and *Ngn2* are involved in the specification of the glutamatergic neurotransmitter phenotype and single

and double knockout mice for *Ngn1/2* show a striking phenotype, where the vast majority of cortical neurons express GABA as a neurotransmitter instead of glutamate [Fode et al., 2000; Schuurmans et al., 2004].

Interestingly, in other parts of the nervous system, for example, the spinal cord, expression of a specific neurotransmitter is partially determined by a combinatorial expression of transcription factors [reviewed in Jessell, 2000]. However, a recent report demonstrates that the level of spontaneous activity and the frequency of intracellular Ca<sup>2+</sup> elevations in developing neural circuitry can have dramatic long-term consequences on the number of neurons expressing a given type of neurotransmitter [Borodinsky et al., 2004]. This important finding raises the possibility that transcription factors restrict the type of neurotransmitter that a neuronal precursor can express but that the level of spontaneous activity may play an important role in fine tuning the final number of neurons expressing a given neurotransmitter phenotype [Spitzer et al., 2004]. Therefore, the balance between the number of neurons expressing glutamate and GABA is probably the results of complex interactions between intrinsic (transcription factor expression) and extrinsic (spontaneous activity and calcium signaling) activity during development.

### GABA

As discussed earlier, another neurochemical abnormality hypothesized to explain the pathophysiology of autism is suppressed GABAergic inhibition [Husman, 2001; Rubenstein and Merzenich, 2003; Belmonte et al., 2004], which may be due, at least in part, to reduced expression of GAD65 and GAD67 [Fatemi et al., 2002], the two isoforms of the GABA biosynthetic enzyme glutamate decarboxylase. Reduced GABAergic neurotransmission may also be caused by deletional mutations in chromosome 15q11-q13, the locus of genes encoding subunits of the GABA<sub>A</sub> receptor (*GABRB3*, *GABRA5*, and *GABRG3*). In Angelman's syndrome (AS), such deletions lead to reduced expression of GABA<sub>A</sub>/BZD receptors [Holopainen et al., 2001a, 2001b; Luddens et al., 1995]. AS patients exhibit a behavioral phenotype consistent with compromised GABAergic inhibitory neurotransmission, including hyperactivity, hyperkinesis, seizures, and sleep disturbances as well as severe mental retardation, lack of motor coordination, craniofacial abnormalities, and autism [Holopainen et al., 2001a, 2001b]. As discussed in the pre-

vious section, much work needs to be done to explore (1) whether specific GABAergic neuron subpopulations are misplaced or absent in specific cortical regions, (2) whether there is a global numerical decrease of the number of GABAergic interneuron per unit of cortical microcolumns, or (3) whether the number and localization of cortical interneurons is normal in autism but characterized by a functional deficit in GABA expression at the synaptic level as recently shown in another neurodevelopmental pathology, schizophrenia [Lewis and Levitt, 2002].

### Developmental Origin of GABAergic Cortical Interneurons

In rodents, the vast majority of cortical interneurons are generated in approximately the same time window as cortical pyramidal neurons [E11 to E17 in the mouse; E13 to E19 in the rat; Miller, 1985, 1986]. However, cortical interneurons are generated by a distinct set of precursors located in the ventral telencephalon, more specifically in the medial and caudal parts of the ganglionic eminence [Fig. 1A; MGE and CGE, respectively; Anderson et al., 1997; Tamamaki et al., 1997; Lavdas et al., 1999; Wichterle et al., 2001; Nery et al., 2002; Polleux et al., 2002]. At the genetic level, the generation of GABAergic cortical interneurons is under the control of distinct transcription factors: the bHLH transcription factor *Mash1* and the homeodomain-containing transcription factors *Dlx1* and *Dlx2* [Anderson et al., 1997; Casarosa et al., 1999]. Interestingly, interneurons generated in the ventral telencephalon have to undergo a "long journey" to reach their dorsal destination in the cortex through a tangential mode of migration, which, by definition, does not involve fasciculation along the radial glial scaffold until they reach their final destination [Marin and Rubenstein, 2001].

Recently, experiments performed in human telencephalic tissue have shown that, unlike in rodents, a specific subset of dorsal progenitors seems to be competent to produce a subpopulation of cortical interneurons in a *Mash1*-dependent manner [Letinic et al., 2002]. This suggests that, in humans, a subpopulation of cortical dividing precursors are directly involved in the generation of more than 60% of cortical GABAergic interneurons. Although some differences might exist between the mode of generation of cortical interneurons in rodents and primates, the important concept emerging is that (1) specific sets of precursors along

the ventro-dorsal axis of the telencephalon are specialized with regard to their competence to generate glutamatergic or GABAergic cortical neurons and (2) they undergo a drastically different migration pathway to invade the cortex and to differentiate in the appropriate layer.

