Published in final edited form as:

Int Rev Neurobiol. 2013 ; 113: 61–96. doi:10.1016/B978-0-12-418700-9.00003-4.

Identifying Essential Cell Types and Circuits in Autism Spectrum Disorders

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Abstract

Autism spectrum disorder (ASD) is highly genetic in its etiology, with potentially hundreds of genes contributing to risk. Despite this heterogeneity, these disparate genetic lesions may result in the disruption of a limited number of key cell types or circuits –information which could be leveraged for the design of therapeutic interventions. While hypotheses for cellular disruptions can be identified by postmortem anatomical analysis and expression studies of ASD risk genes, testing these hypotheses requires the use of animal models. In this review, we explore the existing evidence supporting the contribution of different cell types to ASD, specifically focusing on rodent studies disrupting serotonergic, GABAergic, cerebellar and striatal cell types, with particular attention to studies of the sufficiency of specific cellular disruptions to generate ASD-related behavioral abnormalities. This evidence suggests multiple cellular routes can create features of the disorder, though it is currently unclear if these cell types converge on a final common circuit. We hope that in the future, systematic studies of cellular sufficiency and genetic interaction will help to classify patients into groups by type of cellular disruptions which suggest tractable therapeutic targets.

Keywords

autism; serotonin; GABA; cerebellum; striatum; rodent behavior; conditional deletion

Introduction to Cell Types and Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a pervasive developmental disorder, with prevalence rates now estimated at more than 1 in 100 (Kirov et al., 2012). While often co-morbid with a variety of other medical problems and behavioral difficulties, including epilepsy, ADHD, and intellectual disability, a diagnosis of ASD is defined by key deficits in social interaction and communication, as well as restricted interests, stereotyped behaviors, and resistance to change. Twin and family studies suggest that a substantial proportion of the risk for developing ASD is heritable (Bailey et al., 1995; Hallmayer et al., 2011), with monozygotic twins displaying 60–90% concordance in ASD diagnoses. However, genetic studies indicate a remarkable heterogeneity, with recent estimates suggesting hundreds of different genes may contribute to this disorder (Klei et al., 2012). Yet, individuals with these different genetic etiologies share a common symptomatology. This suggests that these distinct genetic lesions must be converging on a discrete set of cell types or circuits in the brain that mediate these behavioral disruptions.

The brain contains hundreds of distinct cell types, each a wide range of morphological, anatomical, and molecular features. However, pathological conditions can be caused by disruption of just a single cell type. For example, destruction of dopaminergic neurons of the substantia nigra is sufficient to generate most of the characteristic symptoms of Parkinson's disease. Likewise, removal of hypocretin neurons is sufficient to generate the behavioral features of narcolepsy with cataplexy (Peyron et al., 2000; S. Zhang et al., 2007). This suggests that individual neurological symptoms can be mapped to deficiencies in particular cell types or circuits. A key question for the neurobiology of ASD is which cellular disruptions are sufficient to create the symptoms. Importantly, an understanding of cellular and circuit level disruptions may permit the identification of novel avenues for treatment – if individuals with distinct genetic lesions share a common circuit level pathology, then the circuit becomes the target of treatment, rather than the gene. For example, although the genetics of risk for idiopathic Parkinson's are not understood for most cases, most cases are responsive to dopamine replacement therapies. It is our hope that identification of cellular disruptions that are sufficient to generate ASD symptomatology will lead to similar insights for treatment that will be broadly applicable.

How can one identify these cellular disruptions? There are several complimentary approaches. Most directly, human brain imaging of patient populations and post-mortem anatomical experiments allow for identification of regional and or cellular abnormalities from human patients, respectively. More indirectly, one can examine the expression patterns of known ASD risk genes to identify the cells and regions most likely to be impacted by their disruption. For example, if an ASD risk gene is only expressed in one population of cells in the brain, then these cells must almost certainly mediate the effect of the gene's mutation. These approaches allow for the generation of hypotheses regarding cellular deficits, but testing these hypotheses requires the use of animal models. Therefore, an essential complement to patient-oriented approaches is the use of animal models to test the sufficiency of disruptions in particular cell types to recreate key symptoms of the disorder. This can be done genetically in the mouse either by the deletion of a gene only employed by a single cell type in the brain, or by creating conditional knockouts using the Cre-Lox system (Nagy, 2000).

Here, we are going to focus on reviewing the existing data, particularly from conditional mouse models, regarding the sufficiency of certain cell types for the generation of ASD-like behavioral features. We will briefly overview the genetics of the disorder with a focus on using the expression of ASD-associated genes to guide us towards circuits that may be most disrupted by their loss. We will then briefly discuss behavioral methods of assessment of the ASD-like symptoms in rodents, as a preview to presenting the current knowledge regarding the cell types that may mediate ASD-like behaviors. We will focus on the four regions or cell types that have received substantial attention in ASD thus far: serotonin producing neurons, GABAergic interneurons, the cerebellum, and the striatum.

Genetics of Autism Spectrum Disorder

The genetics of ASD are thoroughly reviewed in recent publications (Berg & Geschwind, 2012; Geschwind, 2011; Persico & Napolioni, 2013). Briefly, the genetic variation contributing to risk in ASD has been investigated in one of several ways. These have included identification of genes for classic ASD-associated syndromes, common variant association studies, and rare variant analyses. ASD-associated syndromes, such as Fragile-X Syndrome, Rett Syndrome, and tuberous sclerosis are typically caused by highly penetrant, loss-of-function mutations in single genes (*FMRI*, *MECP2* and *TSC1* or 2, respectively). Some of these genes have been shown to have regionalized patterns of expression in the brain, such as *SHANK3* which is involved in Phelan-McDermid Syndrome and the

expression of which is enriched in the striatum. However, ASD-associated syndromes account for less than 10% of ASD cases. Genetic variation contributing to risk in idiopathic cases was initially explored through genome-wide association studies (GWAS) of common variants using case-control and family studies (Anney et al., 2012; Berg & Geschwind, 2012; K. Wang et al., 2009; Weiss, Arking, Daly, & Chakravarti, 2009). Common variation has been shown to contribute significantly to risk in a multiple hit, or oligogenic, model (Klei et al., 2012) though the contribution of each gene is quite small. Currently no loci have been reproducibly associated, yet estimates are that several hundred genes will be implicated once enough subjects have been collected for sufficient statistical power. Variants currently approaching significance are near genes previously suggested to be ASD-associated (Anney et al., 2012). There has also been considerable effort in the last several years in understanding the contribution of rare or *de novo* single nucleotide and copy number variants (SNVs and CNVs) in contributing to ASD risk. In a landmark set of publications in 2012, whole exome sequencing (WES) was performed to identify the burden of non-synonymous variants in ASD cases compared to controls (Chahrour et al., 2012; Iossifov et al., 2012; Neale et al., 2012; O'Roak et al., 2012; Sanders et al., 2012). These reports make use of families, which include trios (two parents and ASD proband) as well as quartets (trios with unaffected sibling). Some of these reports have benefited greatly from the latter (Iossifov et al., 2012; O'Roak et al., 2012; Sanders et al., 2012) as the burden in probands can be compared directly to their siblings at the same loci. These reports identify about 120 novel risk genes, only 8 of which were previously suggested by GWAS (SETBP1, SHANK2, DYRK1A, SLC7A7, RPS6KA3, RELN, NRXN1, and *GRIN2B*).

Many reports, of both common & rare variation, attempt to make sense of these genes by analysis of protein-protein interaction networks, or analyses for enrichment in particular biological processes or molecular functions, as defined by Gene Ontologies (GO). However, it has long been recognized that the data used to generate protein interaction networks suffer from a high false discovery rate (Deane, Salwinski, Xenarios, & Eisenberg, 2002), and GO term assignments also exhibit biases (du Plessis, Skunca, & Dessimoz, 2011). It may be helpful to augment these networks with information regarding overlapping spatial and temporal patterns of expression. A few of the genes recently identified by WES are known to have regionalized patterns of expression, and these are summarized in Table 1. The more we learn about the spatial and temporal dynamics of ASD risk genes, the better poised we are to make testable hypotheses about underlying cellular and molecular mechanisms in the disorder. Currently lacking is a method akin to GO, which provides statistical analysis for the enrichment of candidate genes in particular regions or cell types. We have recently developed such a method, cell-type specific expression analysis (CSEA), and application of this method to lists of previously implicated genes in ASD (Basu, Kollu, & Banerjee-Basu, 2009) suggests a modest enrichment in genes found in cortical interneurons and the striatum (not shown). These analyses will likely be more informative as the number of candidate genes increases.

Furthermore, with advances in genome editing technologies (Joung & Sander, 2013; H. Wang et al., 2013), it will become easier to model the effects of targeted mutations, in single and multiple genes, and study the effects of these on behavior. While the relevance to ASD risk has been explored in mouse knockout models for many genes, targeted knock-out studies in specific cell populations have not been performed for the overwhelming majority of candidate genes, with the exception of genes for some ASD-related syndromes such as *Tsc1* (Reith et al., 2013) and *Mecp2* (Adachi, Autry, Covington, & Monteggia, 2009; Alvarez-Saavedra, Saez, Kang, Zoghbi, & Young, 2007; Chao et al., 2010; Fyffe et al., 2008; Michaelson et al., 2012; Samaco et al., 2009).

Mouse models of ASD risk genes provide powerful tools for both exploring the cellular phenotype that results when these genes are disrupted, as well as the larger phenotype of the behaving animal, often mirroring the symptoms observed in humans. In the subsequent sections we will first review how ASD-like behaviors are identified and interpreted in mice. Then we will proceed to discuss the existing evidence in several mouse models, with a focus on conditional deletions and ASD genes that implicate certain circuits by their spatially or temporally restricted patterns of expression.

Brief review of rodent behavioral assays relevant to ASD symptoms

Mouse models provide an important complement to environmental and genetic studies of ASD risk in humans. Mouse models serve three essential functions. First, models mimicking human genetic polymorphisms – particularly rare deleterious variants – provide valuable experimental support for the causality of the genetic mutation. If a rare variant in humans implicates a particular gene, even when there are not sufficient cases available for statistical association, recapitulation of ASD-like features in the mouse provides strong causal inference for the role of the gene in ASD-like behaviors (Abrahams & Geschwind, 2008). Disruptions of genes responsible for human syndromes with some association to ASD, such as Rett or Fragile X, result in similar syndromes in mice (Consortium, 1994; Shahbazian et al., 2002). Second, mice provide experimental opportunities to dissect the neurobiological mechanisms mediating both normal social behaviors, as well as cellular disruptions resulting from particular genetic manipulations. Given the large degree of heterogeneity in human causes of ASD, identification of common neurobiological features across models will be essential to development of broadly applicable treatments.

ASD in humans is defined by deficits in social behavior and communication, as well as stereotypies and resistance to change. While any human disease cannot be perfectly modeled in a mouse, both social and communicative behavior, as well as resistance to change, can be operationally assayed in the mouse by a variety of behavioral paradigms. In this first section, the most common assays used to evaluate ASD-relevant behaviors will be briefly discussed as they are reviewed elsewhere in greater detail (Crawley, 2012; Moy, Nadler, Magnuson, & Crawley, 2006; Silverman, Yang, Lord, & Crawley, 2010; Wohr & Scattoni, 2013). Then we will discuss what cell- or region-specific genetic alterations reveal about the neurobiology of ASD symptoms.

As mice are social creatures, several assays have been developed to assess disruptions to social behaviors. Key social behavior paradigms include social interaction, juvenile play and resident intruder, which evaluate reciprocal social behaviors during full contact between the mice (Pellis & Pasztor, 1999; Scattoni, Martire, Cartocci, Ferrante, & Ricceri, 2012; Scattoni et al., 2008) and the social approach assay, which is designed to measure sociability initiated by the test mouse only (Moy et al., 2004). Abnormal social behaviors can include decreased or increased sociability measured by proximity to or contact with another mouse, agonistic behaviors indicating increased aggression, or increased socio-positive behaviors like following or allogrooming. Evaluation of a genetic model in multiple social assays will allow for a more complete understanding of the nature of the social deficit.

