Published in final edited form as:
Biol Psychiatry. 2007 February 15; 61(4): 521–537. doi:10.1016/j.biopsych.2006.09.021.

Early pharmacological treatment of autism: a rationale for developmental treatment

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Abstract

Autism is a dynamic neurodevelopmental syndrome in which disabilities emerge during the first three postnatal years and continue to evolve with ongoing development. We briefly review research in autism describing subtle changes in molecules important in brain development and neurotransmission, in morphology of specific neurons, brain connections and in brain size. We then provide a general schema of how these processes may interact with particular emphasis on neurotransmission. In this context, we present a rationale for utilizing pharmacologic treatments aimed at modifying key neurodevelopmental processes in young children with autism. Early treatment with selective serotonin reuptake inhibitors (SSRIs) is presented as a model for pharmacologic interventions because there is evidence in autistic children for reduced brain serotonin synthesis during periods of peak synaptogenesis; serotonin is known to enhance synapse refinement; and exploratory studies with these agents in autistic children exist. Additional hypothetical developmental interventions and relevant published clinical data are described. Finally, we discuss the importance of exploring early pharmacologic interventions within multiple experimental settings in order to develop effective treatments as quickly as possible while minimizing risks.

Keywords

autism; neurodevelopment; serotonin; GABA; glutamate; treatment

Autism is the prototypical neurodevelopmental disorder. It is characterized by qualitative alterations in three behavioral areas: social reciprocity, communication, and breadth of interests manifest by repetitive behaviors or restricted interests. Individuals with autism also experience a number of frequently associated behavioral problems such as hyperactivity, impulsivity, anxiety, irritability, and aggression, which are often the focus of pharmacologic interventions (Findling 2005; Sikich 2001). General cognitive deficits are common with approximately 60 – 70% of affected individuals considered mentally retarded (Bertrand et al 2001; Chakrabarti and Fombonne 2005; Fombonne et al 2003) and notable within subject variability in specific cognitive abilities (Joseph et al 2002). Four times as many males as females are affected (Chakrabarti and Fombonne 2005). The extreme diversity of clinical presentations is striking. Signs of the disorder begin to emerge during the first three years of life and sufficient symptoms are frequently present in the core diagnostic areas to reliably diagnose the disorder by age two

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years (Lord et al 2006). Yet, the specific behavioral manifestations of autism change across the life span suggesting ongoing developmental effects that impact prognosis.

Despite intensive research using a variety of techniques, the pathophysiology of autism remains largely unknown. Although individual investigations from various research fields have produced intriguing results, none of the studies have been definitive and discrepant findings are often present among different populations and protocols. Some of these discrepancies may be related to etiologic heterogeneity. For instance, distinct genetic loci have been implicated among males and females with clinically indistinguishable autism (Schellenberg et al 2006). Further genetic models suggest that multiple genes may interact to result in the full phenotype (Pickles et al 1995; Risch et al 1999). Taken together, these findings suggest common developmental pathways may be disrupted at multiple points and that multiple disruptions may be needed to result in the full clinical manifestations of autistic syndrome. Because autism is highly heritable (Bailey et al 1995; Folstein and Piven 1991; Folstein and Rosen-Sheidley 2001), considerable efforts have been expended to identify vulnerability genes (Klauck 2006; Muhle 2004). Several genes with neurodevelopmental functions have been proposed as candidate genes (Polleux and Lauder 2004). In addition, several molecules relevant to neurodevelopment and neurotransmission appear to vary between some individuals with autism and controls using a variety of assessment methods including positron emission transmission scanning (PET), blood and urine analyses, and examination of postmortem samples. Limited neuropathologic data has identified subtle differences in morphology and organization of neurons in various brain regions of some autistic individuals that suggest prenatal or early postnatal maturational problems (Palmen et al 2004). Neuroimaging and head circumference data suggest that the brain increases significantly more in size between 12 and 48 months in toddlers with autism than in typically developing children (Courchesne and Pierce 2005).

The findings above and the typical clinical presentation strongly suggest that brain development is aberrant during early postnatal life in individuals with autism. Although the primary developmental disruptions have not been identified, several factors suggest that early interventions may be valuable even if they do not address autism's etiology. These factors include the redundancy of neurodevelopmental processes, their sensitivity to regulation by a variety of environmental factors, and evidence of cortical plasticity resulting in compensation for early developmental alterations. Behavioral treatments that modify the experiences of affected individuals likely are effective as a consequence of brain plasticity (Dawson and Zanolli 2003; Kasari et al 2006; Kashinath et al 2006; Whalen et al 2006). Several factors suggest early interventions may be valuable even if they do not address autism's etiology. Neurodevelopment is regulated by multiple environmental factors. In addition, there are many examples of the cortex being modified to compensate for earlier development alterations. Pharmacologic interventions provided to young children with autism might have similar benefits if they are able to 1) target the regulation of early neurodevelopmental processes, 2) increase opportunities for plasticity, or 3) enhance the affected child's ability to respond to behavioral treatments. Several researchers have advocated for pharmacologic treatments that capitalize on early neural plasticity (Chugani 2005; Rubenstein and Merzenich 2003; Sikich 2001; Whitaker-Azmitia 2001). However, despite the clear value placed on early behavioral interventions for autism and suggestions to develop developmentally focused pharmacologic treatments, there has been little enthusiasm for, or study of, early pharmacologic interventions in autism.

In this review, we seek to move the field forward by presenting a rationale for pharmacologic treatments that aim to capitalize on brain plasticity in order to compensation for earlier aberrant development. Further, we describe a multi-pronged approach for future research in this area. This review is not exhaustive. There are several complementary reviews (Buitelaar and

Willemsen-Swinkels 2000; Chugani 2005; Courchesne and Pierce 2005; Keller 2003; Levitt 2003; Palmen et al 2004; Polleux and Lauder 2004; Rubenstein and Merzenich 2003) that provide a more detailed analysis of individual components synthesized in this review.

NEURODEVELOPMENTAL PROCESSES IMPLICATED IN AUTISM

Reelin

Reelin is a glycoprotein that has a fundamental neurodevelopmental role in the laminar and columnar organization of the cortex. It interacts with brain-derived neurotrophic factor (BDNF), to facilitate neuronal and glial migration and organization (Alcantara et al 2006). Normal cortical development and mature function depend on appropriate levels of reelin protein, its receptors, and its cytoplasmic adapter, disabled-1 (Dab1) (Deguchi et al 2003). Reelin levels are very high during late fetal life and gradually decline during late childhood to achieve a plateau during adolescence (Forster et al 2006). Appropriate levels of serotonin facilitate the release of reelin. In contrast, excessive serotonin has been shown to reduce reelin levels leading to disorganized radial accumulations of cortical cells (Janusonis et al 2004). Interestingly, mice lacking the gene for reelin (*RELN*) have reduced numbers of cerebellar Purkinje cells, which is the most frequent neuropathologic finding in autism.

A broad region of chromosome 7 that includes *RELN* has been linked to autism in several genome scans (International Molecular Genetic Study of Autism Consortium 2001; Lamb et al 2005; Schellenberg et al 2006){(Ashley-Koch et al 1999; Hong et al 2000). Evidence of linkage is heightened in male only families and individuals with language delays. Multiple genetic association studies have also pointed to a relationship between *RELN* and autism (Persico et al 2001; Serajee et al 2006; Skaar et al 2004; Zhang et al 2002a). However, other investigations have failed to identify a relationship between the RELN gene and autism (Bonora et al 2003; Devlin et al 2004; Devlin et al 2005; Krebs et al 2002; Li et al 2004; Zhang et al 2002b). Support for reelin's involvement in autism include finding of decreased *RELN* mRNA, decreased reelin protein, decreased mRNA for Dab1, and increased mRNA for one of reelin's receptors – the very low density lipoprotein receptor in the frontal and cerebellar cortex of adults with autism (Fatemi et al 2005; Fatemi et al 2004). Reduced plasma levels of reelin have been reported in individuals with autism and, their families (Fatemi et al 2002c).