Interestingly, several extracellular cues have been found to control the motility of cortical interneurons migrating from the basal forebrain to the cortex. Among them are two types of ligands that activate receptor tyrosine kinases (RTK) expressed by migrating interneurons: hepatocyte growth factor (HGF), which activates the MET RTK, and NT4, which binds TRK [Powell et al., 2001; Polleux et al., 2002]. Mice carrying a targeted mutation of the gene encoding urokinase plasminogen activator (uPAR; a component of HGF activation) and knockout mice for *trkB* both show a reduced number of cortical interneurons compared to controls at late embryonic/early postnatal stages [Powell et al., 2001; Polleux et al., 2002]. Interestingly, uPAR knockout mice display a regional defect in which approximately 50% of calbindin-positive cortical interneurons are absent from frontal and parietal cortical areas [reviewed in Levitt et al., 2004]. The uPAR knockout mice display behavioral deficits ranging from scattered and frequent seizures and, more interestingly, increased anxiety-like behaviors [Powell et al., 2003]. The extent to which this mouse model recapitulates some behavioral traits characterizing autism remains to be explored, but this at least provides evidence that a mutation in a single gene controlling cortical interneuron migration and differentiation can have complex consequences on mouse behavior [Levitt et al., 2004]. In summary, another important concept regarding the link between interneuronal migration and the potential etiology of autism (and other neurodevelopmental disorders potentially) is the fact that this long-range migration of cortical interneurons (several millimeters in humans) might be more vulnerable than radial migration of pyramidal neurons to genetic or environmental alterations during early development because of the relative complexity of the cell-cell interactions involved in tangential migration [Marin and Rubenstein, 2001; Levitt et al., 2004].

### Serotonin

One feature of autism is high levels of serotonin (5-HT) in blood platelets [hyperserotonemia; Anderson et al., 1990]. To what extent such elevated

plasma levels reflect a change in central brain levels remains to be explored. Interestingly, polymorphisms in the 5-HT transporter (5-HTT) promoter have been proposed as an underlying cause of autism [Cook et al., 1997; Klauck et al., 1997]. This is presently a controversial topic, as several studies have recently provided conflicting evidence of a potential link between 5-HTT and autism [Anderson et al., 1990; Maestrini et al., 1999; Persico et al., 2000; Yirmiya et al., 2001; Betancur et al., 2002; Persico et al., 2002; Coutinho et al., 2004]. Interestingly, finding evidence for transmission of polymorphic alleles of the 5-HTT may depend on the extent of social and communication deficits in the autism proband, suggesting that the alleles may modify severity of autistic traits rather than conveying risk for autism per se [Tordjman et al., 2001]. Further support for 5-HTT comes from functional MRI studies showing that individuals with one or two copies of a short allele of the gene had greater neuronal activity than controls in the amygdala, a brain region implicated in autism [Hariri et al., 2002; Hariri and Weinberger, 2003]. In addition, multiple single polymorphisms in tryptophan 2,3 dioxygenase (TDO2), the rate-limiting enzyme in L-tryptophan catabolism, have been associated with autism. Such mutations could increase levels of 5-HT throughout the body and brain [Nabi et al., 2004].

The developing serotonergic system may be dysregulated in autism. Brain 5-HT synthesis is normally high in young children, followed by a gradual decline to adulthood. This dynamic is disrupted in autism, such that 5-HT levels are initially lower than normal, but gradually increase to a greater extent than adult levels by 2 to 15 years of age [Chugani et al., 1999; Chugani, 2002]. Positron emission tomography (PET) imaging with radiolabelled L-tryptophan has demonstrated asymmetric 5-HT synthesis in the dentate-thalamo-cortical pathway of autistic boys [Chugani et al., 1997; Chugani, 2002]. Consequences of elevated levels of 5-HT in the developing somatosensory system have been analyzed in a variety of animal models, providing evidence for disruption in the formation of thalamo-cortical sensory circuits [reviewed by Luo et al., 2003]. This is not all that surprising, since accumulated evidence shows 5-HT to be a critical regulator of key events in neural and glial development, including cell proliferation, differentiation, migration, apoptosis, and synaptogenesis [reviewed by Lauder, 1990, 1993; Whitaker-Azmitia, 2001].

Continued interest in the serotonergic system comes from the relative efficacy of serotonergic drugs in the symptomatic treatment of some cases of autism [Volkmar, 2001]. Pharmacological treatment studies in autism are complicated by various factors, including a tremendous range of syndrome expression, a lack of robust animal models of the disorder, and various methodological problems. Theories have tended to follow treatments, and various neurochemical systems have been the focus of study. Treatments developed are effective relative to certain disabling symptoms, but "core" problems (e.g., deficits in social relatedness and communication) appear less responsive to medications. Among these pharmacotherapies, especially in children and adolescents, include selective serotonin reuptake inhibitors (SSRIs), 5-HT<sub>2A</sub> receptor antagonists, tricyclic antidepressants, or mixed dopamine/5-HT receptor antagonists [Carlsson, 1998; Carlsson et al., 1999; McDougale and Posey, 2002; Veenstra-VanderWeele et al., 2000]. Although the reasons underlying the efficacy of these drugs is poorly understood, it is possible that such treatments may counteract developmental defects in serotonergic pathways and/or abnormal dynamics of 5-HT synthesis, catabolism, or transport.