While mice do not use language, mice do employ vocal systems of communication which are socially-conditioned, allowing for analysis of communication deficits. Mice emit ultrasonic vocalizations (USVs) in response to certain social stimuli, such as maternal separation, a possible sexual partner, or a territorial intruder. In the maternal separation paradigm, frequently used in the ASD literature, mouse pups emit USVs in response to separation from the dam. While this is used as a measure of communication (Hofer, Shair, & Brunelli, 2002), factors like anxiety levels can greatly impact this behavior as well.

Additionally, mice produce vocalizations during juvenile social encounters, though this assay has rarely been employed in mouse models of ASD (Cheh et al., 2006; McFarlane et al., 2008; Panksepp et al., 2007; Scattoni, Ricceri, & Crawley, 2011). Deficits in USVs during juvenile social encounters in mice may have more face validity for communication deficits seen in children with ASD during peer interactions.

Aside from vocalization, urinary scent marking behaviors, such as time near another animal's urinary mark or frequency of urinary marks in response to another animal, can serve as a measure of social communication (Kane et al., 2012). This may be a more ethologically representative assay of communicative behaviors as mice rely heavily on olfaction as a mode of communication (Arakawa, Blanchard, Arakawa, Dunlap, & Blanchard, 2008), but does not have a clear human analog.

A variety of assays exist to evaluate the different aspects of restricted behaviors or resistance to change. Interpretation of stereotyped or even repetitive behaviors can be more straightforward than that of assays assessing resistance to change. Assays include quantification of spontaneous stereotyped behaviors such as self-grooming, digging in bedding, or locomotor activities like circling or flipping, (Silverman et al., 2010) and repetitive behaviors such as increased marble burying or nestlet shredding in the homecage (Kane et al., 2012; Thomas et al., 2009). Resistance to change also is measured by failure to exhibit a wild type (WT)-like change in behavior during reversal tasks such as rewarded alternation T-maze or reversal trials in the Morris water maze. These require the mouse to extinguish a previously learned response in favor of a new one (Kirsten et al., 2012; Moy et al., 2006). Performance in exploratory tasks can be used to evaluate resistance to change such as the spontaneous alternation T-maze or holeboard exploration/olfactory preference test which measure the tendency of the mouse to repeatedly explore the same arm sequentially (Silverman et al., 2010) or change hole-poking behavior following familiarization with a food reward (Dougherty et al., 2013; Moy, Nadler, Poe, et al., 2008), respectively. It is valuable to evaluate mice in multiple assays to understand the full range of the model's behavioral disinhibition phenotype.

The multiple comorbidities associated with ASD can also be tested in rodents. Hyperactivity can be assessed in open field assays, learning and memory with the classic Morris water maze or Barnes maze, and epilepsy is readily apparent by EEG studies of the rodent cortex. In addition, though it is not part of the classical diagnostic criteria for ASD, children with ASD often show profound deficits in motor behavior (reviewed by Chukoskie, this volume Chapter 7), which can also be assessed readily in the mouse using sensorimotor batteries as well as rotarod assays.

As with all analyses of complex behaviors, performance in tests relevant to ASD requires some baseline motor capacity. Therefore, it is important to evaluate the locomotor activity levels and sensorimotor abilities of the mice in the proper control tasks to permit appropriate interpretation of more complex behavioral results (Dougherty et al., 2013; Moy, Nadler, Young, et al., 2008). The behavioral assays listed above do not exhaust those applicable to ASD-related behaviors. Comprehensive and informative reviews on the subject are available and should be consulted when designing a study of an ASD model (Crawley, 2012; Moy et al., 2006; Silverman et al., 2010; Wohr & Scattoni, 2013). Nonetheless, the assays discussed are the most widely used in the studies highlighted below characterizing the cellular mediators of ASD behaviors.

ASD models involving serotonergic neurons

Serotonin (5-HT) neurons were one of the earliest suspected cell types to be disrupted in individuals with ASD. Although their cell bodies are restricted to the raphe nuclei of the

midbrain and hindbrain, 5-HT neurons project widely throughout the neuraxis and play a profound neuromodulatory role in the behavior of many other circuits and cell types. 5-HT has long been implicated in regulation of normal behaviors such as sleep and arousal, in addition to potentially being involved in a range of psychiatric disorders. Disruption of the serotonergic system is clearly sufficient to induce abnormal social behaviors, with increased aggression being the most frequently reported throughout the animal literature (See (Miczek et al., 2004) for a review). A number of lines of evidence from clinical populations, pharmacotherapy studies, and genetic mouse models suggest that abnormalities in the serotonergic system may also contribute to the etiology of ASD. Primarily, it is widely replicated that at least 25% of ASD patients have elevated levels of 5-HT in whole blood platelets, not due to possible artifacts such as diet (Anderson et al., 1987; Betancur et al., 2002; E. H. Cook & Leventhal, 1996; Schain & Freedman, 1961). While 5-HT in the blood does not derive from the central nervous system (CNS), the 5-HT transporter protein (SLC6A4) is responsible for the uptake of 5-HT into the blood platelets as well as terminals in the brain (Lesch, Wolozin, Murphy, & Reiderer, 1993). Blood platelet hyperserotonemia could result from increased SLC6A4 activity in ASD individuals, which would also deplete synapses of 5-HT more quickly, ultimately reducing 5-HT activity in the brain. Examination of human postmortem tissue revealed increased SLC6A4 immunoreactivity in the brains of autistic subjects (Azmitia, Singh, & Whitaker-Azmitia, 2011). This is further supported by studies showing that decreasing 5-HT activity through tryptophan depletion, the 5-HT precursor acquired through diet, can exacerbate repetitive thoughts and behaviors, aggression, anxiety and irritability in ASD adults (E. H. Cook & Leventhal, 1996; McDougle et al., 1993). These findings suggest a role for low synaptic levels of 5-HT in a subset of ASD cases.

Drugs that act to increase 5-HT activity in the brain have been investigated as pharmacotherapies for ASD symptoms. Clinical trials investigating the use of Selective Serotonin Reuptake Inhibitors (SSRIs) in the treatment of ASD symptoms have yielded mixed results. A small but significant effect in the treatment of repetitive behaviors with SSRIs is suggested in the published literature (Carrasco, Volkmar, & Bloch, 2012). However, the effect may be due to publication bias if studies which find a lack of support for SSRI therapy in ASD remain unpublished (Carrasco et al., 2012). This indicates that inhibition of SLC6A4 and the resulting increase in synaptic 5-HT is not sufficient as a treatment for ASD symptoms. However, if serotonergic dysfunction only results in a subset of ASD cases, as the hyperserotonemia results suggest, then complete efficacy of SSRIs in the treatment of all ASD individuals is not expected. Current FDA-approved drugs for the treatment of ASD include the atypical antipsychotics aripiprazole (Abilify) and risperidone (Risperdal). These drugs act as antagonists or inverse agonists at many 5-HT receptors and SLC6A4, as well as other neuromodulatory receptors such as dopaminergic, adrenergic, histaminergic, and muscarinic receptors. These drugs reduce irritability, hyperactivity, and stereotypies/repetitive behaviors in children and adolescents with ASD. This is a similar reduction as seen with SSRI treatment, but with a more rapid onset (Canitano & Scandurra, 2011; Ching & Pringsheim, 2012; E. H. Cook & Leventhal, 1996). These findings implicate the serotonin system in the symptoms of resistance to change or repetitive behaviors, at least in regard to acute response to pharmacological treatments. Genetic models which globally disrupt genes whose expression is specific to the serotonin system, or which conditionally disrupt ASD-associated genes in serotonergic neurons, serve as tools to dissect the role played by the serotonin system in ASD-related behaviors.

Thus far, there are very few 5-HT-specific disruptions of broadly expressed ASD-associated genes in animal models. One method for doing so uses the promoter for the *Fev* gene (also known as *Pet1*) to drive expression of Cre recombinase. *Fev* is an Ets-family transcription factor shown to be necessary for early specification of the 5-HT neurons (Hendricks et al.,

2003). From among genes associated with ASD risk, to date, this has only been employed to disrupt the Rett syndrome gene *Mecp2* (behavioral features of Rett syndrome will be discussed in greater detail below). Serotonergic-specific disruption of this gene results in a decrease of the serotonin synthesis enzyme tryptophan hydroxylase 2 (Tph2) and a concomitant decrease in 5-HT levels. These mice demonstrate increased aggressive behaviors, but no evidence of repetitive behaviors. *Mecp2* deletion in 5-HT neurons was clearly not sufficient to recreate the entire Rett syndrome phenotype as these mice also did not show motor deficits, breathing irregularities, or heightened anxiety (Samaco et al., 2009). In contrast to this conditional deletion, there are a fair number of deletions of genes specific to serotonin cells, such as *Tph2* and *Slc6a4* (the serotonin transporter), which can also serve to more broadly elucidate the sufficiency of serotonergic disruption in generating ASD-like behaviors.

Many mutations of 5-HT-cell-specific genes result in ASD-like behaviors (see Table 2). Complete depletion of brain 5-HT by deletion of the gene encoding Tph2, the rate-limiting enzyme in the synthesis of CNS 5-HT, results in abnormal social behaviors, communication deficits and repetitive behaviors (Alenina et al., 2009; Angoa-Perez et al., 2012; Kane et al., 2012; Mosienko et al., 2012). A knock-in mouse model expressing a mutant, low-activity form of Tph2, equivalent to a rare human variant, also exhibits abnormal social behavior and an approximate 80% reduction in brain 5-HT (Beaulieu et al., 2008). Likewise, mice null for *Slc6a4*, exhibit abnormal social behaviors and repetitive behaviors as well as a loss of about half of the serotonin-expressing neurons and reduced overall brain 5-HT levels (Kalueff, Fox, Gallagher, & Murphy, 2007; Moy et al., 2009). While an increase in 5-HT concentration has been reported in specific brain areas like the striatum of *Slc6a4*^{-/-} mice (Mossner, Simantov, Marx, Lesch, & Seif, 2006) it is likely due to compensatory 5-HT uptake by the dopamine transporter in these areas (Zhou, Lesch, & Murphy, 2002) and not reflective of an overall increase in brain 5-HT. Heterozygous *Slc6a4* mutants display ASD-like behaviors to a lesser degree than *Slc6a4*^{-/-} mice, however 5-HT levels were not reported in these mice (Kyzar et al., 2012; Moy et al., 2009). Mice expressing a high activity *Slc6a4* variant, Ala56, have unchanged overall 5-HT levels but do exhibit increased 5-HT clearance rates (Veenstra-VanderWeele et al., 2012). Disrupted social and communicative behaviors as well as increased stereotyped behaviors are demonstrated by these mice. Interestingly, both mice lacking *Slc6a4* and those expressing a high activity *Slc6a4* variant exhibit ASD-like phenotypes. Both models would be predicted to have a decrease of synaptic serotonin overall – the Ala56 variant due to more rapid clearance, and the knockout due to long-term depletion of serotonin from the presynapse in the absence of the ability to efficiently recycle the transmitter. Finally, mice mutant for the *Itgb3* gene, which encodes a protein that interacts with *Slc6a4*, show slight social behavior deficits and repetitive behaviors as well as a reduced volume of the serotonergic-expressing neurons of the dorsal raphe nucleus (Carter et al., 2011; Ellegood, Henkelman, & Lerch, 2012).

Building on these findings we employed Translating Ribosome Affinity Purification (TRAP) to identify additional gene transcripts enriched in the serotonergic system and screened for polymorphisms in patients that may be related to ASD symptoms (Dougherty et al., 2013). Of the transcripts identified, we found that polymorphisms in *CELF6*, which is thought to code for an RNA-binding protein, may contribute to ASD risk in patients. Global disruption of the murine orthologue of *CELF6* resulted in a 30% decrease in levels of 5-HT extracted from brain tissue, early communicative deficits, and evidence for resistance to change. Overall, this suggests polymorphisms in the *Celf6* gene may contribute to ASD-related behaviors in mice and humans.

Though it is difficult to measure directly, ostensibly all of these mutations appear to reduce the levels of synaptic 5-HT activity. Thus, taken together, the above studies suggest

disruptions of 5-HT neurons that result in reduced synaptic 5-HT are sufficient to generate some ASD-related behaviors. This is supported by a study that restored 5-HT levels in *Tph2*^{-/-} mice through administration of the immediate 5-HT precursor, 5-hydroxytryptophan, and reported rescue of social behaviors and partial rescue of repetitive behaviors (Angoa-Perez et al., 2012). Yet, questions remain as to the exact neurobiological mechanism by which these disruptions lead to ASD like behaviors, and whether a 5-HT-related mechanism accounts for as substantial a proportion of human cases that the blood findings would suggest. Since rescue of 5-HT levels in mice only partially alleviates disrupted behaviors (Angoa-Perez et al., 2012), this suggests reduced 5-HT levels may also have durable developmental consequences that may also contribute to ASD symptoms. Likewise, as a largely neuromodulatory system, 5-HT mediated behavior disruptions must be transmitted through other circuits that are more directly wired as executors of behavior.