BDNF

BDNF appears to have several developmentally important roles (Galuske et al 1999). BDNF promotes GABAergic interneuron neurite growth and stimulates the synthesis and release of GABA (Collazo et al 1992; Marty et al 1996; Matsumoto et al 2006; Nawa et al 1994; Widmer and Hefti 1994). BDNF also regulates the strength of synaptic inhibition (Rutherford et al 1997; Rutherford et al 1998). BDNF is increased by synaptic activity such that appropriate synaptic activity increases BDNF release, which further enhances synaptic activity (Castren et al 1992; Isackson et al 1991; Patterson et al 1992). Each of these BDNF actions favors maturation of cortical neurons. Excess BDNF leads to premature closure of cortical critical periods (Huang et al 1999). Thus, excessive levels of BDNF are likely to lead to precocious maturation, limiting the brain's ability to refine synaptic processes in response to relevant experiences (Hanover et al 1999; Huang et al 1999). Such precocious maturation would be expected to limit a person's ability to recognize salient stimuli in the environment.

Increases in BDNF have been demonstrated in three separate samples of autistic individuals relative to typically developing or non neurologically impaired children(Connolly et al 2006; Miyazaki et al 2004; Nelson et al 2001). Further, both Nelson and Miyazaki found similar increases in developmentally delayed comparison groups and Nelson also found increases in other neurotrophic factors (vasoactive intestinal peptide and neurotrophin 4/5). It is notable

that a follow-up study by Nelson and colleagues, using a double-antibody technique, did not replicate their original finding (Nelson et al 2006). Overall, this data suggests that findings of increased BDNF may be incidental or reflect a compensatory response to an earlier developmental problem, but are unlikely to be etiologically specific for autism.

Cholinergic system

Acetylcholine has two main types of receptors, muscarinic and nicotinic, with different functions. Muscarinic receptors inhibit the release of gamma-aminobutyric acid (GABA) from GABAergic interneurons (parvalbumin positive) that regulate background cortical activity. Nicotinic receptors excite different GABAergic interneurons (cholecystokinin positive) that fine tune the response of pyramidal cells to specific stimuli (phasic actitvity) (Freund 2003). Acetylcholine levels appear to gradually increase during childhood, reaching maximal levels toward the end of the first decade of life and then remain stable (Diebler et al 1979; Herlenius and Lagercrantz 2004).

Postmortem studies suggest acetylcholine neurotransmission may be abnormal in autistic adults. In the cortex, binding to both types of acetylcholine receptors is reduced. There is ~30% less binding to muscarinic M1 receptors and ~70% less binding to nicotinic (nAch) receptors (Perry et al 2001). Further investigations have also noted reduced mRNA and reduced protein expression of the α4 subunit of the nAch receptor (Martin-Ruiz et al 2004) (Lee et al 2002).

The cholinergic reductions reported in adults with autism would be expected to perturb GABAergic signaling, which would have a ripple effect in multiple neurotransmitter systems. Background (tonic) excitatory activity may increase. Pyramidal cell responses to stimulation would likely be less well modulated. Disruption of the balance between the GABAergic and glutamatergic systems would be expected to have significant developmental effects. The critical and complex interactions between neurotrophins and neurotransmitters are schematically depicted in Figure 1.

Glutamate neurotransmitter system

Glutamate is the predominant excitatory neurotransmitter in the brain and comprises about half of all synapses in the forebrain (Herlenius and Lagercrantz 2004). Glutamate has two main families of receptors: the metabotropic, which are single protein, G-protein coupled receptors, and the ionotropic receptors comprised of multiple subunits that form ion channels. Although not examined specifically in autism, dysregulation of the metabotropic glutamate receptor, mGluR5, has been hypothesized to play an important role in the pathophysiology of Fragile X syndrome, another neurodevelopmental disorder in which autism is very common (Bear et al 2004). In autism, the iontropic receptors, which include the *N*-methyl-D-aspartate (NMDA) receptor, the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (*AMPA),* and kainate receptor, have been studied most extensively.

During early development NMDA receptors predominate, whereas in adults, AMPA and kainate receptors are more active. NMDA receptors, particularly those containing the NR2B receptor subunit allow increased calcium influx and are more sensitive to stimulation than AMPA or kainate receptors. Further both excessive activation via excitotoxicity and inhibition of NMDA receptors have been related to increased cell death (Herlenius and Lagercrantz 2004; Olney 1994). Glutamatergic terminals are over produced during the early postnatal period and seem to reflect overproduction of synapses that will later be pruned. Through separate mechanisms, activity at NMDA and kainate glutamatergic receptors promotes neurite outgrowth and branching (Monnerie and Le Roux 2006; Nguyen et al 2001). In addition, glutamate's effects on long-term potentiation via NMDA and long-term depression via AMPA are likely to play critical roles in synapse development and enhancement. At the neuronal level,

nonsynaptic release of glutamate facilitates the migration of GABAergic interneurons that subsequently release GABA which promotes the development and migration of primordial glutamatergic cells (Manent et al 2006). These processes may augment the development of excitatory neurotransmission.

There have been multiple reports of linkage disequilibrium to chromosomal regions on chromosomes 16, 6, and 2 that contain genes important in glutamatergic functioning (International Molecular Genetic Study of Autism Consortium 2001; Jamain et al 2002; Shuang et al 2004). 6q21 contains the gene for a kainate receptor, *GRIK2*, while 16p13 contains the gene for the NMDA receptor. Chromosome 2 contains the gene *SLC25A12*, the mitochondrial aspartate/glutamate carrier. Some association studies also provide evidence of a relationship between autism and *SLC25A12* (Ramoz et al 2004; Segurado et al 2005), while others do not (Rabionet et al 2006).

Increased plasma levels of glutamate have been found in adults with autism relative to healthy controls and appear to correlate with levels of social impairment (Shinohe et al 2006). Multiple amino acids including glutamate were increased in a small sample of children with autism, their parents and their siblings (Aldred et al 2003). One study of postmortem brain tissue from adults with autism has demonstrated increased mRNA levels of several glutamate-related genes: excitatory amino acid transporter 1 (*EAAT1*) and three of the AMPA receptor subunits. Interestingly, the increase in AMPA receptor message does not lead to an increase in AMPA binding. In fact less AMPA binding is observed in the brains of individuals with autism (Purcell et al 2001). This suggests at least two possibilities; the message is not translated or the subunit proteins do not function properly for ligand binding. Increased functional *EAAT1* would result in more rapid removal of glutamate from extracellular spaces, which might reduce excitation at glutamate receptors. Alternatively, increased *EAAT1* could reflect upregulation in response to other factors such as excessive extracellular glutamate.

GABAergic system

GABA binds to two major classes of receptors ($GABA_A$ and $GABA_B$), which exhibit diverse functional activities as a result of multiple subunit combinations. During early periods of neurogenesis, GABA is present throughout the developing brain. During the first two years of postnatal life, GABA levels increase dramatically to about twice adult levels, then GABA levels gradually fall to below adult levels during puberty. During adolescence, GABA levels increase more slowly to achieve adult levels (Diebler et al 1979; Johnston and Coyle 1981). Estradiol has been shown to enhance the physiologic response of $GABA_A$ receptors during development (Nunez et al 2005). GABA acts through $GABA_A$ receptors to exert neurotrophic effects on progenitor cells, excitatory neurons, and glia (reviewed (Barker et al 1998; Lauder et al 1998; Nguyen et al 2001). One type of GABAergic interneuron, the Cajal-Retzius cell, is an early target of serotonergic afferents. When stimulated by serotonin, Cajal-Retzius cells produce and secrete reelin (Janusonis 2004). GABA also stimulates the migrations of specific cortical neurons expressing $GABA_B$ receptors (Lopez-Bendito et al 2003).