Dysregulation of the developing serotonergic system could occur by various mechanisms, including mutations in genes encoding transcription factors involved in specification and patterning of 5-HT receptors or neurons (discussed below). Another possibility is that altered expression of genes regulating cholesterol biosynthetic or metabolic pathways could compromise functioning of key players in the serotonergic system, such as the 5-HTT [Scanlon et al., 2001], VMAT2, MAO-A, or 5-HT receptors [Hillbrand et al., 2000]. This possibility is supported by hypertrophic development of raphe 5-HT neurons in mice with targeted disruption of *Dhcr7*, a gene encoding the last enzyme in cholesterol biosynthesis, which is also mutated in Smith-Lemli-Opitz Syndrome [Waage-Baudet et al., 2003].

### Catecholamines

Evidence for possible involvement of dopamine and norepinephrine in autism comes from evidence of decreased activity of dopamine  $\beta$ -hydroxylase (DBH) and increased levels of norepinephrine in serum from autistic children and their parents [Lake et al., 1977] and changes in catecholamine metabolites in such children [Martineau et al., 1994].

Recently, the DBH gene on chromosome 9q34 has been investigated as a possible candidate locus using an affected sib-pair method. Although no association was found for polymorphic alleles in sib-pairs, there was a significantly higher frequency in mothers, raising the possibility that polymorphisms in DBH could increase the risk for having an autistic child [Robinson et al., 2001]. Recent evidence for maternal modifier effects (where the maternal genotype affects the fetal phenotype) at DBH and MAO-A loci [Jones et al., 2004] provides support for this idea and suggests changes in the interuterine environment as a possible risk factor. This is consistent with evidence that both tyrosine hydroxylase (TH) and DBH are required for prenatal development of mouse embryos [Thomas et al., 1995, 1998; Roffler-Tarlov and Rios, 2001; Portbury et al., 2003].

### Developmental Origin of Monoamine Neurons

In the vertebrate embryo, midbrain dopamine (DA) neurons, hindbrain 5-HT neurons of the raphe nuclei, and noradrenergic (NA) neurons of the locus coeruleus (LC) are generated adjacent to the midhindbrain boundary (MHB) or isthmus, an organizing center induced by networks of transcription factors and secreted signals [Ye et al., 2001]. DA neurons are generated rostral to the MHB, in response to intersecting signals, sonic hedgehog (Shh), from the floorplate, and Fgf8 from the MHB. 5-HT neurons form caudal to the MHB in response to the same two signals, but preceded by an earlier signal, Fgf4, from the primitive streak [Ye et al., 1998; Hynes and Rosenthal, 1999]. NA neurons of the LC are also generated caudal to the MHB, in response to signaling by Shh, Fgf8, and BMPs [Crossley et al., 1996; Morin et al., 1997; Guo et al., 1999; Vogel-Hopker and Rohrer, 2002; Jaszai et al., 2003; Lam et al., 2003; Eddison et al., 2004]. As discussed below, specification of neurotransmitter phenotypes of MA neurons requires a complex signaling through networks of transcription factors, which is only beginning to be elucidated [Briscoe et al., 1999; Pattyn et al., 1999, 2000, 2004]. However, it is already clear that these transcriptional networks must include *En1*, *En2*, *Wnt1*, and *Lmx1b* [Smidt et al., 2003].

### Acetylcholine

Since an early study reported evidence of neuropathology in basal forebrain of autistics, the location of cholinergic neurons innervating the cortex, this neuro-

transmitter system has received little attention in the field of autism. Recently, however, the forebrain cholinergic system has come under closer scrutiny, with several studies utilizing neurochemical, immunocytochemical, or molecular biological techniques, providing evidence of abnormal expression of cholinergic receptors in cortex and cerebellum in autism. The first study reported lower levels of muscarinic and nicotinic cholinergic receptors in parietal and frontal cortices, together with elevated levels of the trophic factor BDNF in basal forebrain [Perry et al., 2001]. A subsequent study found evidence for decreased ligand binding to  $\alpha 3/\alpha 4/\beta 2$  nicotinic receptors in cerebellum [Lee et al., 2002]. Recently, reduced expression of  $\alpha 4\beta 2$  nicotinic receptor subunits and receptor binding in parietal cortex of autistic brains, together with increased  $\alpha 7$  receptor binding in the cerebellum, was reported [Martin-Ruiz et al., 2004]. Because the level of evidence implicating the cholinergic circuitry in autism is still poor at the present time, we will not review in detail the mechanisms patterning development of this neurotransmitter system.

### CASE REVIEW OF SPECIFIC CANDIDATE GENES FOR AUTISM

The concept that emerges from analyzing the current literature is that autism obviously constitutes a very heterogeneous neuropathology at the genetic level, which probably means that there are many gene mutations that could independently lead to an "autistic" brain at the functional level. In Table 1A–C, we summarize all of the existing evidence from gene linkage and association studies for genes associated with autism. These tables represent candidate genes identified as confirmed targets in familial forms of autism as well as other genes that are simply candidates within genetic loci implicated in autism through linkage and association studies. In the following sections we perform a case review of several of the most interesting candidates classified into two categories: (1) genes involved in the early patterning of specific regions of the CNS or specific neuronal subpopulations and (2) genes involved in the synapses assembly of specific neuronal circuits.