ASD models involving GABAergic interneurons

γ -Aminobutyric acid (GABA) is the dominant fast-acting inhibitory neurotransmitter in the brain, and GABAergic interneurons have fundamental roles in multiple circuits, including in the cortex, in fine tuning the transmission of information and in suppressing excess excitation. GABAergic interneurons make up only about 20% of cortical neurons, yet these neurons are integral to maintaining proper function and balance in cortical circuits (Markram et al., 2004; Taniguchi et al., 2011). A disturbance in the CNS excitation/inhibition balance between the glutamatergic and GABAergic systems has been suggested in the etiology of ASD (Rubenstein & Merzenich, 2003) and is consistent with the observed high comorbidity with epilepsy. The animal model research suggests the primary factor in the excitation/inhibition imbalance is loss of GABAergic inhibitory control over excitatory neurons. This loss of inhibition appears to occur one of two ways: either disruption in GABAergic neurotransmission at the synaptic level or aberrant organization or loss of GABAergic neurons during development. Mutations in several synaptic genes, such as those encoding neuroligins, members of the SHANK family of proteins at the synaptic density, and neurexins, give rise to ASD-relevant phenotypes in mouse models (see (Persico & Napolioni, 2013) for review), supporting the hypothesis of altered synaptic communication in ASD etiology. And, there is some support for a deficit in cortical interneurons from one human post-mortem transcriptomic study (Voineagu et al., 2011). Below, genetic models of GABAergic perturbation in relation to ASD-relevant behaviors are discussed.

At the synapse, the GABA_A receptor is highly involved in the inhibition of excitatory neural pathways and is expressed early in development (Muhle, Trentacoste, & Rapin, 2004). Cytogenetic abnormalities within the human chromosome 15q11–q13 region, which houses the GABA_A receptor subunit genes *GABRB3*, *GABRA5*, and *GABRG3*, have been associated with ASD susceptibility, as well as the neurodevelopmental disorders Prader-Willi syndrome and Angelman syndrome, which are frequently comorbid with ASD (Buxbaum et al., 2002; E. H. Cook, Jr. et al., 1998; Michaelson et al., 2012; Persico & Napolioni, 2013; Wagstaff et al., 1991).

Mice mutant for the GABA_A receptor shed light on the potential for disrupted GABAergic neurotransmission to generate ASD symptoms. Homozygous *Gabrb3* knockouts, and to a lesser extent heterozygous knockouts display EEG abnormalities and epilepsy along with sensory disturbances (DeLorey et al., 1998; Liljelund, Handforth, Homanics, & Olsen, 2005; Ugarte, Homanics, Firestone, & Hammond, 2000). Behavioral phenotyping relevant to ASD symptoms revealed repetitive behaviors, behavioral disinhibition and abnormal social behaviors in *Gabrb3* deficient mice (DeLorey et al., 1998; DeLorey, Sahbaie, Hashemi, Homanics, & Clark, 2008). These results indicate that either disruption of inhibitory control

directly results in ASD behaviors or the ensuing hyperexcitability disrupts the homeostasis of other systems in the brain controlling these behaviors.

Other mouse models provide support for perturbations of the GABAergic system in ASD etiology, by conditional deletion of broadly expressed genes. For example, mice completely deficient for *Mecp2*, a mouse model of Rett syndrome, develop normally until about 5 weeks of age and then exhibit physical and behavioral declines (R. Z. Chen, Akbarian, Tudor, & Jaenisch, 2001). These mice demonstrate hyperactivity, abnormal social behaviors, motor deficits, irregular breathing, stereotypic and repetitive behaviors, decreased weight, anxiety, and premature lethality (Chao et al., 2010; Guy, Hendrich, Holmes, Martin, & Bird, 2001; Schaevitz, Moriuchi, Nag, Mellot, & Berger-Sweeney, 2010). In the CNS, they show decreased brain weight and brain cell size with a decrease in cortical activity resulting from a shift in the excitation/inhibition balance (Dani et al., 2005). The diminished inhibitory rhythmic activity renders circuits like the hippocampal CA3 circuit prone to hyperexcitability (L. Zhang, He, Jugloff, & Eubanks, 2008). Abnormal sensorimotor behaviors are reversed in *Mecp2* null mice with ketamine treatment (Kron et al., 2012) suggesting the consequent hyperexcitability from *Mecp2* deletion is primary in the behavior etiology.

Overall, only partial recapitulation of the Rett syndrome phenotype is observed with conditional deletions of *Mecp2* using the Cre-Lox system to target the glutamatergic pyramidal cell layer of the forebrain (Alvarez-Saavedra et al., 2007), dopaminergic cells (Samaco et al., 2009), serotonergic cells (Samaco et al., 2009), hypothalamic cells (Fyffe et al., 2008), and amygdalar cells (Adachi et al., 2009). Of these, only the glutamatergic conditional knockout demonstrates abnormal social interaction and the serotonergic and hypothalamic conditional knockouts display increased aggression, although no other behaviors relevant to the core ASD symptoms were observed. The dopaminergic and amygdalar conditional knockouts did not exhibit any of the phenotypes relevant to the core ASD symptoms.

In contrast, disruption of the GABAergic system is sufficient to generate the Rett syndrome phenotype in *Mecp2*^{-/-} mice. The use of the GABA vesicular transporter (*Viaat*) as the promoter region driving the expression of Cre recombinase results in specific depletion of *Mecp2* from greater than 90% of GABA-expressing neurons and a complete recapitulation of the Rett syndrome phenotype (Chao et al., 2010). When *Mecp2* is deleted specifically from the GABA-expressing neurons only in the forebrain using a *Dlx5/6-Cre*, the core ASD-relevant behaviors are still observed including repetitive behaviors, abnormal social behaviors, and impaired sensorimotor gating (Chao et al., 2010). For Rett syndrome at least, these disruptions seem to be due to acute loss of *Mecp2*, and not to abnormal circuit formation during development; inducible deletion of *Mecp2* in the adult mouse was sufficient to recapitulate some of the behavioral features of the germline mutation (although social behaviors were not assessed) (McGraw, Samaco, & Zoghbi, 2011). This suggests the imbalance of inhibitory control over excitation induced by the absence of *Mecp2* in the brain may be reversible.

In *Mecp2*, more than in any other model, multiple groups have attempted postnatal 'rescue' experiments by variously expressing *Mecp2* under the control of various cell specific and ubiquitous promoters. These are an important complement to the cell-specific deletion experiments. Deletions indicate which cell types are sufficient to disrupt the behavior. Cell-specific rescue experiments indicate which cell types are sufficient for normal behavior, and also provide some indication if the deficits are due to acute loss of the protein, or permanent abnormalities that are a consequence of the absence of *Mecp2* during development. They also serve as a model for potential treatment strategies. However, the interpretation of these

studies is complicated by the potentially non-physiological levels of expression of *Mecp2* from exogenous promoters, and may account for the differences seen across studies (Giacometti, Luikenhuis, Beard, & Jaenisch, 2007; Guy, Gan, Selfridge, Cobb, & Bird, 2007; Jugloff et al., 2008; Luikenhuis, Giacometti, Beard, & Jaenisch, 2004). Thus far, an interneuron-specific *Mecp2* rescue has not been demonstrated.

Other mutant models have provided evidence that disruption of GABAergic inhibitory neurotransmission can result in an ASD-like phenotype. The mouse model of the ASD related syndrome, Dravet's syndrome, which is caused in humans by heterozygous loss-of-function mutations in the *SCN1A* gene, exhibits a 20–50% reduction in the α -subunit of the brain voltage-gated Na^+ channels. This is the primary Na^+ channel in GABAergic interneurons and thus is critical for action potentials in these neurons (Han et al., 2012). GABAergic-specific deletion of *Scn1a* using the *Dlx1/2 Cre*, revealed that ASD-relevant behaviors in *Dlx1/2-Scn1a*^{+/-} mice, particularly abnormal social behaviors, are due to decreased GABAergic neurotransmission specifically in the forebrain. These behaviors were reversed with benzodiazepine administration. This study not only strongly implicates the sufficiency of the loss of inhibitory control in the forebrain for abnormal social behaviors but also further suggests abnormal social behaviors in some ASD patients may not be irreversible consequences of neural development and may, in fact, be treated in some manner with anticonvulsants or anxiolytics. Benzodiazepines are often prescribed to individuals with ASD (Oswald & Sonenklar, 2007), although typically for management of epilepsy and co-morbid anxiety disorder, and not explicitly for social behaviors.

However, it is clear that aberrant developmental organization of the GABAergic neurons may also result in disrupted GABA inhibition of excitatory neurons. Reeler mice, which lack the *Reln* gene that encodes a large glycoprotein secreted by GABAergic interneurons and glutamatergic cerebellar neurons, show extreme cell positioning abnormalities in the lamina of the neocortex and cerebellar cortex (Goffinet, 1984). Reeler mice also exhibit abnormal social behaviors and sensorimotor gating, and repetitive behaviors (Persico et al., 2001; Salinger, Ladrow, & Wheeler, 2003). The dysfunction resulting from the aberrant cell organization may be ameliorated, however. Reintroduction of Reelin into an adult Reeler mouse brain has been shown to alter dendritic spine morphology and alleviate associative learning deficits (J. T. Rogers et al., 2013). Whether this can rescue abnormal social behaviors has yet to be investigated. These studies suggest that brain plasticity may be the key to therapies for ASD symptoms, particularly social deficits, stemming from excitation/inhibition imbalance.

Finally, altered inhibition through GABAergic dysfunction may be a mechanism by which ASD-related behaviors develop in other, non-GABA-specific models of ASD. For example, many interneurons express 5-HT receptors (Willins, Deutch, & Roth, 1997), and *Tph2*^{-/-} mice exhibit alterations in GABA levels in areas of the forebrain (Waider et al., 2013). This suggests 5-HT levels, either acutely or during development, may influence the overall inhibitory control of excitatory neurons. Given that genetic ASD models specific to the serotonergic and GABAergic systems independently express similar behavioral phenotypes, a similar etiological mechanism is possible. It may be that the ASD-like behaviors in 5-HT models are ultimately due to GABA-dependent deficits in inhibitory control. Genetic interaction studies, such as are common in *Drosophila*, may prove fruitful in addressing this question. If crossing 5-HT-related ASD models with GABA-related ASD models provides no further exacerbation of the phenotype, the suggestion would be that they are in the same genetic pathway.

ASD models involving the cerebellum

A variety of clinical studies have reported cerebellar abnormalities in autistic brains. For example, reduced cerebellar gray matter in autistic subjects was correlated with Autism Diagnostic Interview-Revised and Autism Diagnostic Observation Schedule-Generic Scores in a voxel-based morphometry study (Riva et al., 2013). Imaging studies have reported increased cerebellar activation during a motor task (Allen, Muller, & Courchesne, 2004) and cerebellar hypoplasia in autistic subjects relative to controls (Courchesne, Yeung-Courchesne, Press, Hesselink, & Jernigan, 1988). The most often reported cerebellar abnormality is a reduction in Purkinje cells, as demonstrated by post-mortem studies (Bailey et al., 1998; Ritvo et al., 1986; Wegiel et al., 2013), though few cerebellar alterations were detected at the transcriptional level (Voineagu et al., 2011). However, the clinical observations have led to a hypothesis that cerebellar pathology may play a role in the etiology of some cases of ASD. Because the involvement of the cerebellum in the ASD discussed in depth in Becker et al. of this volume, Chapter 1, it is only briefly covered here.

Classically, the behaviors involving cerebellar function are often thought of as limited to those involving motor coordination and motor learning (Trouillas et al., 1997). However, behaviors outside of the motor domain have been shown to depend on an intact cerebellum, such as those involved in behavioral modification (Peterson et al., 2012). Furthermore, individuals with cerebellar lesions exhibit what has been termed cerebellar cognitive affective syndrome which is characterized by impaired executive functions, disrupted spatial cognition, blunted affect, inappropriate behavior, and language deficits (Schmahmann & Sherman, 1998). This indicates the cerebellum likely influences non-motor behaviors through its connections with other brain regions.