However, GABA's most important developmental role may relate to its involvement in synapse maturation. Prior to maturation of glutamatergic synapses, GABA has both excitatory and inhibitory actions, as opposed to its exclusively inhibitory actions later (Barker et al 1998). The differential activity of GABA during fetal life and once glutamatergic synapses have matured during the early neonatal period may be mediated by the changing composition of $GABA_A$ receptors on cell bodies and dendrites (Ramos et al 2004). The balance between GABA and glutamate is critical and complex with many cortical cells receiving GABAergic and glutamatergic inputs at the same postsynaptic site. However, during early development there are frequent inaccuracies between the presynaptic outputs and their postsynaptic receptors (Rao et al 2000). GABAergic activity promotes enhanced GABAergic fidelity and indirectly

reduces glutamatergic mismatch by not reinforcing inappropriate glutamatergic synapses. Appropriate development of excitatory (glutamate) and inhibitory (GABA) neurotransmission appears to rely on experience-dependent signaling with a bias towards GABAergic synapse fidelity (Anderson et al 2004b). Finally, individual types of GABAergic interneurons, which have unique patterns of input and output, are able to differentially modulate cortical activity in response to neurotransmitters and neuromodulators (Freund 2003). The coordination between GABA and glutamate creates and sustains an environment conducive to proper neurodevelopment. It is essential for appropriate synapse elimination, assembly of architectural units of cells into mini- and macro-columns, and the development of integrated brain systems.

Changes in GABAergic function have been associated with autism by multiple studies using different methodologies. Multiple groups have found linkage disequilibrium near or with *GABRB3* (Bass et al 2000; Buxbaum et al 2002; Cook et al 1998; Martin et al 2000). Others have been unable to replicate these findings (Menold et al 2001; Salmon et al 1999). In addition, about 3% of some clinical samples of individuals with autism show cytogenetic abnormalities of chromosome 15q11-q13, which contains genes for three of the $GABA_A$ receptor subunits (*GABRB3*, *GABRA5*, *GABRG3*) (Cook et al 1997b; Dykens et al 2004). These genetic findings have been extended in a single-nucleotide polymorphism (SNP) study of 14 GABA receptor genes, which found strong support for the involvement of *GABRA4* through interaction with *GABRB1* in vulnerability to autism (Ma, 2005#70). Decreases in GAD65, which converts glutamate to GABA, and reduced binding of agonists and antagonists to $GABA_A$ sites have been noted (Blatt et al 2001; Fatemi et al 2002b).

Serotonergic system

Serotonin has at least 15 different receptors, which appear to have unique functional activities and spatial distributions. Specific 5-HT receptor subtypes play pivotal roles during development. Activation of $5HT_{1A}$ receptors reduces the length of dendrites and the number of dendritic spines in hippocampus (Sikich et al 1990). These findings are consistent with the dendritic changes reported in hippocampal neurons in autism (Bauman and Kemper 1985; Bauman and Kemper 2005; Raymond et al 1996). $5HT_{2C}$ receptors appear to be involved in long-term potentiation in the hippocampus (Tecott et al 1998). $5HT_{2A}$ receptors are involved in neuronal differentiation, dendritic maturation and modulation of levels of brain derived neurotrophic factor (BDNF). The influence of $5HT_{2A}$ on BDNF may underlie the anti-apoptotic effect of $5HT_2$ agonists observed in vitro (Dooley et al 1997; Vaidya et al 1997).

Serotonin has multiple roles that may be important in experience-dependent organization. During development, serotonin modulates the activity of GABAergic interneurons, particularly the reelin-releasing Cajal-Retzius cells. In addition, serotonin released by axons projecting from the thalamus has been demonstrated to play a critical role in the establishment of appropriate thalamocortical connections. Although thalamic neurons cannot synthesize serotonin, they transiently express the serotonin transporter, internalize extracellular serotonin, and incorporate it into synaptic vesicles along with their primary neurotransmitter, glutamate (Lebrand et al 1996). The function of this serotonergic uptake and co-release remains unclear. Possible functions include 1) serving as a borrowed transmitter; 2) removing excess serotonin from the extracellular space until the raphe and glial networks become fully mature, and 3) creating a gradient of serotonin between the raphe projections and thalamic afferents to guide neurite extension (Gaspar et al 2003).

In the cortex, serotonin modulates the release of glutamate in response to incoming neuronal activity by acting both directly on glutamatergic neurons and indirectly on GABAergic interneurons (Laurent et al 2002; Mooney et al 1994). Serotonin is necessary for the maturation of thalamic afferents, cortical dendrites and axons. However, too much serotonin results in immature, widely dispersed dendritic branches (Mooney et al 1994; Salichon et al 2001). Too

little serotonin results in fewer dendritic spines, abnormally small dendritic arbors and somatosensory barrels, and reduced synaptic density (Bennett-Clarke et al 1994; Mazer et al 1997; Osterheld-Haas and Hornung 1996; Yan et al 1997). This finding is consistent with the observation that minicolumns in individuals with autism are narrower than those of individuals with typical development (Casanova et al 2002b).

One of the earliest and most consistent findings in autism research has been the presence of plasma and platelet hyperserotonemia in a significant portion of children and adolescents with autism (Abramson et al 1989; Anderson et al 1987; Leboyer et al 1999; Levy and Bicho 1997; Piven et al 1991; Ritvo et al 1970; Rolf et al 1993; Takahashi et al 1977). Subsequently, the serotonin transporter gene, *SERT* or *SLC6A4* located on chromosome 17q11.1–12 has been widely examined. Most studies have focused on the 5-HT transporter gene-linked polymorphic region (5-HTTLPR), in which the short allele is associated with lower expression of the serotonin transporter (5-HTT). The specific results of these studies are frequently inconsistent with one another with some studies finding linkage (IMGSAC 2001; International Molecular Genetic Study of Autism Consortium 1998; Yonan et al 2003) and others not (Auranen et al 2000; Barrett et al 1999; Betancur et al 2002; Buxbaum et al 2001). Association studies have had equally confusing results with some observing preferential transmission of the short allele, which is associated with lower expression of the serotonin transporter (Conroy et al 2004; Cook et al 1997a; Devlin et al 2005; McCauley J. L. 2004) and others finding preferential transmission of the long allele (Klauck et al 1997; Yirmiya et al 2001). Although these discrepancies are not well understood they may be related to differences in the phenotype of the samples, differences in interactions with other genes prevalent in the particular ethnic groups studied, or variability in genotyping procedures (Yonan et al 2006). It is also possible that vulnerability to autism is conferred by more subtle and rare alleles within the gene (Sutcliffe et al 2005). Further, the autism-related effects of *SERT* may be evident only if interactions with other genes or specific environmental conditions are present (D'Amelio et al 2005; Prasad et al 2005).

Perhaps the most relevant evidence for serotonergic involvement in autism has arisen from Positron Emission Tomography (PET) studies, which allow visualization of serotonin synthesis in living individuals with autism. PET scans have revealed altered spatial patterns of serotonin synthesis in a pathway connecting the cerebellum and frontal cortex (Chugani et al 1997). Even more intriguing is the finding of differences in the developmental pattern of serotonin synthesis capacity between individuals with autism and controls with typical (Chugani et al 1999). Normally, serotonin synthesis is 200% the adult levels until about five years of age, and then gradually falls to adult levels over the next several years (Chugani et al 1999; Herlenius and Lagercrantz 2004). However, in individuals with autism, serotonin synthesis is very low until approximately nine years of age and then increases to about 150% of the normal adult levels. The typical developmental pattern of serotonin synthesis is very similar to the developmental variations in the number of cortical synapses. Tremendous synaptogenesis occurs during early childhood followed by gradual synapse elimination during adolescence (Huttenlocher 1979; Huttenlocher 1990). These relationships are illustrated in Figure 2. There are also parallels with the temporal pattern of GABA levels (Diebler et al 1979).

Evidence for Altered Brain Morphology in Autism

Neuropathologic studies in autism have been limited by the very small numbers of postmortem specimens available. Further, many of the donors had comorbid disorders such as epilepsy that may also be associated with abnormalities in brain structures. Although most studies show some subtle abnormalities, there are many inconsistencies and several key findings have not yet been replicated. Observations that require replication include reduced size and increased packing density of neurons in the limbic system, reduced dentritic trees in hippocampal

neurons, apparently age-related changes in the diagonal band of Broca and the inferior olive, agenesis of the facial nucleus, and minicolumn pathology (Bauman and Kemper 1985) (Bauman and Kemper 1985; Casanova et al 2002a; Casanova et al 2002b; Kemper and Bauman 1993; Raymond et al 1996; Rodier et al 1996). There is more agreement about the presence of neocortical disorganization in a subset of cases, various olivary abnormalities in 2 studies (67% of cases) and decreased number and/or size of cerebellar Purkinje cells in 72% of cases (Arin et al 1991; Bailey et al 1998; Bauman and Kemper 1985; Casanova et al 2002a; Casanova et al 2002b; Casanova et al 2002c; Fatemi et al 2002a; Fehlow et al 1993; Guerin et al 1996; Ritvo et al 1986; Rodier et al 1996; Williams et al 1980). Reduction in the number of cerebellar Purkinje cells, coupled with relatively few abnormalities in the inferior olive, has been interpreted to imply that the initial brain changes in autism occur prior to birth (Bauman and Kemper 1985; Bauman and Kemper 2005). Demonstrations of more significant inferior olive abnormalities have raised questions about whether these abnormalities might be occurring during early postnatal life (Bailey et al 1998; Harding and Copp 1997; Kern 2003).