### Genes Patterning the Central Nervous System (CNS)

#### *Engrailed and cerebellar neurodevelopmental defects*

Recently, association studies have provided evidence for *Engrailed-2* (*En2*) as

a susceptibility locus for autistic spectrum disorder [Gharani et al., 2004]. The highly conserved homeodomain-proteins ENGRAILED-1 and ENGRAILED-2 are specifically expressed in the MHB region during distinct periods of embryogenesis. This spatio-temporal pattern of *engrailed* gene expression coincides with the generation of cerebellar precursor cells, which has led to the suggestion that they may be important for establishing correct cell numbers in the cerebellum [Kuemmerle et al., 1997; Baader et al., 1998]. In support of this view; disruption of *En-1* in mice results in a deletion of the cerebellar anlage and midbrain [Wurst et al., 1994] and perinatal death of these mice. *En2* knockout mice are viable but show reduced cerebellar size and mildly abnormal foliation [Millen et al., 1994]. Given the likely functional redundancy of the two engrailed genes, *En1* may compensate for the loss of *En2*, thus explaining the mild phenotypic changes in *En2* knockout mice [Joyner et al., 1991], especially since *En2* is expressed in cerebellar Purkinje cells (PCs) throughout embryonic development but is down-regulated in these cells after birth.

#### *Engrailed and differentiation of dopaminergic neurons*

It has recently been shown that *En1* and *En2* are essential for the maintenance of DA neurons in the substantia nigra (SN) and ventral tegmentum (VT) [Simon et al., 2003]. In the mouse, both engrailed genes are expressed in mesencephalic DA (mDA) neurons from E11 to adulthood. In engrailed 1/2 double mutants, mDA neurons are normally induced and express several differentiation markers, but then fail to mature and are lost by E14.

The association study reporting *En2* as a susceptibility locus for autism spectrum disorder [Gharani et al., 2004], together with the phenotype observed in *En1/2* knockout mice [Simon et al., 2003], correlates well with the well-documented cerebellar phenotype in autism, including cerebellar hypoplasia and decreased number of PCs (see Neuroanatomical Abnormalities in Autism). Furthermore, the role of Engrailed in the patterning of dopaminergic neurons is also compatible with some findings implicating a dopaminergic defect in autism [Ernst et al., 1997].

#### *Wnt is a patterning gene also involved in activity-dependent dendritic development*

Several reports have provided evidence for *Wnt2* as a susceptibility gene for autism [Wassink et al., 2001; however, see also McCoy et al., 2002]. Sev-

**Table 1A. Autism Candidate Genes**

Marker	Symbol	Description	Chromo-	References
			somes X-2	
			Chromosome	
GDB:191610	MAOA	monoamine oxidase A	Xp11.4-p11.3	Cohen et al., 2003
Hs.438877	NLGN3	Neuroigin 3	Xq13.1	Jamain et al., 2003
KIAA1260	NLGN4	Neuroigin 4	Xp22.33	Jamain et al., 2003
PMC153509P1	AGTR2	angiotensin II receptor, type 2	Xq22-q23	Thomas et al., 1999; Vervoort et al., 2002
DXS7069	MECP2	methyl CpG binding protein 2 (Rett syndrome)	Xq28	Muhle et al., 2004; Longo et al., 2004
DXS1047	MRXS11	mental retardation, X-linked, syndromic 11	Xq26-q27	Liu et al., 2001; Shao et al., 2003
DXS6789	PTOS2	Ptois, hereditary congenital 2	Xq24-q27.1	Liu et al., 2001; Shao et al., 2003
DXS6789	CHDS3	Coronary heart disease, susceptibility to, 3	Xq23-q26	Liu et al., 2001; Shao et al., 2003
DXS6789	MTBS	Mycobacterium tuberculosis, susceptibility to infection by	Xq	Liu et al., 2001; Shao et al., 2003
D1S1675	GGAA20F08	NA (moderate effect)	1p12	Risch et al., 1999
D1S1656	SCZD9	schizophrenia disorder 9	1q21-1q22	Buxbaum et al., 2004; Risch et al., 1999
D1S534				
D1S2141	KCNK2	potassium channel, subfamily K, member 2	1q41	Buxbaum et al., 2004
G65049	DISC1	disrupted in schizophrenia 1	1q42.1	Buxbaum et al., 2004
D1S3462	SLEB1	systemic lupus erythematosus susceptibility 1	1q41-q42	Buxbaum et al., 2004
D1S547	KMO	kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	1q42-q44	Buxbaum et al., 2004
WI-20654	KCNK1	potassium channel, subfamily K, member 1	1q42-q43	Buxbaum et al., 2004
606058	cAMP-GEFII	cAMP-regulated guanine nucleotide exchange factor 1 (also mutations in TBR-1; GAD1; DLX1; DLX2; CHN1; ATF2; HOXD1, NEUROD1)	2q21-q33	Bacchelli et al., 2003
D2S142	PSDA	Phrase speech delay, autism-related	2q	Buxbaum et al., 2004
SHGC-82894	SCN2A2	sodium channel, voltage-gated, type II, alpha 2	2q23-q24	Weiss et al., 2003
RH79252	SCN3A	sodium channel, voltage-gated, type III, alpha	2q24	Weiss et al., 2003
A006O14	SCN1A	sodium channel, voltage-gated, type I, alpha	2q24.3	Weiss et al., 2003
D2S364	MPRM	Myopathy, proximal, with early respiratory muscle involvement (Edstr)	2q24-q31	Buxbaum et al., 2004
D2S2188	MMDK	Mesomelic dysplasia, Kantaputra type	2q24-q32	IMGSAC, 2001
D2S2716	CHRNA1	cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle)	2q24-q32	Agulhon et al., 1999; Martin-Ruiz, et al., 2004
G54269	AGC1	mitochondrial aspartate-glutamate carrier protein	2q24-q33	Ramos et al., 2004
G16421	INPP1	inositol polyphosphate-1-phosphatase	2q32	Serajee et al., 2003
RH68995	ERBB4	receptor for NDF/hereregulin	2q33.3-q34	Pescucci et al., 2003
RH66377	ADAM23	disintegrin and metalloproteinase domain 23	2q33	Pescucci et al., 2003
RH15615	KLF7	Kruppel-like factor 7	2q33.3-q34	Pescucci et al., 2003
RH65625	GPR1	G protein-coupled receptor 1	2q33.3	Pescucci et al., 2003
PMC16008P2	FZD5	frizzled homolog 5	2q33-q34	Pescucci et al., 2003
G62667	MAP2	microtubule-associated protein 2	2q34-q35	Pescucci et al., 2003
D2S2128	NRP2	neuropilin 2	2q33.3	Pescucci et al., 2003
D2S2634	CREB1	cAMP responsive element binding protein 1	2q34	Pescucci et al., 2003