Further supporting a role for cerebellar dysfunction is the many genetic animal models of ASD that exhibit cerebellar abnormalities. Rare mutations in the *RELN* gene has been identified in individuals with ASD (Neale et al., 2012), and mice mutant for this gene demonstrate extreme cell positioning abnormalities in the cerebellar cortex (Goffinet, 1984) and ASD-relevant abnormal social, communicative and repetitive behaviors (Mullen, Khialeeva, Hoffman, Ghiani, & Carpenter, 2013). Mice lacking Engrailed-2, a transcription factor associated with ASD in human genetics studies, exhibit impaired social behaviors with disrupted cerebellar foliation and gene expression (Brielmaier et al., 2012; Gharani, Benayed, Mancuso, Brzustowicz, & Millonig, 2004; Joyner, Herrup, Auerbach, Davis, & Rossant, 1991; Millen, Wurst, Herrup, & Joyner, 1994; Sen et al., 2010; Sillitoe, Stephen, Lao, & Joyner, 2008). Despite these cerebellar phenotypes, few studies have attempted to clarify the cerebellar contribution to ASD pathogenesis by studying genetic models in a cerebellum-specific manner.

These studies have mostly employed the Purkinje cell protein 2 (*Pcp2*) sequence as a promoter driving Cre recombinase for cerebellar-specific deletion of ASD-relevant genes. *Pcp2-Fmr1*^{-/-} mice exhibit altered dendritic morphology in Purkinje cells and recapitulate the attenuated eyeblink conditioning observed in global *Fmr1*^{-/-} mice and Fragile X patients, who have a mutation in *FMRI* the gene (Koekkoek et al., 2005). The *Pcp2-Fmr1*^{-/-} mice also show impaired sensorimotor gating, but other ASD-relevant behavior testing was not reported. Cerebellar-specific deletion of either *Tsc1* or *Tsc2*, genes inactivated in the ASD-related syndrome Tuberous Sclerosis, results in a progressive loss of Purkinje cells due to apoptosis possibly induced by neuronal stress (Reith et al., 2013; Tsai et al., 2012). Surprisingly, these mice also display decreased social behaviors and increased repetitive behaviors and ultrasonic vocalizations. The loss of Purkinje cells and the abnormal behaviors were prevented with postnatal-onset of rapamycin treatment, which rectifies the dysregulation of mTOR signaling downstream of *Tsc1* or *2*. These cerebellar-specific

genetic deletions suggest a role for Purkinje cells in ASD-relevant behaviors, likely resulting from the influence of these cells on the excitation/inhibition balance in other brain areas.

The loss of Purkinje cells may alter the functioning of the frontal cortex. An association was reported between early signs of ASD and dorsolateral prefrontal cortex volume in premature infants with cerebellar injury (Limperopoulos et al., 2012). The Purkinje cells receive excitatory input from glutamatergic granule cells and provide GABAergic inhibition to other areas of the cerebellum, particularly deep cerebellar nuclei. These nuclei then send projections to the thalamus and cerebral cortex (Gonzalo-Ruiz & Leichnetz, 1990; Middleton & Strick, 2001; Saab & Willis, 2003; Sarna & Hawkes, 2003; Yamamoto, Yoshida, Yoshikawa, Kishimoto, & Oka, 1992). Therefore, disruption of GABAergic inhibition in the Purkinje cells can influence functioning in thalamo-cortical circuits. Reduced Purkinje cell function has been suggested to ultimately produce reduced cerebellar modulation of dopamine release in the medial prefrontal cortex (T. D. Rogers et al., 2013). It is possible that loss of Purkinje cells ultimately leads to an imbalance of the excitation/inhibition ratio in the cortex, which, as discussed above, is hypothesized as an underlying mechanism of ASD.

However, there is also evidence suggesting that the Purkinje cells are a particularly vulnerable population. For example, neonatal exposure to toxins like alcohol or nicotine can reduce Purkinje cell numbers (W. J. Chen, Parnell, & West, 1998). Thus it is possible that the cerebellar abnormalities seen in individuals with ASD are simply indicators of a broader developmental deficit influencing many systems.

ASD models involving the striatum

The striatum forms the largest nucleus of the basal ganglia, receiving input from both cortical and thalamic structures (Middleton & Strick, 2000). The dorsal striatum is composed of the caudate and putamen in humans, which is a single structure in mice. Made up of mainly GABAergic projection neurons (medium spiny neurons), which synapse onto neurons of the substantia nigra pars reticulata (SNPr)/globus pallidus interna (GPi) in addition to the globus pallidus externa (GPe), the output of the circuit leads to inhibition or disinhibition of regions of the thalamus and descending pathways (Gerfen, 1992; Kemp & Powell, 1970; Middleton & Strick, 2000; Stocco, Lebiere, & Anderson, 2010). The ventral striatum is composed of the nucleus accumbens and the olfactory tubercle. The nucleus accumbens has been considered a reward-processing center, receiving inputs from the amygdala and the dopaminergic neurons of the ventral tegmental area (VTA) (Gregorios-Pippas, Tobler, & Schultz, 2009; Ubeda-Banon et al., 2007). A number of functional imaging studies have linked underactivation or overactivation in the dorsal striatum (specifically, the head of the caudate) to symptomology of certain psychiatric disorders, such as obsessive-compulsive disorder (OCD), by looking at fMRI BOLD signal at rest between affected individuals and controls (Whiteside, Port, & Abramowitz, 2004), as well as during tasks of motor inhibition (Nakao et al., 2005; Page et al., 2009), implicit learning (Rauch et al., 1997), and planning tasks (van den Heuvel et al., 2005). Because of the association to striatal dysfunction in OCD, it has been attractive to propose that such dysfunction could be causal to ritualistic, OCD-like behaviors observed in patients with ASD (Sears et al., 1999). Resting state activity in autistic children appears elevated in both the dorsal and ventral striatum compared to controls in at least one report (Di Martino et al., 2011). A few reports show increased volume of the caudate in autistic patients compared to controls and correlate this change (Hollander et al., 2005; Langen et al., 2009; Rojas et al., 2006; Sears et al., 1999), to scores of repetitive or other autistic-like behavior such as the Autism Diagnostic Interview – Revised (ADI-R) (Rutter, & Le Couteur, 1994) or Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) scores. However, the data are

conflicting with the correlation being either positive (Hollander et al., 2005; Rojas et al., 2006) or negative (Sears et al., 1999) and not all reports control for total brain size nor for the administration of neuroleptic medications.

Several ASD risk genes have enriched expression in the striatum and are important for striatal function. These include the forkhead box transcription factors *FOXP1* (Ferland, Cherry, Preware, Morrissey, & Walsh, 2003; Tamura, Morikawa, Iwanishi, Hisaoka, & Senba, 2004) and *FOXP2* (Takahashi, Liu, Hirokawa, & Takahashi, 2003), the dopamine receptor *DRD3* (Staal, de Krom, & de Jonge, 2012), and the post-synaptic density scaffolding protein, *SHANK3* (Peca et al., 2011). While several disruptions in *FOXP1* are linked to ASD (Hamdan et al., 2010; O'Roak et al., 2011; Talkowski et al., 2012), *Foxp1* null mice have not yet been assessed for behaviors relevant to ASD-like symptoms. *FOXP2* is considered a potential risk gene for ASD primarily due to its apparent role in speech and language (Newbury & Monaco, 2010), as well as its regulation of downstream genes *MET* and *CNTNAP2* which have been associated previously with ASD risk (Arking et al., 2008; Bakkaloglu et al., 2008; Mukamel et al., 2011; Vernes et al., 2011). Reports on *FOXP2* have focused on motor function and production of ultrasonic vocalization (Fisher & Scharff, 2009), the latter of which has had some conflicting evidence – either reporting a deficit in amount of vocalization (Shu et al., 2005) or lack thereof, with a subtler phenotype in amplitude of vocalization (Gaub, Groszer, Fisher, & Ehret, 2010). Mice deficient in *Foxp2* protein have not yet been assessed on other ASD-like measures, such as the three-chambered test of sociability.

Among genes important to striatal function modeled in mice, perhaps one of the most well documented in relation to ASD risk is *SHANK3*. *SH3* and multiple ankyrin repeat domains 3 (*Shank3*) is a scaffolding protein associated with the post-synaptic density, which links receptors and ion channels at the post synaptic terminus to the cytoskeleton and downstream molecular signaling pathways (Sheng & Kim, 2000). Mice null for *Shank3* protein show ASD-like behaviors in a number of behavioral assays as well as disrupted cortico-striatal neuronal transmission (Bozdagi et al., 2010; Folstein, Dowd, Mankoski, & Tadevosyan, 2003; Verpelli et al., 2011; X. Wang et al., 2011; Yang et al., 2012). *Shank3* mutant mice display stereotyped motor behaviors, which has been proposed as correlated to deficits in striatal function (Peca et al., 2011). Specifically, they show excessive grooming (but not allogrooming) which leads to facial lesions (Peca et al., 2011). In the same report, *Shank3*^{-/-} mice were found to have striatal hypertrophy – both in the surface area and dendritic length of medium spiny neurons (MSNs) – a finding which the authors suggest may mirror human reports of increased volume in the caudate nucleus in autistic patients. Whole cell patch clamp recording of *Shank3*^{-/-} mice showed reduced frequency and amplitude to MSN AMPAR-mediated mEPSCs. Peca and colleagues in that report argue that this dysfunction is restricted to the striatum, based upon lack of such deficits in transmission in the hippocampus, as well as normal reversal learning in the Morris water maze task (Peca et al., 2011). However, because *Shank3* is also expressed in the cerebellum (Welch, Wang, & Feng, 2004), and because the cerebellum may also have a role in the expression of autistic-like phenotypes, it is not clear that there is not also cerebellar dysfunction in the *Shank3* null mouse. Furthermore, more recently Yang and colleagues (Yang et al., 2012) have shown reduced glutamatergic synaptic transmission in the hippocampus and a deficit in long-term potentiation in *Shank3*^{-/-} mice.

Overall, in the models described, it is difficult to assess the contribution of striatal dysfunction to the observed phenotype, as genes such as *SHANK3* are not exclusive to the striatum. Furthermore, many other ASD risk genes which have more global expression, may have a particularly crucial role to play in the striatum that has yet been undiscovered. To address these problems, it will be useful to look at specific striatal disruption of these genes.

There are a number of transgenic mice, expressing Cre recombinase under the control of different gene promoters, which can be used to mediate disruption in the striatum. The promoters driving Cre expression are as described in (Gong et al., 2007) (and on gensat.org) and their genes are summarized in Table 3. Novel methodologies, such as translational profiling of cell populations (Doyle et al., 2008; Heiman et al., 2008), have the potential to uncover highly specific markers of different cell types, which can be used to benefit future genetic manipulations.

Other regions and cell types

We focused our review on four systems and cell types that had previously received wide attention particularly using conditional deletion strategies in model organisms, yet these are certainly not the only systems hypothesized to have a role in ASD. Indeed, it is difficult to identify a cell type or region that has not previously been suggested to be involved in ASD. For some of these, such as the hippocampus, the experimental tractability of the system may in part be responsible for the amount of work that has been focused there. For other potential cellular mechanisms, such as immune-mediated neurodevelopmental abnormalities, there is accumulating evidence these may play a role in some cases (reviewed by Hsiao in this volume, Chapter 9), but less work has been done thus far into an understanding of the consequences on particular neuronal cell types in the brain. And beyond neurons, there are certainly emerging hypotheses regarding the role of glia (Ballas, Lioy, Grunseich, & Mandel, 2009; Maezawa, Swanberg, Harvey, LaSalle, & Jin, 2009) and neural stem cells (Amiri et al., 2012). In depth analyses of the sufficiency of these cell types to create ASD-like behavior disruptions are certainly needed. Determination of sufficiency in conditional deletion experiments must take into account that drivers of recombination have varying levels of specificity (Gofflot et al., 2011). Ultimately, converging lines of evidence, from multiple mouse models and human neuroanatomy will help to define the cell types and circuits which form the basis of ASD symptoms.