Minicolumns in autism

Minicolumns are viewed by some to be the fundamental functional unit within the brain, while others view macrocolumns, which are typically comprised of 60–80 minicolumns, as the more fundamental functional unit (Casanova et al 2003; Mountcastle 1997; Rockland 2004). During typical development, multiple minicolumns are organized into macrocolumns such as the somatosensory barrel fields or ocular dominance columns in which the minicolumns within a macrocolumn receive afferent activity that is highly coordinated with respect to spatial location or physiologic function (Casanova et al 2003; Rubenstein and Merzenich 2003).

One study has found an increase in the number of minicolumns in postmortem specimens from individuals with autism. The minicolumns were observed to be narrower overall but have a broader excitatory core with fewer and more widely spaced cells and a significantly reduced area of peripheral neuropil than is typical (Casanova et al 2002b). These differences suggest problems both prenatally during neurogenesis with specification of the number of minicolumns and later during inhibitory interneuron maturation, and dendritic process refinement. These results seem compatible with Raymond's observations of poorly elaborated dendritic arbors in the hippocampus, which also seem likely to reflect difficulties with initial process formation and maintenance of synaptic contacts.

In autism, the reduction of inhibitory neuropil surrounding the minicolumn core would impair the minicolumn's ability to influence its neighbors. Specifically, excitation of narrow minicolumns will excite a slightly larger number of adjacent minicolumns but will not inhibit any more distant minicolumns. Consequently, organization into discrete, functionally related macrocolumns is likely to be more difficult since the edges of these columns will not be readily detected. In contrast, excitation of typically proportioned minicolumns will lead to excitation of the adjacent minicolumns and inhibition of those at an intermediate distance so that discrete functional macrocolumns are formed (Casanova et al 2003). The observation that individuals with autism often are very detail focused but fail to recognize the broader context of information may be a functional consequence of narrower minicolumns.

Brain Volume in Autism

Over the past decade, magnetic resonance imaging (MRI) and head circumference studies of children with idiopathic autism have demonstrated a rapid and developmentally inappropriate increase in brain volume between 12 and 48 months, that exceeds the typical increases in volume by 5–10%. Afterwards, there is significant slowing of brain growth at a time when typically developing youth show significant increases in brain volume (Aylward et al 2002; Courchesne et al 2003; Gillberg and de Souza 2002; Hazlett 2005; Lainhart et al 1997; Sparks

et al 2002)reviewed in (Redcay 2005). MRI studies show increases in both gray and white matter, with white matter changes sometimes appearing more robust with some regional differences evident. Recent data from MRI and neuropsychological studies suggest a relative underconnectivity between fronto-parietal regions and interhemispheric loci (Just et al 2006; Nyden et al 2004; Vidal et al 2006).

A number of potential explanations of the early increase in brain size observed in autism have been proposed including excessive neurons or glia, an absence of developmentally appropriate dendritic pruning or overexuberant dendritic arborization. However, the underlying mechanism has not yet been clarified. It will be particularly important to determine whether development is simply precocious or whether it is atypical in other ways as well.

The consequences of these early brain changes are not clear. Courchesne and colleagues (Courchesne and Pierce 2005; Redcay 2005) have hypothesized that the impact will be greatest on neurons whose development occurs over an extended period, such as those in the frontal cortex, in contrast to those that mature earlier and more rapidly (primary sensory cortices). Thus, the effects may be greatest on large integrative neurons, which integrate information from brain regions that mature earlier and are responsible for higher order cognitive functions. Aberrations in the development of such integrative neurons might lead to more use of local processing strategies rather than contextual processing strategies. Such shifts could theoretically underlie the impaired processing of complex information reported in some individuals with autism (Minshew et al 2002; Williams et al 2006). Perhaps more importantly, since these changes in brain volume and connectivity occur at approximately the same time that the manifestations of autism are becoming apparent, it is possible that therapeutic interventions provided during this period might be particularly beneficial.

CORTICAL PLASTICITY

The previous sections have summarized many of the molecular events and interactions essential for typical development and their presumptive relationships to autism. Under optimal developmental conditions, these processes act in a well integrated manner to organize the cortex so it effectively discerns salient stimuli, places these stimuli in an appropriate context and acts upon the resulting information. This key developmental process involves two primary steps (see reviews (Grossman et al 2003; Levitt 2003; Rubenstein and Merzenich 2003). First, the inherent width of dendritic arbors appears largely predetermined and supports the establishment of typical but unrefined topographic patterns that are large, imprecise and inefficient (Greenough 1987). Cellularly, this is reflected by overproduction of neurons, neuronal processes and synapses (reviewed by Levitt (2003). During this period, neural connections form at the rate of almost 40,000 synapses per second.

In the second step, the size, precision and efficiency of these topographic patterns are refined in a process dubbed *"experience-dependent" organization.* Such refinement requires meaningful, coordinated activity between thalamocortical excitatory afferents and both excitatory and inhibitory intracortical connections to improve signal detection and reduce extraneous noise (Belmonte et al 2004; Polleux F 2004; Rubenstein and Merzenich 2003). At a cellular level, in areas of appropriate stimulation, existing synapses are strengthened and the new synapses and neuritic processes are formed. Concurrently, in areas that receive fewer or less appropriate inputs, synapses and neuritic processes are eliminated. In nonprimates, the periods of synapse refinement appear significantly more restricted than in primates who have subsequent plateau and regressive phases of cortical remodeling (Levitt 2003). The initial plateau phase in humans has not yet been well defined, but is estimated to be greatest between two and seven years, with ongoing reorganization prominent through adolescence. There are marked regional variations in the timing of enhanced organization (Huttenlocher 1979;

Huttenlocher and Dabholkar 1997). Myelination typically follows the initial periods of synapse refinement. As discussed earlier, several trophic factors and neurotransmitters, including BDNF, glutamate, GABA, and serotonin, play critical molecular roles in experience-dependent organizational refinement.

In addition, synaptic refinement continues to be possible throughout life under limited conditions (Hensch 2004; Pizzorusso et al 2002; Werker and Tees 2005). When activation of cholinergic neurons in nucleus basalis is coordinated with meaningful sounds, adult primary auditory cortex is capable of significant plasticity (Kilgard and Merzenich 1998). Similarly, if a sensory stimuli is paired with activation of dopaminergic neurons from the ventral tegmental area, cortical reorganization occurs both in the primary sensory area and in interconnected secondary auditory cortex of adults (Bao et al 2001).

Sensitive Periods

The concept of critical periods, which may also be known as sensitive periods or optimal periods, is intricately tied to the processes of structural and functional cortical organization described above (reviewed by Hensch (Hensch 2004). Initially described by Konrad Lorenz, critical periods were thought to be well demarcated timepoints when aberrant experiences resulted in disruption of subsequent typical behavior (Lorenz 1958). This concept has also been applied to teratology research to define periods when permanent developmental damage occurs in response to the smallest doses of a toxin (Rice and Barone 2000). Later, the concept was extended to reflect periods when cortical organization was altered in response to abnormal stimulation (Hubel and Wiesel 1970; LeVay et al 1980; Wiesel and Hubel 1963; Wiesel and Hubel 1965). The onset and offset of these periods of plasticity in response to environmental stimuli were often used to infer the timing of typical maturation of specific functions and cortical regions. As further work has been done, it has become clear that there may be remarkable dissynchrony between 1) the time course of a region's typical maturation, 2) the period in which its structural organization can be disrupted by abnormal experiences and 3) the period during which alterations in its structure induced by prior aberrant experiences may be reversed or overcome. For instance, in visual cortex, the normal development of cortical columns sensitive to global motion extends to six years. However, the period in which this organization can be perturbed by deprivation is limited to the first few months of life. In contrast, visual acuity also normally develops through age six, but is sensitive to damage from visual deprivation until age eleven. Repair of deprivation induced changes is only possible up until age seven (Lewis and Maurer 2005). The time course of a region's sensitive period may be much shorter than overall development (Johnson 2005) but may also extend well beyond the window for typical development (Hensch 2005).