eral mutations affecting the Wnt2 coding sequence were initially found to segregate with autism in families containing multiple affected individuals [Wassink et al., 2001].

The *Wingless-Int (Wnt)* genes encode a large family of cysteine-rich secreted glycoproteins that regulate diverse cell behaviors during embryonic development [reviewed in Wodarz and Nusse, 1998]. Nineteen *Wnt* genes have been identified in the murine and human genome. The secreted Wnt signals positively through the Frizzled family of receptors and can be antagonized by secreted Frizzled-related pro-

teins (sFRPs) as one of several unrelated protein families that negatively regulate receptor binding by Wnts.

In the developing mammalian brain, Wnts have been implicated in the patterning of the CNS along the antero-posterior axis, including patterning of the telencephalon, diencephalon, and mesencephalon. The signaling mechanisms underlying Wnt function during CNS patterning include many cytoplasmic proteins, including Dishevelled and  $\beta$ -catenin. Specifically, overexpression of  $\beta$ -catenin increases cortical size by positively regulating the generation of neural precursors [Chenn and Walsh,

2002], while loss-of-function mutations in individual Wnts cause deletions or malformations of distinct brain regions [Brault et al., 2001; Lyuksytova et al., 2003; Zhou et al., 2004]. Spatial and temporal expression patterns of WNT genes show striking similarity between human and mouse, suggesting that the developmental roles of these genes may be highly conserved [Grove et al., 1998; Abu-Khalil et al., 2004].

Recent investigation of the role of Wnt signaling during neuronal differentiation has revealed a surprising late role in the regulation of activity-