Conclusions

From the current review of the consequences of conditional deletions and deletions of genes enriched in certain cell types, it is clear there are multiple cellular disruptions that are sufficient to recreate some ASD-like symptoms in the mouse. What does this suggest to us about the likely cellular mechanisms of human ASD? Some things are becoming clearer.

First, there are mutations that lead to broad deficits in the early organization of the brain such as in the gene *RELN* (Goffinet, 1984; Neale et al., 2012), or *CNTNAP2* (Penagarikano et al., 2011). These mutations disrupt many different circuits and lead to multiple deficits including intellectual disability, epilepsy, motor coordination difficulties, and finally ASD. These deficits may be more difficult to treat with a single strategy, and may represent a class of developmental disorders that need to be considered differently than other diagnoses of ASD (Gillberg, 2010).

Second, even amongst those individuals without broad cellular disorganization of CNS development, it seems likely that, much like the heterogeneity of ASD genetics, there is likely to be some heterogeneity of cellular mechanisms as well. Thus, compared to Parkinson's disease, it seems unlikely that all ASD patients will share a single common cellular pathology. Yet, it may still be the case that there are a limited number of distinct cellular pathologies leading to the disorder. For example, it is possible that a subset of patients develop ASD as a consequence of serotonergic abnormalities, while another subset as a consequence of disrupted social reward processing in the striatum. If the ASD cases can be clustered by cellular deficits, then at least within these clusters, patients with distinct

genetic causes may still respond to a single treatment strategy. Genetic interaction experiments as well as conditional deletion of a variety of ASD risk genes across different cell types, in conjunction with careful and consistent phenotyping, are going to be key to understanding whether such a clustering of cellular mechanisms indeed exists.

Acknowledgments

This work was supported by NINDS (4R00NS067239-03) to JDD, and an NIMH ACE Network Grant (9R01MH100027-06). MAR was supported by Kirschstein-NRSA (5T32GM007067-38).

References

- Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. *Nat Rev Genet.* 2008; 9(5):341–355. [PubMed: 18414403]
- Adachi M, Autry AE, Covington HE 3rd, Monteggia LM. MeCP2-mediated transcription repression in the basolateral amygdala may underlie heightened anxiety in a mouse model of Rett syndrome. *Journal of Neuroscience.* 2009; 29(13):4218–4227. [PubMed: 19339616]
- Alenina N, Kikic D, Todiras M, Mosienko V, Qadri F, Plehm R, Bader M. Growth retardation and altered autonomic control in mice lacking brain serotonin. *Proc Natl Acad Sci U S A.* 2009; 106(25):10332–10337. [PubMed: 19520831]
- Allen G, Muller RA, Courchesne E. Cerebellar function in autism: functional magnetic resonance image activation during a simple motor task. *Biol Psychiatry.* 2004; 56(4):269–278. [PubMed: 15312815]
- Alvarez-Saavedra M, Saez MA, Kang D, Zoghbi HY, Young JL. Cell-specific expression of wild-type MeCP2 in mouse models of Rett syndrome yields insight about pathogenesis. *Human Molecular Genetics.* 2007; 16(19):2315–2325. [PubMed: 17635839]
- Amiri A, Cho W, Zhou J, Birnbaum SG, Sinton CM, McKay RM, Parada LF. Pten deletion in adult hippocampal neural stem/progenitor cells causes cellular abnormalities and alters neurogenesis. *The Journal of neuroscience : the official journal of the Society for Neuroscience.* 2012; 32(17):5880–5890. [PubMed: 22539849]
- Anderson GM, Freedman DX, Cohen DJ, Volkmar FR, Hoder EL, McPhedran P, Young JG. Whole blood serotonin in autistic and normal subjects. *J Child Psychol Psychiatry.* 1987; 28(6):885–900. [PubMed: 3436995]
- Angoa-Perez M, Kane MJ, Briggs DI, Sykes CE, Shah MM, Francescutti DM, Kuhn DM. Genetic depletion of brain 5HT reveals a common molecular pathway mediating compulsivity and impulsivity. *J Neurochem.* 2012; 121(6):974–984. [PubMed: 22443164]
- Anney R, Klei L, Pinto D, Almeida J, Bacchelli E, Baird G, Devlin B. Individual common variants exert weak effects on the risk for autism spectrum disorders. *Hum Mol Genet.* 2012; 21(21):4781–4792. [PubMed: 22843504]
- Arakawa H, Blanchard DC, Arakawa K, Dunlap C, Blanchard RJ. Scent marking behavior as an odorant communication in mice. *Neurosci Biobehav Rev.* 2008; 32(7):1236–1248. [PubMed: 18565582]
- Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Chakravarti A. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. *Am J Hum Genet.* 2008; 82(1):160–164. [PubMed: 18179894]
- Azmitia EC, Singh JS, Whitaker-Azmitia PM. Increased serotonin axons (immunoreactive to 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology.* 2011; 60(7–8):1347–1354. [PubMed: 21329710]
- Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, Rutter M. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med.* 1995; 25(1):63–77. [PubMed: 7792363]
- Bailey A, Luthert P, Dean A, Harding B, Janota I, Montgomery M, Lantos P. A clinicopathological study of autism. *Brain.* 1998; 121(Pt 5):889–905. [PubMed: 9619192]

- Bakkaloglu B, O'Roak BJ, Louvi A, Gupta AR, Abelson JF, Morgan TM, State MW. Molecular cytogenetic analysis and resequencing of contactin associated protein-like 2 in autism spectrum disorders. *Am J Hum Genet.* 2008; 82(1):165–173. [PubMed: 18179895]
- Ballas N, Liroy DT, Grunseich C, Mandel G. Non-cell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. *Nature neuroscience.* 2009; 12(3):311–317.
- Basu SN, Kollu R, Banerjee-Basu S. AutDB: a gene reference resource for autism research. *Nucleic Acids Research.* 2009; 37:D832–D836. [PubMed: 19015121]
- Beaulieu JM, Zhang X, Rodriguiz RM, Sotnikova TD, Cools MJ, Wetsel WC, Caron MG. Role of GSK3 beta in behavioral abnormalities induced by serotonin deficiency. *Proc Natl Acad Sci U S A.* 2008; 105(4):1333–1338. [PubMed: 18212115]
- Berg JM, Geschwind DH. Autism genetics: searching for specificity and convergence. *Genome Biol.* 2012; 13(7):247. [PubMed: 22849751]
- Betancur C, Corbex M, Spielesoy C, Philippe A, Laplanche JL, Launay JM, Leboyer M. Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Mol Psychiatry.* 2002; 7(1):67–71. [PubMed: 11803447]
- Bozdagi O, Sakurai T, Papapetrou D, Wang X, Dickstein DL, Takahashi N, Buxbaum JD. Haploinsufficiency of the autism-associated Shank3 gene leads to deficits in synaptic function, social interaction, and social communication. *Mol Autism.* 2010; 1(1):15. [PubMed: 21167025]
- Briellmaier J, Matteson PG, Silverman JL, Senerth JM, Kelly S, Genestine M, Crawley JN. Autism-relevant social abnormalities and cognitive deficits in engrailed-2 knockout mice. *PLoS One.* 2012; 7(7):e40914. [PubMed: 22829897]
- Buxbaum JD, Silverman JM, Smith CJ, Greenberg DA, Kilifarski M, Reichert J, Vitale R. Association between a GABRB3 polymorphism and autism. *Mol Psychiatry.* 2002; 7(3):311–316. [PubMed: 11920158]
- Canitano R, Scandurra V. Psychopharmacology in autism: an update. *Prog Neuropsychopharmacol Biol Psychiatry.* 2011; 35(1):18–28. [PubMed: 21034789]
- Carrasco M, Volkmar FR, Bloch MH. Pharmacologic treatment of repetitive behaviors in autism spectrum disorders: evidence of publication bias. *Pediatrics.* 2012; 129(5):e1301–1310. [PubMed: 22529279]
- Carter MD, Shah CR, Muller CL, Crawley JN, Carneiro AM, Veenstra-VanderWeele J. Absence of preference for social novelty and increased grooming in integrin beta3 knockout mice: initial studies and future directions. *Autism Res.* 2011; 4(1):57–67. [PubMed: 21254450]
- Chahrour MH, Yu TW, Lim ET, Ataman B, Coulter ME, Hill RS, Walsh CA. Whole-exome sequencing and homozygosity analysis implicate depolarization-regulated neuronal genes in autism. *PLoS Genet.* 2012; 8(4):e1002635. [PubMed: 22511880]
- Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, Zoghbi HY. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature.* 2010; 468(7321):263–269. [PubMed: 21068835]
- Cheh MA, Millonig JH, Roselli LM, Ming X, Jacobsen E, Kamdar S, Wagner GC. En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. *Brain Res.* 2006; 1116(1):166–176. [PubMed: 16935268]
- Chen RZ, Akbarian S, Tudor M, Jaenisch R. Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat Genet.* 2001; 27(3):327–331. [PubMed: 11242118]
- Chen WJ, Parnell SE, West JR. Neonatal alcohol and nicotine exposure limits brain growth and depletes cerebellar Purkinje cells. *Alcohol.* 1998; 15(1):33–41. [PubMed: 9426835]
- Ching H, Pringsheim T. Aripiprazole for autism spectrum disorders (ASD). *Cochrane Database Syst Rev.* 2012; 5:CD009043. [PubMed: 22592735]
- Consortium, T. D.-B. F. X. Fmr1 knockout mice: a model to study fragile X mental retardation. *Cell.* 1994; 78(1):23–33. [PubMed: 8033209]
- Cook EH Jr. Courchesne RY, Cox NJ, Lord C, Gonen D, Guter SJ, Courchesne E. Linkage-disequilibrium mapping of autistic disorder, with 15q11–13 markers. *Am J Hum Genet.* 1998; 62(5):1077–1083. [PubMed: 9545402]

- Cook EH, Leventhal BL. The serotonin system in autism. *Curr Opin Pediatr*. 1996; 8(4):348–354. [PubMed: 9053096]
- Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. Hypoplasia of cerebellar vermal lobules VI and VII in autism. *N Engl J Med*. 1988; 318(21):1349–1354. [PubMed: 3367935]
- Crawley JN. Translational animal models of autism and neurodevelopmental disorders. *Dialogues Clin Neurosci*. 2012; 14(3):293–305. [PubMed: 23226954]
- Dani VS, Chang Q, Maffei A, Turrigiano GG, Jaenisch R, Nelson SB. Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A*. 2005; 102(35):12560–12565. [PubMed: 16116096]
- Deane CM, Salwinski L, Xenarios I, Eisenberg D. Protein interactions: two methods for assessment of the reliability of high throughput observations. *Mol Cell Proteomics*. 2002; 1(5):349–356. [PubMed: 12118076]
- DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, Olsen RW. Mice lacking the beta3 subunit of the GABAA receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. *Journal of Neuroscience*. 1998; 18(20):8505–8514. [PubMed: 9763493]
- DeLorey TM, Sahbaie P, Hashemi E, Homanics GE, Clark JD. Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: a potential model of autism spectrum disorder. *Behav Brain Res*. 2008; 187(2):207–220. [PubMed: 17983671]
- Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, Mairena MA, Milham MP. Aberrant striatal functional connectivity in children with autism. *Biol Psychiatry*. 2011; 69(9):847–856. [PubMed: 21195388]
- Dougherty JD, Maloney SE, Wozniak DF, Rieger MA, Sonnenblick L, Coppola G, Heintz N. The disruption of Celf6, a gene identified by translational profiling of serotonergic neurons, results in autism-related behaviors. *Journal of Neuroscience*. 2013; 33(7):2732–2753. [PubMed: 23407934]
- Doyle JP, Dougherty JD, Heiman M, Schmidt EF, Stevens TR, Ma G, Heintz N. Application of a translational profiling approach for the comparative analysis of CNS cell types. *Cell*. 2008; 135(4):749–762. [PubMed: 19013282]
- du Plessis L, Skunca N, Dessimoz C. The what, where, how and why of gene ontology-a primer for bioinformaticians. *Briefings in Bioinformatics*. 2011; 12(6):723–735. [PubMed: 21330331]
- Ellegood J, Henkelman RM, Lerch JP. Neuroanatomical Assessment of the Integrin beta3 Mouse Model Related to Autism and the Serotonin System Using High Resolution MRI. *Front Psychiatry*. 2012; 3:37. [PubMed: 22557981]
- Ferland RJ, Cherry TJ, Preware PO, Morrissey EE, Walsh CA. Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain. *J Comp Neurol*. 2003; 460(2):266–279. [PubMed: 12687690]
- Fisher SE, Scharff C. FOXP2 as a molecular window into speech and language. *Trends Genet*. 2009; 25(4):166–177. [PubMed: 19304338]
- Folstein SE, Dowd M, Mankoski R, Tadevosyan O. How might genetic mechanisms operate in autism? *Novartis Found Symp*. 2003; 251:70–80. discussion 80–73, 109–111, 281–197. [PubMed: 14521188]
- Fyffe SL, Neul JL, Samaco RC, Chao HT, Ben-Shachar S, Moretti P, Zoghbi HY. Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. *Neuron*. 2008; 59(6):947–958. [PubMed: 18817733]
- Gaub S, Groszer M, Fisher SE, Ehret G. The structure of innate vocalizations in Foxp2-deficient mouse pups. *Genes Brain Behav*. 2010; 9(4):390–401. [PubMed: 20132318]
- Gerfen CR. The neostriatal mosaic: multiple levels of compartmental organization. *Trends Neurosci*. 1992; 15(4):133–139. [PubMed: 1374971]
- Geschwind DH. Genetics of autism spectrum disorders. *Trends Cogn Sci*. 2011; 15(9):409–416. [PubMed: 21855394]