Over time, there has also been increasing emphasis on the potential for beneficial plasticity during the sensitive periods. The dyssynchrony between typical maturation and sensitive periods and the ability to vary the timing of the optimal plasticity period by environmental manipulations suggests that it may be possible to overcome early atypical development later in life. Further, later interventions might be targeted at different molecular processes than those initially involved in the perturbation. Hence there is great interest in tailoring educational and medical interventions to exploit these periods of heightened plasticity (Ito 2004;Liao et al 2004;Werker and Tees 2005;Wynder 1998).

Neuroscience research efforts have shifted from identifying specific optimal periods to trying to understand the biological factors that underlie them and how these factors differ from those involved in normal developmental processes (Johnson 2005; Katz 1999). There is remarkable variability in the molecules that regulate developmental plasticity related to different cortical functions. However, there is emerging evidence that parvalbumin-positive GABAergic interneurons, whose number and maturation are stimulated by BDNF, play a critical role in the

determining the close of the optimal period. Specifically, enhanced $GABA_A$ function (eg. by BDNF overexpression or benzodiazepine binding) promotes closure of the optimal period of plasticity. Reduced $GABA_A$ function (eg. by sensory deprivation or knocking out Gad 65 synthesis) prolongs the optimal period of plasticity (Fagiolini et al 2004; Hensch et al 1998; Huang et al 1999; Iwai et al 2003; Morales et al 2002). In addition, there is strong evidence that disruption of the extracelluar matrix around neuronal spines is required for plasticity (reviewed in Hensch, 2005). Release of Tissue plasminogen activator (tPA) in response to environmental stimuli appears to break down the extracellar matrix (Mataga et al 2004; Mataga et al 2002; Oray et al 2004).

NEURODEVELOPMENTALLY BASED INTERVENTIONS FOR AUTISM

In the absence of revolutionary discoveries that elucidate the pathophysiology of autism and lead to accurate diagnosis in utero or infancy, treatments will focus on halting further abnormal brain development and compensating for prior aberrations. This approach is comparable to almost all neuropsychiatric disorders whose pathophysiologies are poorly understood as well as to most somatic medical disorders such as hypertension and diabetes. Conceptually, animal and human studies of sensitive periods indicate that strategies focused on overcoming early problems with brain development may be efficacious for the treatment of autism. Early intensive behavioral interventions (EIBI), which have become the standard of care in autism and appear to influence overall development and reduce the intensity of core symptomatology, are based on this strategy, (Aman 2005; McEachin et al 1993; Smith et al 2000).

There are indications that pharmacologic manipulations are equally as capable as behavioral intervention of producing benefits if provided during sensitive periods. For instance, in the drosophila model of Fragile X syndrome (FRAX), the mGluR antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP) can reverse associated abnormalities in both brain structure and behavior if provided during early development, but has no anatomical effects if given later (McBride et al 2005; Yan et al 2005). In humans with Smith-Lemli-Opitz Syndrome (SLOS), in whom autism is extremely prevalent, cholesterol supplementation prior to age five reduces the risk of autism spectrum disorders four-fold (22% versus 88%) compared to later supplementation (Tierney et al 2001). These two disorders are intriguing because up to 25% of males with FRAX and 50% of individuals with SLOS meet diagnostic criteria for autism (Hatton et al 2006; Sikora et al 2006). Thus, treatments that impact experience-dependent brain organization and are provided during periods of heightened plasticity might compensate for some developmental abnormalities observed in autism.

Identifying effective treatments

The optimism related to developmental treatments must be tempered by the recognition that we do not know which pharmacologic interventions will be effective. Further, given the apparent etiologic heterogeneity of autism, it is possible that different interventions will be efficacious in different autistic subgroups. Interventions related to molecules or processes demonstrating clear developmental differences in autism as compared to typical development may be particularly effective. However, it is also possible that the developmental periods when such interventions would have been effective will have ended prior to diagnosis of autism. In that case, it will be important to evaluate treatments targeted toward molecules known to be important in the brain's response to developmental perturbations or toward enhancing plasticity instead. The multiplicity of molecules involved in typical neurodevelopment and the complexity of their interactions suggest several potential areas in which interventions might be developed for use in autism.

In designing developmentally-based interventions, it is important to remember several key challenges. First, the intervention attempts to modulate function in an already perturbed system.

The levels of almost all neurodevelopmental molecules are precisely regulated in development and some sort of disruption has already occurred. Investigators cannot assume that provision of a molecule to autistic individuals during development will have the same impact as provision of the same molecule to individuals without pre-existing perturbations in the molecule's signaling, transport and spatial distribution, or synthesis. For instance, mice with reduced serotonin synthesis due to 1473G tryptophan hydroxylase-2 homozygosity, show typical responses to a SSRI only if provided with exogenous tryptophan (Cervo et al 2005). Secondly, it seems essential to base intervention strategies on brain rather than peripheral findings. For instance, initial observations of hyperserotonemia led to the conclusion that brain levels of serotonin were likely to be inappropriately high. Therefore, attempts were made to reduce brain serotonin through the use of fenfluramine and L-tryptophan depletion. In both these cases, peripheral serotonin was reduced, but there was no clear benefit from treatment despite several studies (Campbell et al 1988). Instead, irritability was common with fenfluramine and there was clear exacerbation of autistic symptoms with tryptophan depletion (McDougle et al 1996). As interventions are proposed and developed, it will be essential to evaluate both efficacy and safety and tolerability.

Serotonergic interventions have promise

Interventions that enhance serotonergic neurotransmission during early childhood development appear to have the most immediate potential for eliciting clinically important, adaptive brain changes in children with autism(Chugani 2002; Chugani 2005). Serotonin plays a critical role in the development of cortical columns and experience-dependent organization. Animal work suggests that fluoxetine treatment can prevent functional brain damage from hypoxic injuries (Chang et al 2006). Further, there is evidence of a developmental abnormality in serotonin synthesis in some young children with autism. Equally important, FDA-approved medications which are likely to enhance serotonergic neurotransmission are currently available. These agents appear to have relatively few adverse effects in human children even when exposure occurs *in utero* or during early infancy (Barbey and Roose 1998; Gentile 2005; Isacsson et al 2005; Levinson-Castiel et al 2006; Malm et al 2005; Misri et al 2006; Moses-Kolko et al 2005; Safer and Zito 2006). The adverse effects that have been noted in humans are related to withdrawal syndromes, high serum levels or overdoses (Knoppert et al 2006), and possible activation and increased suicidality. Further, the long-term impact from perinatal and/or early childhood exposure to selective serotonin inhibitors (SSRIs) is not yet known. It should be noted that three rodent studies in which developing animals were treated with high doses of SSRI for extended periods observed various late emerging side effects such presumed anxiety in adult mice (Ansorge et al 2004). In contrast, animals treated with 67% lower doses from PND1-7 showed no late emerging behavioral effects utilizing the same assessments (Chang et al 2006).

SSRIs increase availability of serotonin in the synaptic cleft. A recent review of limited data in the pediatric autism population suggests that the SSRI may have some benefits and appear safe, but definitive studies do not exist (Kolevzon et al 2006). The most rigorous study, demonstrated clinical benefit of fluoxetine in the treatment of repetitive behaviors in children and adolescents with autism (Hollander et al 2004). There is also case series data suggesting this SSRI may have developmental effects, particularly on language, in young children with autism (DeLong et al 2002; DeLong et al 1998). In response to the need to move forwards with the evaluation of promising early interventions, two centers within the NIH-funded Studies to Advance Autism Research and Treatment (STAART) network have initiated a pioneering study to examine the developmental impact of fluoxetine treatment in preschool children with autism. This developmental trial builds upon an initial feasibility study initiated by Drs. Sikich and DeLong in 1999.