**Table 1B. Autism Candidate Genes**

Marker	Symbol	Description	Chromosomes 3-13	
			Chromosome	References
D3S3680	KIAA0121	KIAA0121 gene product	3p25.2	IMGSAC 2001
D3S3037	AUTS2	Autism, susceptibility to, 2	3q25-27	Auranen et al. 2002
D3S3037	AUTS3	Autism, susceptibility to, 3	3q25.1-3q25.2	Auranen et al. 2002
D3S2418	DFNA44; FGF12	deafness, autosomal dominant; fibroblast growth factor 12	3q28-29	Risch et al. 1999
D4S2366	HLN2	Huntington-like neurodegenerative disorder 2	4p15.3	Risch et al. 1999
D4S2366	SLEB3	systemic lupus erythematosus susceptibility 3	4p16-15.2	Risch et al. 1999
D4S2366	PPP2R2C	protein phosphatase 2 (2A), regulatory subunit B (PR 52), gamma isoform	4p16.1	Risch et al. 1999
SHGC-50349	GLRB	glycine receptor, beta	4q31.3	Ramanthan et al. 2004
PMC310777P9	NPY5R	neuropeptide Y receptor Y5	4q31-q32	Ramanthan et al. 2004
NPY1R_956	NPY1R	neuropeptide Y receptor Y1	4q31.3-q32	Ramanthan et al. 2004
D4S3293	TDO2	tryptophan 2,3-dioxygenase	4q31-q32	Nabi et al. 2004
SHGC-67298	GRIA2	glutamate receptor, ionotropic, AMPA 2	4q32-q33	Samanthan et al. 2004
GDB:373727	GRIK2	glutamate receptor, ionotropic, kainate 2 (GluR6 kainate receptor)	6q16.3-q21	Jamain et al. 2002
D6S283	IDDM15	insulin-dependent diabetes mellitus 15	6q21	Philippe et al. 1999
D7S640	AUTS1	autism susceptibility to, 1	7q	Ashley-Koch et al. 1999; Barrett et al. 1999; IMGSAC 2001
KIAA0442	AUTS2	autism, susceptibility to, 2	7q11.22-q11.23	Sultana et al. 2002
D7S2204	CMT2F	Charcot-Marie-Tooth disease, axonal, F	7q11-q21	IMGSAC 1998
D7S3120	RELN	reelin	7q22	Hutcheson et al. 2003
D7S501-D7S2847	FOXP2, SPCH1; WNT2; RAY1; NRCAM; SMO; CAV1,2; GPR22; CFTR; MEST/PEG1; MEK2; PAX4		7q22.1-q33	Hutcheson et al. 2003; IMGSAC 2001; Adres 2002
sWSS3754	GMR8	glutamate receptor, metabotropic 8	7q31-q33	Serajee et al. 2003
STS-X83368	PIK3CG	phosphoinositide-3-kinase, catalytic, gamma polypeptide	7q22.3	Serajee et al. 2003
D7S23	WNT2	wingless-type MMTV integration site family member 2	7q31	Wassinck et al. 2001
D7S684	CHDM	Chordoma (malignant tumors derived from notochordal remnants)	7q33	IMGSAC 1998
D7S495	OTSC2	otosclerosis 2 (sclerosis of labyrinthine capsule; hearing impairment)	7q34-q36	Shao et al. 2003
RH102827	EN2	engrailed homolog 2	7q36	Gharani et al. 2004
D9S1826	SPG19	spastic paraplegia 19 (autosomal dominant)	9q	IMGSAC 2001
GDB:551079	DBH	dopamine beta-hydroxylase	9q34	Robinson et al. 2001
D9S158	JBTS1	Joubert syndrome 1	9q34.3	IMGSAC 2001
D9S1826	DFNB33	deafness, autosomal recessive 33	9q34-qtel	IMGSAC 2001
D11S2371	SCZD2	schizophrenia disorder 2	11q14-q21	Risch et al. 1999
D12S1901	AVPR1A	arginine vasopressin receptor 1A	12q14-q15	Wassinck et al. 2004
608049	AUTS3	Autism, susceptibility to, 3	13q14-q22	Bradford et al. 2001
HTR2A-112F	HTR2A-2	5-hydroxytryptamine (serotonin) receptor 2A	13q14-q21	Andres et al. 2002; Veenstra-VanderWeele et al. 2002
D13S779	SCZD7	schizophrenia disorder 7	13q32	Risch et al. 1999

dependent arborization of dendrites in hippocampal pyramidal neurons. Yu and Malenka [2003] have shown that overexpression of  $\beta$ -catenin (and other members of the cadherin/catenin complex) enhances dendritic arborization, whereas sequestering endogenous  $\beta$ -catenin causes a decrease in dendritic branch tip number and prevents the enhancement of dendritic morphogenesis caused by neural activity [Yu and

Malenka, 2003]. This study also revealed that the release of Wnt, which occurs during normal neuronal development, is enhanced by manipulations that mimic increased activity and that Wnt contributes to the effects of neural activity on dendritic arborization. These results show that  $\beta$ -catenin is an important mediator of dendritic morphogenesis and that Wnt/ $\beta$ -catenin signaling is likely to be important for activ-

ity-dependent dendritic differentiation [Yu and Malenka, 2003].

Interestingly, inactivation of Dishevelled-1 (*Dvl1*) in the mouse leads to abnormal development of social behaviors, such as differences in whisker trimming, deficits in nest building, reduced huddling contact during cage sleeping, and subordinate responses in a social dominance test [Lijam et al., 1997]. Notably, sensorimotor gating was abnormal

**Table 1C. Autism Candidate Genes**

Marker	Symbol	Description	Chromosomes	References
			15–20	
			Chromosome	
D15S652	OTSC1; LCS1	otosclerosis 1; lymphedema-cholestasis syndrome 1	15q25–q26	Risch et al. 1999
D7S530	AUTS1	autism susceptibility 1	15q11–q13	IMGSAC 1998
PMC311424P2	HERC2	hect domain and RLD 2	15q13	Smith et al. 2000
STS-L08485	GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	15q11.2–q12	Shao et al. 2003; Longo et al. 200
RH11160	GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	15q11.2–q12	Shao et al. 2003; Longo et al. 200
PMC24540P1	NDN	NECDIN [necdin (mouse) homolog]	15q11–q13	Chibuk et al. 2001
D15S10	UBE3A	ubiquitin protein ligase E3A (human papilloma virus E6-associated protein, Angelman syndrome)	15q11–q13	Nurmi et al. 2003
GDB:593257	GABRG3	gamma-aminobutyric acid (GABA) A receptor, gamma 3	15q11–q13	Shao et al. 2003; Long et al. 2004
RH44910	PWCR1	Prader-Willi syndrome chromosome region 1	15q11.2	Veltman et al. 2004
RH121309	SNURF	SNRPN upstream reading frame	15q12	Mann and Bartolomei 1999
GDB:626138	SNRPN	small nuclear ribonucleoprotein polypeptide N	15q12	Mann and Bartolomei 1999
PMC64493P3	NDNL2/ MAGE-G	necdin-like 2/Melanoma-associated antigen F1	15q13.1	Chibuk et al. 2001
G10644	PTPN9	protein tyrosine phosphatase, non-receptor type 9	15q23	Smith et al. 2000
G06258	SLP-1, hUNC-24	stomatin (EPB72)-like 1	15q24–q25	Smith et al. 2000
D16S403	RP22	retinitis pigmentosa 22 (autosomal recessive)	16p12.3–p12.1	Risch et al. 1999
GDB:378419	TSC2; PKD1*	tuberous sclerosis 2; polycystic kidney disease 1	16p13.3	Serajee et al. 2003
D16S407	GRIN2A	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	16p13.2	IMGSAC 1998
G67509	SSTR5	somatostatin receptor 5	16p13.3	Lauritsen et al. 2003
SLC6A4	HTT, HTTINT2	Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4; brain 5-HT transpo	17q11.1–q12	IMGSAC 2001
D17S1298	FIMG1	myasthenia gravis, familial infantile, 1	17p13	Risch et al. 1999
D19S587	HSCRS3	Hirschsprung disease, short-segment, 3	19q12	Liu et al. 2001
PMC310963P4	APOE	apolipoprotein E	19q13.2	Persico et al. 2004
D20S482	CHED2	corneal endothelial dystrophy 2 (autosomal recessive)	20p13	Risch et al. 1999