- Gharani N, Benayed R, Mancuso V, Brzustowicz LM, Millonig JH. Association of the homeobox transcription factor, ENGRAILED 2, 3, with autism spectrum disorder. *Mol Psychiatry*. 2004; 9(5):474–484. [PubMed: 15024396]
- Giacometti E, Luikenuis S, Beard C, Jaenisch R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *Proc Natl Acad Sci U S A*. 2007; 104(6):1931–1936. [PubMed: 17267601]
- Gillberg C. The ESSENCE in child psychiatry: Early Symptomatic Syndromes Eliciting Neurodevelopmental Clinical Examinations. *Res Dev Disabil*. 2010; 31(6):1543–1551. [PubMed: 20634041]
- Goffinet AM. Events governing organization of postmigratory neurons: studies on brain development in normal and reeler mice. *Brain Res*. 1984; 319(3):261–296. [PubMed: 6383524]
- Gofflot, F.; Wendling, O.; Charatoire, N.; Birling, M-C.; Warot, X.; Auwerx, J. Characterization and Validation of Cre-Driver Mouse Lines. 2011.
- Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, Gerfen CR. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *Journal of Neuroscience*. 2007; 27(37):9817–9823. [PubMed: 17855595]
- Gonzalo-Ruiz A, Leichnetz GR. Connections of the caudal cerebellar interpositus complex in a new world monkey (*Cebus apella*). *Brain Res Bull*. 1990; 25(6):919–927. [PubMed: 2289174]
- Gregorios-Pippas L, Tobler PN, Schultz W. Short-term temporal discounting of reward value in human ventral striatum. *J Neurophysiol*. 2009; 101(3):1507–1523. [PubMed: 19164109]
- Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of neurological defects in a mouse model of Rett syndrome. *Science*. 2007; 315(5815):1143–1147. [PubMed: 17289941]
- Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat Genet*. 2001; 27(3):322–326. [PubMed: 11242117]
- Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Risch N. Genetic heritability and shared environmental factors among twin pairs with autism. *Arch Gen Psychiatry*. 2011; 68(11):1095–1102. [PubMed: 21727249]
- Hamdan FF, Daoud H, Rochefort D, Piton A, Gauthier J, Langlois M, Michaud JL. De novo mutations in FOXP1 in cases with intellectual disability, autism, and language impairment. *Am J Hum Genet*. 2010; 87(5):671–678. [PubMed: 20950788]
- Han S, Tai C, Westenbroek RE, Yu FH, Cheah CS, Potter GB, Catterall WA. Autistic-like behaviour in *Scn1a*^{+/-} mice and rescue by enhanced GABA-mediated neurotransmission. *Nature*. 2012; 489(7416):385–390. [PubMed: 22914087]
- Heiman M, Schaefer A, Gong S, Peterson JD, Day M, Ramsey KE, Heintz N. A translational profiling approach for the molecular characterization of CNS cell types. *Cell*. 2008; 135(4):738–748. [PubMed: 19013281]
- Hendricks TJ, Fyodorov DV, Wegman LJ, Lelutiu NB, Pehek EA, Yamamoto B, Deneris ES. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. *Neuron*. 2003; 37(2):233–247. [PubMed: 12546819]
- Hofer MA, Shair HN, Brunelli SA. Ultrasonic vocalizations in rat and mouse pups. *Curr Protoc Neurosci*. 2002; Chapter 8(Unit 8):14. [PubMed: 18428567]
- Hollander E, Anagnostou E, Chaplin W, Esposito K, Haznedar MM, Licalzi E, Buchsbaum M. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol Psychiatry*. 2005; 58(3):226–232. [PubMed: 15939406]
- Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Wigler M. De novo gene disruptions in children on the autistic spectrum. *Neuron*. 2012; 74(2):285–299. [PubMed: 22542183]
- Joung JK, Sander JD. TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol*. 2013; 14(1):49–55. [PubMed: 23169466]
- Joyner AL, Herrup K, Auerbach BA, Davis CA, Rossant J. Subtle cerebellar phenotype in mice homozygous for a targeted deletion of the *En-2* homeobox. *Science*. 1991; 251(4998):1239–1243. [PubMed: 1672471]
- Jugloff DG, Vandamme K, Logan R, Visanji NP, Brotchie JM, Eubanks JH. Targeted delivery of an *Mecp2* transgene to forebrain neurons improves the behavior of female *Mecp2*-deficient mice. *Human Molecular Genetics*. 2008; 17(10):1386–1396. [PubMed: 18223199]

- Kalueff AV, Fox MA, Gallagher PS, Murphy DL. Hypolocomotion, anxiety and serotonin syndrome-like behavior contribute to the complex phenotype of serotonin transporter knockout mice. *Genes Brain Behav.* 2007; 6(4):389–400. [PubMed: 16939636]
- Kane MJ, Angoa-Perez M, Briggs DI, Sykes CE, Francescutti DM, Rosenberg DR, Kuhn DM. Mice genetically depleted of brain serotonin display social impairments, communication deficits and repetitive behaviors: possible relevance to autism. *PLoS One.* 2012; 7(11):e48975. [PubMed: 23139830]
- Kemp JM, Powell TP. The cortico-striate projection in the monkey. *Brain.* 1970; 93(3):525–546. [PubMed: 4990231]
- Kirov G, Pocklington AJ, Holmans P, Ivanov D, Ikeda M, Ruderfer D, Owen MJ. De novo CNV analysis implicates specific abnormalities of postsynaptic signalling complexes in the pathogenesis of schizophrenia. *Molecular Psychiatry.* 2012; 17(2):142–153. [PubMed: 22083728]
- Kirsten TB, Chaves-Kirsten GP, Chaible LM, Silva AC, Martins DO, Britto LR, Bernardi MM. Hypoactivity of the central dopaminergic system and autistic-like behavior induced by a single early prenatal exposure to lipopolysaccharide. *J Neurosci Res.* 2012; 90(10):1903–1912. [PubMed: 22714803]
- Klei L, Sanders SJ, Murtha MT, Hus V, Lowe JK, Willsey AJ, Devlin B. Common genetic variants, acting additively, are a major source of risk for autism. *Mol Autism.* 2012; 3(1):9. [PubMed: 23067556]
- Koekkoek SK, Yamaguchi K, Milojkovic BA, Dortland BR, Ruigrok TJ, Maex R, De Zeeuw CI. Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD, enlarges spines, and attenuates cerebellar eyelid conditioning in Fragile X syndrome. *Neuron.* 2005; 47(3):339–352. [PubMed: 16055059]
- Kron M, Howell CJ, Adams IT, Ransbottom M, Christian D, Ogier M, Katz DM. Brain activity mapping in Mecp2 mutant mice reveals functional deficits in forebrain circuits, including key nodes in the default mode network, that are reversed with ketamine treatment. *Journal of Neuroscience.* 2012; 32(40):13860–13872. [PubMed: 23035095]
- Kyzar EJ, Pham M, Roth A, Cachat J, Green J, Gaikwad S, Kalueff AV. Alterations in grooming activity and syntax in heterozygous SERT and BDNF knockout mice: the utility of behavior-recognition tools to characterize mutant mouse phenotypes. *Brain Res Bull.* 2012; 89(5–6):168–176. [PubMed: 22951260]
- Langen M, Schnack HG, Nederveen H, Bos D, Lahuis BE, de Jonge MV, Durston S. Changes in the developmental trajectories of striatum in autism. *Biol Psychiatry.* 2009; 66(4):327–333. [PubMed: 19423078]
- Lesch KP, Wozozin BL, Murphy DL, Reiderer P. Primary structure of the human platelet serotonin uptake site: identity with the brain serotonin transporter. *J Neurochem.* 1993; 60(6):2319–2322. [PubMed: 7684072]
- Liljelund P, Handforth A, Homanics GE, Olsen RW. GABAA receptor beta3 subunit gene-deficient heterozygous mice show parent-of-origin and gender-related differences in beta3 subunit levels, EEG, and behavior. *Brain Res Dev Brain Res.* 2005; 157(2):150–161.
- Limperopoulos C, Chilingaryan G, Sullivan N, Guizard N, Robertson RL, du Plessis AJ. Injury to the Premature Cerebellum: Outcome is Related to Remote Cortical Development. *Cereb Cortex.* 2012
- Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, Rutter M. The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. *J Autism Dev Disord.* 2000; 30(3):205–223. [PubMed: 11055457]
- Lord C, Rutter M, Le Couteur A. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *J Autism Dev Disord.* 1994; 24(5):659–685. [PubMed: 7814313]
- Luikenhuis S, Giacometti E, Beard CF, Jaenisch R. Expression of MeCP2 in postmitotic neurons rescues Rett syndrome in mice. *Proc Natl Acad Sci U S A.* 2004; 101(16):6033–6038. [PubMed: 15069197]

- Maezawa I, Swanberg S, Harvey D, LaSalle JM, Jin LW. Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2009; 29(16):5051–5061. [PubMed: 19386901]
- Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci*. 2004; 5(10):793–807. [PubMed: 15378039]
- McDougle CJ, Naylor ST, Goodman WK, Volkmar FR, Cohen DJ, Price LH. Acute tryptophan depletion in autistic disorder: a controlled case study. *Biol Psychiatry*. 1993; 33(7):547–550. [PubMed: 8513041]
- McFarlane HG, Kusek GK, Yang M, Phoenix JL, Bolivar VJ, Crawley JN. Autism-like behavioral phenotypes in BTBR T+tf/J mice. *Genes Brain Behav*. 2008; 7(2):152–163. [PubMed: 17559418]
- McGraw CM, Samaco RC, Zoghbi HY. Adult neural function requires MeCP2. *Science*. 2011; 333(6039):186. [PubMed: 21636743]
- Michaelson JJ, Shi Y, Gujral M, Zheng H, Malhotra D, Jin X, Sebat J. Whole-genome sequencing in autism identifies hot spots for de novo germline mutation. *Cell*. 2012; 151(7):1431–1442. [PubMed: 23260136]
- Miczek KA, Faccidomo S, De Almeida RM, Bannai M, Fish EW, Debold JF. Escalated aggressive behavior: new pharmacotherapeutic approaches and opportunities. *Ann N Y Acad Sci*. 2004; 1036:336–355. [PubMed: 15817748]
- Middleton FA, Strick PL. Basal ganglia output and cognition: evidence from anatomical, behavioral, and clinical studies. *Brain Cogn*. 2000; 42(2):183–200. [PubMed: 10744919]
- Middleton FA, Strick PL. Cerebellar projections to the prefrontal cortex of the primate. *Journal of Neuroscience*. 2001; 21(2):700–712. [PubMed: 11160449]
- Millen KJ, Wurst W, Herrup K, Joyner AL. Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse *Engrailed-2* mutants. *Development*. 1994; 120(3):695–706. [PubMed: 7909289]
- Mosienko V, Bert B, Beis D, Matthes S, Fink H, Bader M, Alenina N. Exaggerated aggression and decreased anxiety in mice deficient in brain serotonin. *Transl Psychiatry*. 2012; 2:e122. [PubMed: 22832966]
- Mossner R, Simantov R, Marx A, Lesch KP, Seif I. Aberrant accumulation of serotonin in dopaminergic neurons. *Neurosci Lett*. 2006; 401(1–2):49–54. [PubMed: 16638624]
- Moy SS, Nadler JJ, Magnuson TR, Crawley JN. Mouse models of autism spectrum disorders: the challenge for behavioral genetics. *Am J Med Genet C Semin Med Genet*. 2006; 142C(1):40–51. [PubMed: 16419099]
- Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, Crawley JN. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav*. 2004; 3(5):287–302. [PubMed: 15344922]
- Moy SS, Nadler JJ, Poe MD, Nonneman RJ, Young NB, Koller BH, Bodfish JW. Development of a mouse test for repetitive, restricted behaviors: relevance to autism. *Behav Brain Res*. 2008; 188(1):178–194. [PubMed: 18068825]
- Moy SS, Nadler JJ, Young NB, Nonneman RJ, Grossman AW, Murphy DL, Lauder JM. Social approach in genetically engineered mouse lines relevant to autism. *Genes Brain Behav*. 2009; 8(2):129–142. [PubMed: 19016890]
- Moy SS, Nadler JJ, Young NB, Nonneman RJ, Segall SK, Andrade GM, Magnuson TR. Social approach and repetitive behavior in eleven inbred mouse strains. *Behav Brain Res*. 2008; 191(1):118–129. [PubMed: 18440079]
- Muhle R, Trentacoste SV, Rapin I. The genetics of autism. *Pediatrics*. 2004; 113(5):e472–486. [PubMed: 15121991]
- Mukamel Z, Konopka G, Wexler E, Osborn GE, Dong H, Bergman MY, Geschwind DH. Regulation of MET by FOXP2, genes implicated in higher cognitive dysfunction and autism risk. *Journal of Neuroscience*. 2011; 31(32):11437–11442. [PubMed: 21832174]
- Mullen BR, Khialeeva E, Hoffman DB, Ghiani CA, Carpenter EM. Decreased reelin expression and organophosphate pesticide exposure alters mouse behaviour and brain morphology. *ASN Neuro*. 2013; 5(1):e00106. [PubMed: 23298182]