Regardless, this approach is not without risk. A major impetus for treatment with an SSRI is our interpretation that there are inadequate amounts of serotonin in the brains of children with autism. However, this interpretation of Chugani's seminal PET studies may be incorrect since the PET studies provide no information about children younger than two years. Serotonin synthesis in the central nervous system may indeed be inadequate, but may indicate an adaptation to an earlier developmental period of excessive serotonin synthesis or signaling (Whitaker-Azmitia 2005). Subsequent use of an SSRI may impede natural compensatory changes in the developing brain, thereby worsening pathology. Further, serotonin findings in autism may merely indicate dysfunction in one or more of the neurotransmitters and factors that facilitate the development of serotonin neurons, receptors, synthesis, or release **(see** Figure 1). If the autism phenotype is a manifestation of an 'upstream' regulator, it is unclear what the effect of early SSRI treatment will be. Additionally, it is possible with serotonergic treatments, as with any other treatment provided early in development, that there may be late emerging side effects such as have been described in some animal studies. Ultimately, it will be essential to assess the balance between the potential benefits of early treatment on a devastating life long disorder and the potential risks of ineffective treatment or late-emerging adverse effects.

Other potential developmental pharmacologic interventions

Given our understanding of signaling molecule disruptions in autism, there are several potential targets in addition to serotonin for developmental interventions in autism, as summarized in Table 1. The two primary approaches would be to enhance plasticity and promote compensatory experience related changes or to remediate identified imbalances in neurotransmission. In the first case, plasticity could potentially be promoted if sensitive periods were extended or reopened as suggested by Chugani (2005). Reductions in $GABA_A$ activity (e.g. by reduced GABA synthesis, increased degradation or receptor blockade) might have this effect. However, current GABA antagonists are quite toxic. Blockade of BDNF early in development might also have this effect. Another strategy might be to disrupt the extracellular matrix with an agent like tPA in order to create a more permissive environment for synaptic reorganization. Finally, one could try to augment cholinergic or dopaminergic neurotransmission in emulation of the animal studies of auditory cortical areas that demonstrated plasticity by activating the nucleus basalis or ventral tegmental area (Bao et al 2001;Kilgard and Merzenich 1998). Cholinesterase inhibitors or dopamine agonists might act in these ways.

If one takes the approach of trying to address identified disruptions in neurotransmission, evidence most strongly supports targeting the serotonin system, as discussed earlier, or the GABAergic system. The evidence of reduced GABAergic activity coupled with its critical role in experience-dependent brain organization makes it a primary target. Modulation of GABA could influence the complex excitatory:inhibitory balance which appears critical to process and synapse refinement. The availability of approved agents that enhance GABAergic neurotransmission such as valproic acid, benzodiazepines and estradiol suggest that they may be appropriate candidates for treating children with autism. Further, in a mouse model that has deficiencies in *SERT*, female mice and males treated with estradiol show more normal levels of serotonin, more complex hippocampal dendrites and fewer anxiety related behaviors than untreated males or females with ovariectomy or tamoxifen (Ren-Patterson et al 2006). In addition, if autism reflects an increased ratio of excitation to inhibition as suggested by Rubenstein and Merzenich (2003), benefits may also be derived from dampening excitatory neurotransmission. Agents which reduce glutamatergic activity (such as lamotrigine, topiramate or zonisamide) or enhance activity of the EAAT might be beneficial due to such actions.

If on the other hand, autism is related to a hypoglutamatergic state at NMDA receptors as proposed by Carlsson (Carlsson 1998), highly selective NMDA partial agonists might have utility as a treatment. Unfortunately, even a regionally selective, highly specific agonist is likely to be neurotoxic. Carlsson advocates an alternative approach in the augmentation of AMPA neurotransmission through the use of ampakines. Although no ampakines have yet been approved by the FDA, a 6 month pilot study in adults with Fragile X syndrome, did not observe significant adverse effects (Berry-Kravis, personal communication). Carlsson has also suggested that the primary implication of weak NMDA tone is excessive $5HT_{2A}$ activity. Although there is no experimental support from autistic subjects for Carlsson's hypothesis, serotonin synthesis is increased during adolescence in individuals with autism and limited use of serotonergic antagonists, such as the second generation antipsychotics, could be helpful.

Prior medication trials demonstrating some developmental effects in autism

Prior medication trials involving at least 6 children with autism that have demonstrated some benefit for core symptoms of autism are summarized in Table 2. With the exception of DeLong's and Alcami's studies, none of these trials focused on developmentally targeted intervention. Consequently, few young children are included. We would expect that benefits for any of these treatments might be enhanced if younger children were included. It is noteworthy that the only trials that indicate improvement in communication or social behavior are open studies. In contrast, reduction in restricted and repetitive behaviors (RRB) have been demonstrated in multiple trials, the largest of which is a trial of the second-generation antipsychotic, risperidone. It remains unclear whether this reflects the increased difficulty of assessing social and communicative behaviors or if it reflects the limitations associated with brief acute trials and the difficulties maintaining children in double-blind treatment for extended periods. More comprehensive reviews of pharmacologic treatment studies in autism are available (Buitelaar and Willemsen-Swinkels 2000;McDougle 2005)

How should evaluation of potential developmental treatments for autism proceed?

In order to validly assess the impact of developmentally focused interventions, it will be essential to evaluate not only acute effects but also long-term changes in core symptoms and other developmental abilities, acute and mid-range tolerability, and very late emerging adverse effects such as those described in animal studies. Because of the limitations of every system available for study, multiple approaches will be required. The three major approaches that can be utilized at this time are: 1) animal studies; 2) inclusion of children of all ages in clinical trials of agents with potential neurodevelopmental applications, and 3) more protracted trials or post-trial observation periods that allow assessment of long-term consequences of treatment.

Animal models

Although our primary interest is in the initiation of rigorous, developmentally focused medication trials in autism, the need for extensive animal research in this field cannot be overstated. The use of animal models may allow us to characterize developmental windows for treatment and evaluate the appropriate duration of treatment in ways that are impossible in human studies. Further, animal models will provide the opportunity to rigorously define the relationships between treatment and changes in activity of different neuromodulators and in brain structure in ways that are not possible in humans. Such information may facilitate the design of better treatments. The use of different genetic variants (in mice) may allow us to formulate ideas about treatment specificity among subgroups of autistic individuals.

However, there are a number of prerequisites to using such models. First, it will be essential to meticulously define periods of developmental equivalence between mice, nonhuman primates and humans. It will be crucial to examine the events that are the target of interventions: 1) synaptogenesis, 2) synapse refinement, and 3) the development of the integrative pathways

presumed to be impaired in autism. This task is complicated by the disparities between primate and rodent development particularly with regard to extended plateaus of synapse refinement observed only in primates. There is also extremely limited information about the time course of these events in humans. In addition, it will be essential to characterize the comparative pharmacokinetic and pharmacodynamic properties of the candidate agent in animals and children, rather than adults. Further, animal studies should use pharmacologically relevant, rather than excessive, doses of the therapeutic agents that are administered by mouth or transdermally if possible. Blood levels of the medication across species are likely to be more informative than simple mg/kg or mg/body surface area. Although CSF levels would provide the most relevant comparison, it is not realistic to measure such levels in autistic children. Further, it will be important to continue recent efforts to improve the quality of behavioral assessments in animals models (Garner et al 2006; Nadler et al 2004).

The choice of animal models should be based on the presence of behavioral or neurodevelopmental differences that reasonably approximate aspects of the autism phenotype as discussed in several recent reviews (Andres 2002; DiCicco-Bloom et al 2006; Machado and Bachevalier 2003; Moy et al 2006; Sadamatsu et al 2006). Examples of this might include inducible *FRAX* knockouts (Galvez and Greenough 2005), inducible Smith Lemli Opitz Syndrome (SLOS) knockouts (Waage-Baudet et al 2003), mice that have low rates of brain serotonin synthesis (Zhang et al 2004) inbred strains that show impaired sociability (Moy et al 2004), or Garner's set shifting mouse. SLOS models are particularly interesting because they exhibit disrupted serotonin and glutamate pathways (Waage-Baudet et al 2003). However, it seems unlikely that a rodent model will be able to fully capture all of the neurodevelopmental and behavioral abnormalities present in autism.