in these mice, as assessed by prepulse inhibition of acoustic and tactile startle reflexes. This interesting behavioral phenotype has been suggested as a potential model for aspects of several human psychiatric disorders, including autism. Given the developmental mechanisms underlying this interesting behavioral phenotype, it is tempting to speculate that the *Wnt/Dvl1* pathway might control patterning and synaptic assembly and dendritic branching in key cortical and mesencephalic neuronal networks controlling complex social behaviors impaired in autism. Surprisingly, to date, very few studies have explored this possibility, and future investigations should test this hypothesis.

#### *Transcription factors specifying cortical interneuron phenotype*

Aristaless-related proteins (including *Arx*) belong to the Pax family of homeobox transcription factors, several members of which are known to be involved in genetic diseases. A recent study using a gene-targeting approach to identify a pivotal role for the *Arx* transcription factor during brain development has

demonstrated that this gene controls appropriate proliferation of dorsal and ventral forebrain neural precursors, but also the migration and differentiation of GABAergic interneurons from the medial ganglionic eminence to the cortex [Kitamura et al., 2002]. These findings prompted these investigators to ask whether any human diseases are caused by *ARX* inactivation. Their search led them to several individuals suffering from X-linked lissencephaly with abnormal genitalia (XLAG), who turn out to have mutations in *ARX*. Interestingly, consistent with the findings in mice, phenotype/genotype studies in humans suggest that truncating mutations cause X-linked lissencephaly with abnormal genitalia, and insertion/missense mutations result in epilepsy and mental retardation without cortical dysplasia [Sherr, 2003]. Therefore, mutations in the homeobox gene, *ARX*, cause a diverse spectrum of disorders, including cognitive impairment, epilepsy, and, in another group of patients, severe cortical malformations. Although the precise prevalence of *ARX* mutations is unclear, further investigations will undoubtedly test whether dis-

tinct mutations in *ARX* may cause diverse forms of mental retardation, epilepsy, or autism in males [Sherr, 2003].

#### **Genes Controlling Synaptic Assembly and Dendritic Development**

##### *Neurexins (NLGN) 3 and 4*

Recently, the identification of a frameshift mutation (1186insT) in the *NLGN4* gene as well as a cysteine to threonine substitution in position 451 of *NLGN3* have been reported to segregate with autism in two independent Swedish families [Jamain et al., 2003]. Subsequently, another team has confirmed this finding and has found a 2-base-pair deletion in the *NLGN4* gene, leading to a premature stop codon, in both autistic and nonautistic mentally retarded males [Laumonier et al., 2004]. This strongly suggests that *NLGN4* might not only be involved in autism, but also in mental retardation, suggesting that autism and mental retardation might have common genetic origins.

The Neuroligin gene family was initially identified as a component of postsynaptic glutamatergic synapses, which have been shown to play a role in the *trans*-neuronal signaling that controls synapse differentiation through binding of neuroligin- $\beta$  on the presynaptic side [Ichtchenko et al., 1995; Song et al., 1999]. As we were writing this review, Chih and coworkers reported consequences of these disease-associated mutations on neuroligin function in vitro [Chih et al., 2004]. These authors demonstrated that a point mutation in the arginine residue in position 451 and a nonsense mutation in aspartate 396 of *NLGN3* and *NLGN4*, respectively, resulted in intracellular retention of the mutant proteins. Overexpression of wildtype *NLGN3* and *NLGN4* proteins in hippocampal neurons stimulated the formation of presynaptic terminals, whereas the disease-associated mutations resulted in loss of this synaptogenic function. These findings suggest that the previously identified mutations in *Neuroligin* genes are likely to be relevant for the neurodevelopmental defects in autism spectrum disorders and mental retardation, since they impair the function of synaptic cell adhesion molecules [Chih et al., 2004].