- Nagy A. Cre recombinase: the universal reagent for genome tailoring. *Genesis*. 2000; 26(2):99–109. [PubMed: 10686599]
- Nakao T, Nakagawa A, Yoshiura T, Nakatani E, Nabeyama M, Yoshizato C, Kawamoto M. A functional MRI comparison of patients with obsessive-compulsive disorder and normal controls during a Chinese character Stroop task. *Psychiatry Res*. 2005; 139(2):101–114. [PubMed: 15970434]
- Neale BM, Kou Y, Liu L, Ma'ayan A, Samocha KE, Sabo A, Daly MJ. Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature*. 2012; 485(7397):242–245. [PubMed: 22495311]
- Newbury DF, Monaco AP. Genetic advances in the study of speech and language disorders. *Neuron*. 2010; 68(2):309–320. [PubMed: 20955937]
- O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Eichler EE. Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet*. 2011; 43(6):585–589. [PubMed: 21572417]
- O'Roak BJ, Vives L, Girirajan S, Karakoc E, Krumm N, Coe BP, Eichler EE. Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature*. 2012; 485(7397):246–250. [PubMed: 22495309]
- Oswald DP, Sonenklar NA. Medication use among children with autism spectrum disorders. *J Child Adolesc Psychopharmacol*. 2007; 17(3):348–355. [PubMed: 17630868]
- Page LA, Rubia K, Deeley Q, Daly E, Toal F, Mataix-Cols D, Murphy DG. A functional magnetic resonance imaging study of inhibitory control in obsessive-compulsive disorder. *Psychiatry Res*. 2009; 174(3):202–209. [PubMed: 19906516]
- Panksepp JB, Jochman KA, Kim JU, Koy JJ, Wilson ED, Chen Q, Lahvis GP. Affiliative behavior, ultrasonic communication and social reward are influenced by genetic variation in adolescent mice. *PLoS One*. 2007; 2(4):e351. [PubMed: 17406675]
- Peca J, Feliciano C, Ting JT, Wang W, Wells MF, Venkatraman TN, Feng G. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*. 2011; 472(7344):437–442. [PubMed: 21423165]
- Pellis SM, Pasztor TJ. The developmental onset of a rudimentary form of play fighting in C57 mice. *Developmental Psychobiology*. 1999; 34:175–182. [PubMed: 10204093]
- Penagarikano O, Abrahams BS, Herman EI, Winden KD, Gdalyahu A, Dong H, Geschwind DH. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*. 2011; 147(1):235–246. [PubMed: 21962519]
- Persico AM, D'Agruma L, Maiorano N, Totaro A, Militerni R, Bravaccio C, Keller F. Reelin gene alleles and haplotypes as a factor predisposing to autistic disorder. *Mol Psychiatry*. 2001; 6(2):150–159. [PubMed: 11317216]
- Persico AM, Napolioni V. Autism genetics. *Behav Brain Res*. 2013
- Peterson TC, Villatoro L, Arneson T, Ahuja B, Voss S, Swain RA. Behavior modification after inactivation of cerebellar dentate nuclei. *Behav Neurosci*. 2012; 126(4):551–562. [PubMed: 22845704]
- Peyron C, Faraco J, Rogers W, Ripley B, Overeem S, Charnay Y, Mignot E. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nature medicine*. 2000; 6(9):991–997.
- Rauch SL, Savage CR, Alpert NM, Dougherty D, Kendrick A, Curran T, Jenike MA. Probing striatal function in obsessive-compulsive disorder: a PET study of implicit sequence learning. *J Neuropsychiatry Clin Neurosci*. 1997; 9(4):568–573. [PubMed: 9447498]
- Reith RM, McKenna J, Wu H, Hashmi SS, Cho SH, Dash PK, Gambello MJ. Loss of Tsc2 in Purkinje cells is associated with autistic-like behavior in a mouse model of tuberous sclerosis complex. *Neurobiology of disease*. 2013; 51:93–103. [PubMed: 23123587]
- Ritvo ER, Freeman BJ, Scheibel AB, Duong T, Robinson H, Guthrie D, Ritvo A. Lower Purkinje cell counts in the cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. *Am J Psychiatry*. 1986; 143(7):862–866. [PubMed: 3717426]

- Riva D, Annunziata S, Contarino V, Erbetta A, Aquino D, Bulgheroni S. Gray Matter Reduction in the Vermis and CRUS-II Is Associated with Social and Interaction Deficits in Low-Functioning Children with Autistic Spectrum Disorders: a VBM-DARTEL Study. *Cerebellum*. 2013
- Rogers JT, Zhao L, Trotter JH, Rusiana I, Peters MM, Li Q, Weeber EJ. Reelin supplementation recovers sensorimotor gating, synaptic plasticity and associative learning deficits in the heterozygous reeler mouse. *J Psychopharmacol*. 2013; 27(4):386–395. [PubMed: 23104248]
- Rogers TD, Dickson PE, McKimm E, Heck DH, Goldowitz D, Blaha CD, Mittleman G. Reorganization of Circuits Underlying Cerebellar Modulation of Prefrontal Cortical Dopamine in Mouse Models of Autism Spectrum Disorder. *Cerebellum*. 2013
- Rojas DC, Peterson E, Winterrowd E, Reite ML, Rogers SJ, Tregellas JR. Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. *BMC Psychiatry*. 2006; 6:56. [PubMed: 17166273]
- Rubenstein JL, Merzenich MM. Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav*. 2003; 2(5):255–267. [PubMed: 14606691]
- Saab CY, Willis WD. The cerebellum: organization, functions and its role in nociception. *Brain Res Brain Res Rev*. 2003; 42(1):85–95. [PubMed: 12668291]
- Salinger WL, Ladrow P, Wheeler C. Behavioral phenotype of the reeler mutant mouse: effects of RELN gene dosage and social isolation. *Behav Neurosci*. 2003; 117(6):1257–1275. [PubMed: 14674845]
- Samaco RC, Mandel-Brehm C, Chao HT, Ward CS, Fyffe-Maricich SL, Ren J, Neul JL. Loss of MeCP2 in aminergic neurons causes cell-autonomous defects in neurotransmitter synthesis and specific behavioral abnormalities. *Proc Natl Acad Sci U S A*. 2009; 106(51):21966–21971. [PubMed: 20007372]
- Sanders SJ, Murtha MT, Gupta AR, Murdoch JD, Raubeson MJ, Willsey AJ, State MW. De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature*. 2012; 485(7397):237–241. [PubMed: 22495306]
- Sarna JR, Hawkes R. Patterned Purkinje cell death in the cerebellum. *Progress in Neurobiology*. 2003; 70(6):473–507. [PubMed: 14568361]
- Scattoni ML, Martire A, Cartocci G, Ferrante A, Ricceri L. Reduced social interaction, behavioural flexibility and BDNF signalling in the BTBR T+tf/J strain, a mouse model of autism. *Behav Brain Res*. 2012
- Scattoni ML, McFarlane HG, Zhodzishsky V, Caldwell HK, Young WS, Ricceri L, Crawley JN. Reduced ultrasonic vocalizations in vasopressin 1b knockout mice. *Behav Brain Res*. 2008; 187(2):371–378. [PubMed: 18005969]
- Scattoni ML, Ricceri L, Crawley JN. Unusual repertoire of vocalizations in adult BTBR T+tf/J mice during three types of social encounters. *Genes Brain Behav*. 2011; 10(1):44–56. [PubMed: 20618443]
- Schaevitz LR, Moriuchi JM, Nag N, Mellot TJ, Berger-Sweeney J. Cognitive and social functions and growth factors in a mouse model of Rett syndrome. *Physiol Behav*. 2010; 100(3):255–263. [PubMed: 20045424]
- Schain RJ, Freedman DX. Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. *The Journal of pediatrics*. 1961; 58:315–320. [PubMed: 13747230]
- Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain*. 1998; 121(Pt 4):561–579. [PubMed: 9577385]
- Sears LL, Vest C, Mohamed S, Bailey J, Ranson BJ, Piven J. An MRI study of the basal ganglia in autism. *Prog Neuropsychopharmacol Biol Psychiatry*. 1999; 23(4):613–624. [PubMed: 10390720]
- Sen B, Singh AS, Sinha S, Chatterjee A, Ahmed S, Ghosh S, Usha R. Family-based studies indicate association of Engrailed 2 gene with autism in an Indian population. *Genes Brain Behav*. 2010; 9(2):248–255. [PubMed: 20050924]
- Shahbazian M, Young J, Yuva-Paylor L, Spencer C, Antalffy B, Noebels J, Zoghbi H. Mice with truncated MeCP2 recapitulate many Rett syndrome features and display hyperacetylation of histone H3. *Neuron*. 2002; 35(2):243–254. [PubMed: 12160743]