To the extent feasible, advances in lower animals should be extended to non-human primates. Nonhuman primate models allow greater control of environmental factors than human clinical trials. Further, because nonhuman primates mature more quickly than humans, late emerging adverse effects can be detected in a shorter period of time. Thus, nonhuman primate models may be useful to refine the optimal time course of promising human treatments and to define the late-emerging adverse effects. Although the costs of non-primate human studies are great, they are probably less than clinical trials in humans and may pose fewer ethical dilemmas.

Future human clinical trials

Integrating younger children into ongoing and currently planned trials is likely to yield interpretable data about developmental effects most quickly. In trials that include children with autism across the age range (e.g. 18 months to 18 years), it will be possible to examine the correlation between age and both beneficial and adverse effects of treatment. Identification of such relationships will facilitate subsequent trials that more specifically test developmental intervention hypotheses. Trials should be of sufficient size to stratify for known confounding factors such as gender, regression, language, and cognitive phenotypes (Bradford et al 2001; Schellenberg et al 2006). Comprehensive assessment of potential adverse effects will require active review of body systems and developmental processes rather than volunteered reports of side effects. Further, it is essential that these trials develop a mechanism for assessing late emerging adverse effects as well as potential late emerging or enduring benefits.

If an agent is being studied with explicit developmental aims, it is essential that the period of double-blind treatment be sufficiently long to identify developmental changes. Further, it will be important to improve assessments of core symptomatology, designing assessments that are sensitive to change over time. Initial attempts to do this have been undertaken (Cohen et al 2003), but more are needed, particularly assessments that involve direct observation of the child. In addition, it will be extremely valuable to develop biologic markers of treatment response; functional magnetic resonance scans that are temporally linked to treatment may

have promise in this regard. Discontinuation trials that help to define the necessary duration of treatment will be important in minimizing risks and optimizing safety. Developmentally focused, trials in children with single-gene disorders with high prevalence of autism or features of autism, such as Fragile X syndrome, Tuberous Sclerosis, and SLOS, may be particularly useful if sufficient participants can be enrolled. Such trials would have the advantage of etiologic homogeneity, but may not generalize to a majority of individuals with idiopathic autism.

Key ethical issues include: 1) balancing the desire to constrain adjunctive therapies in order to maximize power to detect meaningful drug effects with the need for adjunctive treatments; 2) the use of potent agents in children; and 3) denying potentially effective treatment to participants in the placebo-arm. These concerns are potentially heightened in young children for whom developmentally directed treatments are likely to be most salient. For instance, the potential benefits of adjunctive treatments such as EIBI are likely to be greater for very young children than for older ones because their brains are more plastic. For instance, there are repeated examples of medications having far greater toxicity in the very young, so potential risks may well be increased. Issues related to safety are heightened because very few agents are likely to be approved for use in children and pharmacokinetic testing very seldom includes the youngest children. However, it is also important to remember that there has been a tremendous increase in the use of these agents in children and adolescents with autism clinically despite the absence of any systematic safety information (Witwer and Lecavalier 2005). These issues are likely to be heightened further if a medication's benefits are limited to enhancing development. In that case, it may never be possible to get an indication in adults so that studies in children can proceed in the traditional manner. If phase II/III trials of such agents are to proceed, intensive and unbiased safety monitoring will be required. Additional discussion of the challenges involved in pediatric autism trials is provided in several recent reviews (Hollander et al 2004) (Aman et al 2004) (Anderson et al 2004a).

Potential strategies to minimize the risks to participants exist. Clear discontinuation guidelines in response to clinical deterioration must be in place. Likewise, participants should be informed of their randomization status as soon as their participation is completed by someone who is not involved in study assessments or data interpretation. Further, placebo-arm participants would be guaranteed access to 'active' treatment either at the conclusion of their study regimen or early termination of a placebo-arm in order to insure they had access to the potential benefits of the treatment. It is acknowledged that, if the treatment is only effective during a limited period of development, these benefits may be diminished by the delay in initiating the treatment. In addition, given the clear benefits of early environmental and educational interventions, participants could be allowed access to these therapies as long as there were efforts to match their use in the treatment and control groups and they were carefully documented. Properly designed and executed RCTs provide a safe environment in which interventions can be rigorously evaluated for safety and efficacy (March et al 2004; Sandler 2005) without compromising the best interests of the pediatric participant or quality of the science.

Conclusions

The evidence that autism is a neurodevelopmental disorder which begins *in utero* or during the early postnatal development is extensive. Further, there is increasing awareness of very early childhood changes in autism. Intervening in autism while the brain is still plastic may provide important benefits less likely with later treatment. Indeed, the most widely accepted therapy in autism, early intensive behavioral intervention, is based on this rationale. However, it is essential to develop a broader range of therapeutic options for use during this critical developmental period. Pharmacologic interventions are particularly promising because they

may be more accessible to a larger number of affected children and may be more efficacious in different subgroups, especially those with low-functioning autism. Advances in our understanding of autism and normal neurodevelopment have suggested a number of agents that may positively impact experience-dependent development in autism. Further, there are extensive and expanding networks of investigators available to develop and test the utility of promising interventions. It is essential that we undertake the translational research necessary to make early pharmacologic interventions in autism a reality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by grants from the National Institute of Health (T32-HD40127 to TCB and 1 K23 MH01802-01A2, 1 U54 MH66418 to LS). Dr. Sikich has received support for an investigator initiated study exploring early pharmacologic treatment of autism from Eli Lilly. We are extremely grateful to Emily Williams for her assistance and critical review of this manuscript and Jennifer Richards and Joe Piven for their critical reviews.

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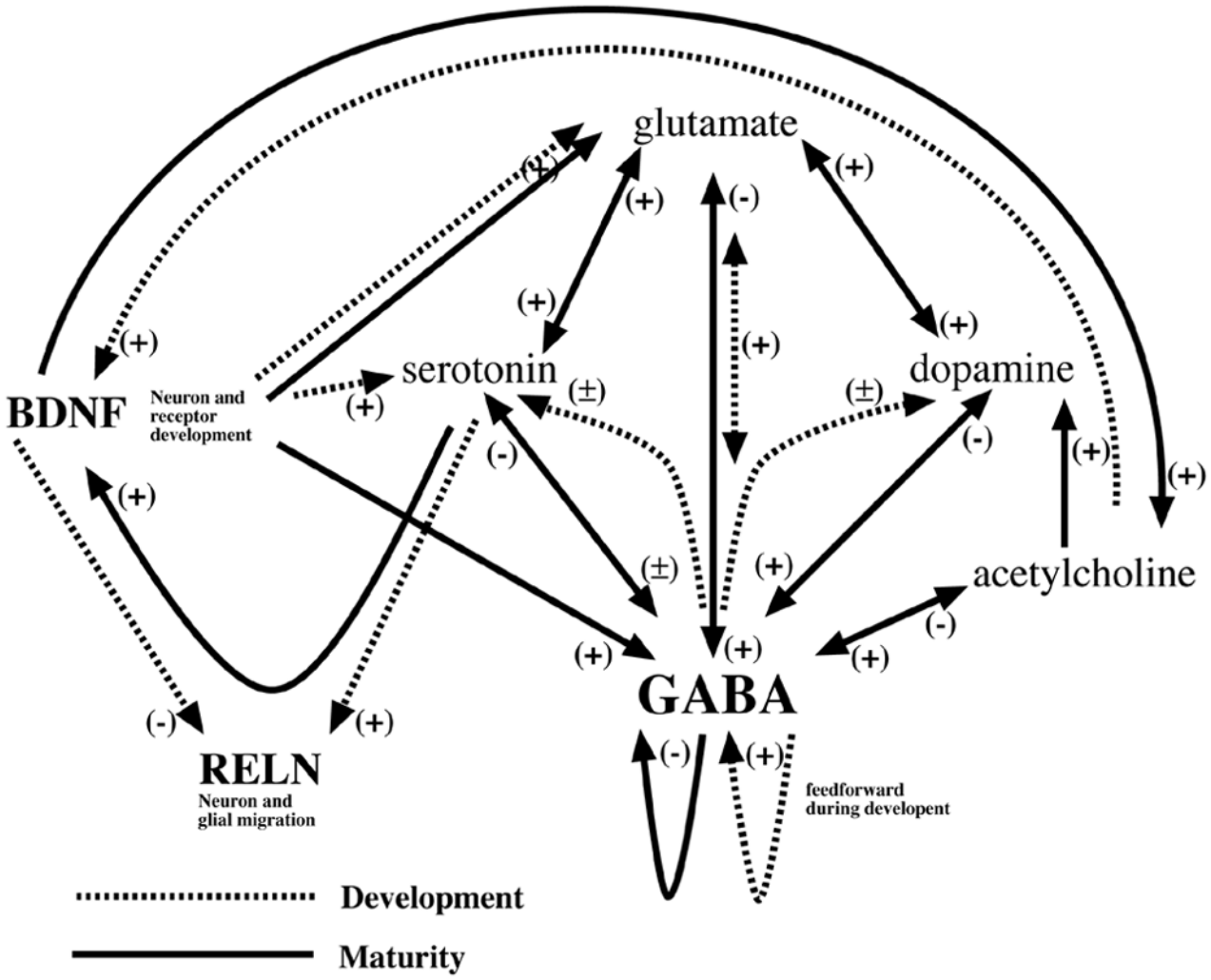
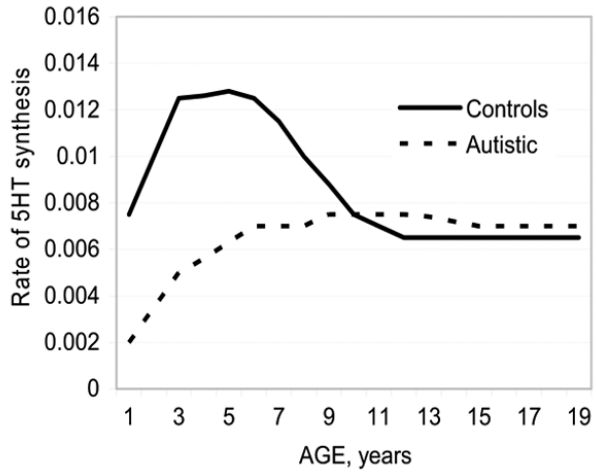