#### *BDNF and MeCP2*

Neurotrophins have multiple functions during peripheral and CNS development, such as controlling neuronal survival, target innervation, and synaptogenesis. Neurotrophins are secreted ligands that exert their biological functions by binding to a high-affinity receptor, the Trk tyrosine kinase receptor. A prominent member of this family of neurotrophins is brain-derived neurotrophic factor (BDNF), which plays multiple roles during neuronal differentiation, including neuronal survival, activity-dependent dendritic and axonal outgrowth/branching, synapse formation, and neuronal plasticity underlying learning and memory [reviewed in Shieh and Ghosh, 1997; Kaplan and Miller, 2000].

In two recent studies monitoring the plasma levels of different neurotrophins on large samples of randomly picked children retrospectively diagnosed with autism spectrum disorder or mental retardation without autism, cerebral palsy or age-matched controls revealed that both NT4 and BDNF (the two *trkB* ligands) are significantly increased in autistic and mentally retarded patients compared to controls [Nelson et al., 2001; Miyazaki et al., 2004]. Interestingly, other neurotrophins such as NGF (*trkA*

ligands) or NT3 (*trkC* ligands) are unchanged compared to controls. Of course one should be careful about translating increased plasma levels with increased CNS levels, but these findings suggest that, during early infancy, *trkB* ligands might specifically be expressed and/or secreted at higher levels in the CNS of autistic or mentally retarded children from a source that remains to be determined (central or peripheral nervous system?). The effect of BDNF and NT4 on activity-dependent dendritic outgrowth and branching is well established during development [McAllister et al., 1996, 1997, 1995] and therefore could be correlated with the transient, early increase of brain growth reported in young autistic babies [Lainhart et al., 1997; Courchesne et al., 2001, 2003]. Future investigations should test whether BDNF-mediated signaling is increased in the early period of postnatal brain development in autism and whether this correlates with a premature increase in dendritic outgrowth of pyramidal neurons, which could in turn have profound consequences on the establishment of cortical networks.

What could cause this increased level of BDNF expression and/or secretion? An interesting possibility lies in the analysis of the role of *MeCP2* in the control of BDNF transcription [Chen et al., 2003]. Mutations in methyl-CpG-binding protein 2 (*MeCP2*), which encodes a protein that has been proposed to function as a global transcriptional repressor, are the cause of Rett syndrome, an X-linked progressive neurological disorder. Interestingly, Rett syndrome is sometime associated with autism. Although the selective inactivation of *MeCP2* in neurons is sufficient to confer a Rett-like phenotype in mice [Shahbazian et al., 2002], the specific functions of *MeCP2* in postmitotic neurons are not known. Chen and coworkers have recently shown that *MeCP2* binds selectively to *BDNF* promoter III and functions to repress expression of the *BDNF* gene [Shahbazian et al., 2002]. Membrane depolarization mimicking sustained levels of neuronal activity triggers the calcium-dependent phosphorylation and release of *MeCP2* from *BDNF* promoter III, thereby facilitating transcription. These studies indicate that *MeCP2* plays a key role in the control of neuronal activity-dependent gene regulation and suggest that the deregulation of this process may underlie the pathology of Rett syndrome.

Rett disorder and autism are pervasive developmental disorders as defined

by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and the International Classification of Diseases, Tenth Revision. Recent studies indicate that at least 80% of Rett disorder cases are caused by mutations in the *MeCP2* gene. Since there is some phenotypic overlap between autistic disorder and Rett disorder [Muhle et al., 2004], a recent study aimed at analyzing large groups of females clinically diagnosed with autistic disorder for the presence of mutations in the *MeCP2* gene. Two females (4%) presenting autistic disorder were found to have de novo mutations in *MeCP2*. These data provide additional evidence of variable expression in the Rett disorder phenotype and, taken together with the role of *MeCP2* in the control of BDNF transcription mentioned above, these findings suggest a potential mechanism whereby *MeCP2* mutations might represent a risk factor for the appearance of autism through regulating BDNF expression and potentially dendritic differentiation in the cortex.

#### CONCLUSION

The concept that emerges from analyzing the current literature is that autism has a strong genetic component, but obviously constitutes a very heterogeneous neuropathology at the genetic level (see Wassink et al., this issue; see also Table 1A–C). In light of current evidence, it seems likely that the etiology of autism involves complex interactions between *environmental factors* and *genetic mutations* controlling either (1) the patterning of neuronal populations critical for the control of inhibition/excitation in the cortex and/or (2) the synaptic assembly of these excitatory and inhibitory neuronal networks and/or the neuronal networks involved in large-scale cortical neuromodulation.

An important concept in developmental neurobiology is that genes involved in the development of the CNS are extremely pleiotropic: i.e., several important genes involved in the early patterning and specification of neuronal subpopulations also act later in development to regulate the proper synaptic assembly of the same or other neuronal populations. Therefore, a mutation in one of the genes reviewed above will undoubtedly have many complex, non-redundant functions during development of the CNS that could lead to a divergent and complex neuropathology such as autism.

We hope that after reading this review the reader will realize that, although

much work needs to be done in the exploration of the neurodevelopmental mechanisms that could underlie autism, we now have new directions to explore. A new hypothesis such as the imbalance between excitation and inhibition in the cortex is attractive because it is based on functional evidence characterizing the autistic brain. The conceptual framework and the molecular tools developed in this emerging field will undoubtedly allow rapid progress in the characterization of the neuronal networks underlying this devastating developmental neuropathology. ■

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