- Sheng M, Kim E. The Shank family of scaffold proteins. *J Cell Sci.* 2000; 113(Pt 11):1851–1856. [PubMed: 10806096]
- Shu W, Cho JY, Jiang Y, Zhang M, Weisz D, Elder GA, Buxbaum JD. Altered ultrasonic vocalization in mice with a disruption in the *Foxp2* gene. *Proc Natl Acad Sci U S A.* 2005; 102(27):9643–9648. [PubMed: 15983371]
- Sillitoe RV, Stephen D, Lao Z, Joyner AL. Engrailed homeobox genes determine the organization of Purkinje cell sagittal stripe gene expression in the adult cerebellum. *Journal of Neuroscience.* 2008; 28(47):12150–12162. [PubMed: 19020009]
- Silverman JL, Yang M, Lord C, Crawley JN. Behavioural phenotyping assays for mouse models of autism. *Nat Rev Neurosci.* 2010; 11(7):490–502. [PubMed: 20559336]
- Staal WG, de Krom M, de Jonge MV. Brief report: the dopamine-3-receptor gene (*DRD3*) is associated with specific repetitive behavior in autism spectrum disorder (ASD). *J Autism Dev Disord.* 2012; 42(5):885–888. [PubMed: 21691864]
- Stocco A, Lebiere C, Anderson JR. Conditional routing of information to the cortex: a model of the basal ganglia's role in cognitive coordination. *Psychol Rev.* 2010; 117(2):541–574. [PubMed: 20438237]
- Takahashi K, Liu FC, Hirokawa K, Takahashi H. Expression of *Foxp2*, a gene involved in speech and language, in the developing and adult striatum. *J Neurosci Res.* 2003; 73(1):61–72. [PubMed: 12815709]
- Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, Heilbut A, Gusella JF. Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. *Cell.* 2012; 149(3):525–537. [PubMed: 22521361]
- Tamura S, Morikawa Y, Iwanishi H, Hisaoka T, Senba E. *Foxp1* gene expression in projection neurons of the mouse striatum. *Neuroscience.* 2004; 124(2):261–267. [PubMed: 14980377]
- Taniguchi H, He M, Wu P, Kim S, Paik R, Sugino K, Huang ZJ. A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron.* 2011; 71(6):995–1013. [PubMed: 21943598]
- Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl).* 2009; 204(2):361–373. [PubMed: 19189082]
- Trouillas P, Takayanagi T, Hallett M, Currier RD, Subramony SH, Wessel K, Manyam B. International Cooperative Ataxia Rating Scale for pharmacological assessment of the cerebellar syndrome. The Ataxia Neuropharmacology Committee of the World Federation of Neurology. *J Neurol Sci.* 1997; 145(2):205–211. [PubMed: 9094050]
- Tsai PT, Hull C, Chu Y, Greene-Colozzi E, Sadowski AR, Leech JM, Sahin M. Autistic-like behaviour and cerebellar dysfunction in Purkinje cell *Tsc1* mutant mice. *Nature.* 2012; 488(7413):647–651. [PubMed: 22763451]
- Ubeda-Banon I, Novejarque A, Mohedano-Moriano A, Pro-Sistiaga P, de la Rosa-Prieto C, Insausti R, Martinez-Marcos A. Projections from the posterolateral olfactory amygdala to the ventral striatum: neural basis for reinforcing properties of chemical stimuli. *BMC Neurosci.* 2007; 8:103. [PubMed: 18047654]
- Ugarte SD, Homanics GE, Firestone LL, Hammond DL. Sensory thresholds and the antinociceptive effects of GABA receptor agonists in mice lacking the beta3 subunit of the GABA(A) receptor. *Neuroscience.* 2000; 95(3):795–806. [PubMed: 10670447]
- van den Heuvel OA, Veltman DJ, Groenewegen HJ, Cath DC, van Balkom AJ, van Hartskamp J, van Dyck R. Frontal-striatal dysfunction during planning in obsessive-compulsive disorder. *Arch Gen Psychiatry.* 2005; 62(3):301–309. [PubMed: 15753243]
- Veenstra-VanderWeele J, Muller CL, Iwamoto H, Sauer JE, Owens WA, Shah CR, Blakely RD. Autism gene variant causes hyperserotonemia, serotonin receptor hypersensitivity, social impairment and repetitive behavior. *Proc Natl Acad Sci U S A.* 2012; 109(14):5469–5474. [PubMed: 22431635]
- Vernes SC, Oliver PL, Spiteri E, Lockstone HE, Puliyadi R, Taylor JM, Fisher SE. *Foxp2* regulates gene networks implicated in neurite outgrowth in the developing brain. *PLoS Genet.* 2011; 7(7):e1002145. [PubMed: 21765815]

- Verpelli C, Dvoretzskova E, Vicidomini C, Rossi F, Chiappalone M, Schoen M, Sala C. Importance of Shank3 protein in regulating metabotropic glutamate receptor 5 (mGluR5) expression and signaling at synapses. *J Biol Chem*. 2011; 286(40):34839–34850. [PubMed: 21795692]
- Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Geschwind DH. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature*. 2011; 474(7351):380–384. [PubMed: 21614001]
- Wagstaff J, Knoll JH, Fleming J, Kirkness EF, Martin-Gallardo A, Greenberg F, et al. Localization of the gene encoding the GABAA receptor beta 3 subunit to the Angelman/Prader-Willi region of human chromosome 15. *Am J Hum Genet*. 1991; 49(2):330–337. [PubMed: 1714232]
- Waider J, Proft F, Langlhofer G, Asan E, Lesch KP, Gutknecht L. GABA concentration and GABAergic neuron populations in limbic areas are differentially altered by brain serotonin deficiency in Tph2 knockout mice. *Histochem Cell Biol*. 2013; 139(2):267–281. [PubMed: 23052836]
- Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R. One-Step Generation of Mice Carrying Mutations in Multiple Genes by CRISPR/Cas-Mediated Genome Engineering. *Cell*. 2013; 153(4):910–918. [PubMed: 23643243]
- Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, Hakonarson H. Common genetic variants on 5p14.1 associate with autism spectrum disorders. *Nature*. 2009; 459(7246):528–533. [PubMed: 19404256]
- Wang X, McCoy PA, Rodriguiz RM, Pan Y, Je HS, Roberts AC, Jiang YH. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. *Hum Mol Genet*. 2011; 20(15):3093–3108. [PubMed: 21558424]
- Wegiel J, Kuchna I, Nowicki K, Imaki H, Yong Ma S, Azmitia EC, Wisniewski T. Contribution of olivofloccular circuitry developmental defects to atypical gaze in autism. *Brain Res*. 2013; 1512:106–122. [PubMed: 23558308]
- Weiss LA, Arking DE, Daly MJ, Chakravarti A. A genome-wide linkage and association scan reveals novel loci for autism. *Nature*. 2009; 461(7265):802–808. [PubMed: 19812673]
- Welch JM, Wang D, Feng G. Differential mRNA expression and protein localization of the SAP90/PSD-95-associated proteins (SAPAPs) in the nervous system of the mouse. *J Comp Neurol*. 2004; 472(1):24–39. [PubMed: 15024750]
- Whiteside SP, Port JD, Abramowitz JS. A meta-analysis of functional neuroimaging in obsessive-compulsive disorder. *Psychiatry Res*. 2004; 132(1):69–79. [PubMed: 15546704]
- Willins DL, Deutch AY, Roth BL. Serotonin 5-HT_{2A} receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse*. 1997; 27(1):79–82. [PubMed: 9268067]
- Wohr M, Scattoni ML. Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments. *Behav Brain Res*. 2013
- Yamamoto T, Yoshida K, Yoshikawa H, Kishimoto Y, Oka H. The medial dorsal nucleus is one of the thalamic relays of the cerebellocerebral responses to the frontal association cortex in the monkey: horseradish peroxidase and fluorescent dye double staining study. *Brain Res*. 1992; 579(2):315–320. [PubMed: 1378349]
- Yang M, Bozdagi O, Scattoni ML, Wohr M, Roulet FI, Katz AM, Crawley JN. Reduced excitatory neurotransmission and mild autism-relevant phenotypes in adolescent Shank3 null mutant mice. *J Neurosci*. 2012; 32(19):6525–6541. [PubMed: 22573675]
- Zhang L, He J, Jugloff DG, Eubanks JH. The MeCP2-null mouse hippocampus displays altered basal inhibitory rhythms and is prone to hyperexcitability. *Hippocampus*. 2008; 18(3):294–309. [PubMed: 18058824]
- Zhang S, Lin L, Kaur S, Thankachan S, Blanco-Centurion C, Yanagisawa M, Shiromani PJ. The development of hypocretin (orexin) deficiency in hypocretin/ataxin-3 transgenic rats. *Neuroscienc*. 2007; 148(1):34–43.
- Zhou FC, Lesch KP, Murphy DL. Serotonin uptake into dopamine neurons via dopamine transporters: a compensatory alternative. *Brain Res*. 2002; 942(1–2):109–119. [PubMed: 12031859]

Table 1
Genes identified by recent whole exome sequencing (WES) studies with regionalized patterns of expression

A minority of genes recently identified by WES studies have patterns of expression which have been previously studied in the brain. Relevant references are listed alongside the regions and cell types implicated.

Gene Symbol	Region; cell type	References
FOXP1	striatum; medium spiny neurons	Ferland, Cherry, Preware, Morrissey, & Walsh, 2003; Tamura, Morikawa, Iwanishi, Hisaoka, & Senba, 2004
GRIN2B	striatum; all cell types	Kuppenbender, et al., 2000
NRXN1	cortex; Ntsr1+ and Cck+ cells cerebellum; granule cells	Dougherty, Schmidt, Nakajima, & Heintz, 2010
PLXNB1	cerebellum; purkinje cells	Fazzari, et al., 2007
RELN	cortex; GABA interneurons hippocampus; GABA interneurons cerebellum; granule cells	Pesold, et al., 1998
TBR1	cortex	Bulfone, et al., 1995; Englund, et al., 2005

Table 2
ASD-related phenotypes of genetic mouse models of the serotonin system

The impact of serotonin-related genetic mutation on 5-HT levels, 5-HT-expressing neurons, and behavioral phenotypes relevant to the core ASD symptoms.

Mutation	5-HT levels	5-HT Neurons	Abnormal Social Behaviors	Communication Deficits	Stereotyped/repetitive behaviors, Resistance to change	References
<i>Celf6</i> ^{-/-}	Reduced (~30%)	--	Normal sociability	Decreased pup USVs	Trend towards failed reversal performance; failure to change hole-poking behavior	Dougherty et al., 2013
<i>Htrb3</i> ^{-/-}	--	Reduced	Normal sociability; lack of preference for social novelty	--	Increased self-grooming in novel environment	Carter et al., 2011; Ellegood et al., 2012
<i>Htrb3</i> ^{+/-}	--	--	Normal sociability and preference for social novelty	--	Slightly increased self-grooming in novel environment	Carter et al., 2011
<i>Slc6a4</i> ^{-/-}	Reduced	Reduced (~50%)	Decreased sociability; Increased sensitivity to social stress; reduced aggression; increased socio-positive behaviors	--	Increased self-grooming in homecage; normal self-grooming in novel environment; normal nest building	Kalueff et al., 2007; Moy et al., 2009;
<i>Slc6a4</i> ^{+/-}	--	--	Normal sociability in males; decreased sociability in females; normal preference for social novelty; slightly reduced aggression	--	Increased self-grooming in homecage; normal self-grooming in novel environment	Page et al., 2009; Kyzar et al., 2012; Moy et al., 2009
<i>Slc6a4</i> Ala56 (high activity variant)	Unchanged with increased 5-HT clearance	--	Decreased sociability; increased submission to social dominance	Decreased pup USVs	Repetitive homecage wire hanging; normal marble burying and self-grooming in homecage.	Veenstra-VanderWeele et al., 2012
<i>Tph2</i> ^{-/-}	Absent	Intact	Postnatal lack of preference for maternal scent; social memory deficits; social odorant disinterest; decreased social interaction time; lack of preference for social novelty; increased aggression	Decreased urinary scent marking episodes and investigation	Increased nestlet shredding, marbling burying and digging in mixed C57BL/6J-129Sv background; decreased marble burying with increased activity in C57BL/6J background; increased motor impulsivity; normal reversal performance	Angoa-Perez et al., 2012; Kane et al., 2012; Mosienko et al., 2012; Alenina et al., 2009
<i>Tph2</i> R441H (low activity variant)	Reduced (~80%)	--	Increased aggression	--	--	Beaulieu et al., 2008

-- indicates behavior was not assessed.

Table 3
Promoters used to drive Cre recombination in the striatum (from GENSAT)

A list of available Cre recombinase-expressing transgenic mouse lines available with expression in the striatum, varying in specificity of expression.

Gene Symbol	Gene	Pattern of Cre expression
Adora2a	adenosine A2A receptor	Drd2+ (striato-pallidal) projection neurons
Dlx5	distal-less homeobox 5	projection neurons (also expressed in GABA interneurons of the cortex, and in the reticular nucleus of the thalamus)
Drd1a	dopamine receptor D1A	Drd1a+ (striato-nigral) projection neurons (some limited expression in cortex and hypothalamus)
Drd2	dopamine receptor D2	Drd2+ (striato pallidal) projection neurons (some limited expression in limbic cortex and hypothalamus)
Drd3	dopamine receptor D3	Drd3+ (ventral striatum) neurons (expression in layers 2&3 of cortex and in the EC of the hippocampus)
Gng7	guanine nucleotide binding protein, gamma 7	Both Drd1a+ and Drd2+ projection neurons (scattered expression in cortex and hippocampus as well)
Vipr2	vasoactive intestinal peptide receptor 2	Both Drd1a+ and Drd2+ (also cortex, layer 5)