Figure 1. Neurotransmitters and neuromodulators associated with autism

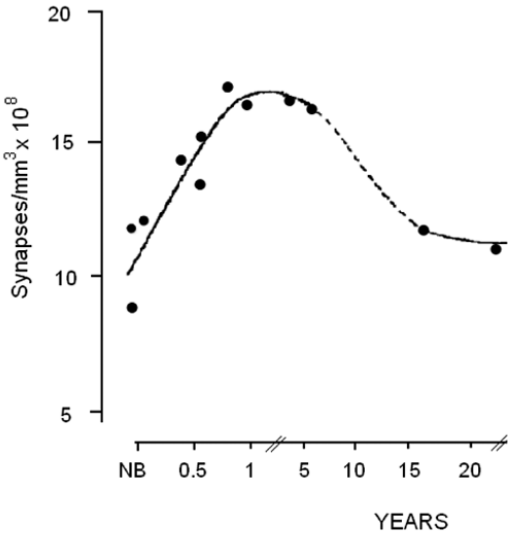
During prenatal and early infant development, neurotransmitters may have trophic, morphogenic, and synaptic signaling roles. 'Maturity' pathways represent the primary communications between neurons capable of appropriate receptor-mediated synaptic neurotransmission. Accordingly, aspects of both pathways may overlap temporally and spatially during both *in utero* and childhood development. The complexity and reciprocal connections of the pathways is notable.

FIGURE 2A. **Brain Serotonin Synthesis**



Schematic based on Chugani, et al 1999

FIGURE 2B. **Cortical Synapses**



From Huttenlocher, 1979

Figure 2. Developmental Similarities in Serotonin Synthesis and Synapse Number

These two schematics depict the capacity of the brain for serotonin synthesis and the number of synapses in frontal cortex. They are based on our interpretation of results from other researchers who examined a limited number of individuals. The serotonin synthesis schematic (A) is based on PET scans of 30 children with autism and 24 controls (8 siblings and 24 children with epilepsy) (Chugani et al 1999). The synapse schematic (B) is based on 12 postmortem specimens (Huttenlocher 1079.) Thus, the exact shapes of the curves is not known.

Table 1

Hypothetical targets for early interventions in autism.

Target Therapeutic Action	Mechanism	Drug Class (Candidate Agent)
Increase Ach signaling	Decrease Ach catabolism	Cholinesterase inhibitor (donepezil)
Increase D_1 signaling	D_1 receptor agonism	D_1 agonist (dihydrexidine)
	Increase dopamine release	valproic acid
	Increase $5HT_{1A}$ activation	valproic acid
Increase $GABA_A$ signaling	Allosteric modulation of $GABA_A$ receptors	Steroids (allopregnanlone, estradiol) Benzodiazepines
	Increase GABA	valproic acid
Decrease glutamate signaling	Decrease glutamate release	lamotrigine
Increase glutamate signaling	AMPA receptor agonist	Ampakines (CX516)
Decrease $5HT_{2A}$ signaling	$5HT_{2A}$ antagonism	
Increase brain serotonin	Decrease 5HT reuptake	SSRIs
Decrease BDNF signaling	Block TrkB receptors	
	Decrease BDNF synthesis	
Increase reelin signaling	Decrease BDNF signaling	
	Increase reelin synthesis	
	Upregulate VLDL and ApoE2 receptors	
More permissive synaptic reorganization	Disrupt extracellular matrix associated with dendrites	tPA

Animal studies suggest certain molecules regulate brain plasticity during development. However, significant caution should be exercised given the spatial selectivity of these processes in development and the global actions of medications. Some agents, like tPA, will likely require extensive tests in animals prior to trials in children. Further, it may be a combination of treatments that will produce maximal benefits, while minimizing toxicity.

Table 2

Published clinical trials with improvement in core autism features.

Drug Class Agent	Presumptive Mechanism	Age (yrs)	n	Design Duration (mo)	Core Symptoms Improvement	Study
SSRI	**block 5HT reuptake**					
fluoxetine		2 – 7	37	Open, 13 – 33	Social, language	DeLong 1998
		3 – 13	12	Open, 52	Language, RRB	Alcami 2000
		2 – 8	129	Open, 5 – 76	Social, language, RRB	DeLong 2002
		5 – 17	44	RCT-crossover, 5	RRB	Hollander 2005
SNRI	**block 5HT reuptake block NE reuptake**					
venlafaxine		3 – 21	10	Open	Social, language, RRB	Hollander 2000
APD	**D_2 antagonist $5HT_{2A}$ antagonist**					
risperidone		5 – 18	18	Open, 3	RRB, social	McDougle 1997
		3 – 7	24	Open, 4	Nonverbal communication	Masi 2001
		5 – 17	101	RCT, 2	RRB	McDougle 2005
		5 – 17	63	Open, 4	RRB	McDougle 2005
		5 – 16	48	Open, 6	Communication, social	Williams 2006
olanzapine		5 – 17	6	Random open, 1.5	RRB, social	Malone 2001
Cholinesterase Inhibitors	**increase synaptic acetylcholine**					
rivastigmine		3 – 12	32	Open, 3	Language, RRB	Chez 2004
AED						
divalproex	**increase NPY** **increase GABA**	5 – 17	13	RCT, 2	RRB	Hollander 2006
Miscellaneous						
cyproheptadine	**$5HT_2$ antagonist**	3 – 11	40	RCT, 2	Social, language	Akhonzadeh 2004
omega-3 FA	**unknown**	5 – 17	13	RCT, 1.5	RRB	Amminger 2006

SSRI: selective serotonin reuptake inhibitor; SNRI: serotonin norepinephrine reuptake inhibitor; APD: antipsychotic drug; AED: antiepileptic drug; NPY: neuropeptide Y; NE: norepinephrine; 5HT: serotonin; $5HT_2$: serotonin receptor type-2; D_2: dopamine receptor type-2

Core symptoms are broadly defined as social deficits, language/communication impairment, and restrictive/repetitive behaviors (RRB). Study designs and outcomes were extremely heterogeneous. Published trials involving 6 or more participants were included if a standard assessment of core symptoms noted statistically significant improvement. Retrospective and ongoing clinical trials were excluded