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Monogenic Mouse Models of Autism Spectrum Disorders: Common Mechanisms and Missing Links

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Abstract

Autism Spectrum Disorders (ASDs) present unique challenges in the fields of genetics and neurobiology because of the clinical and molecular heterogeneity underlying these disorders. Genetic mutations found in ASD patients provide opportunities to dissect the molecular and circuit mechanisms underlying autistic behaviors using animal models. Ongoing studies of genetically modified models have offered critical insight into possible common mechanisms arising from different mutations, but links between molecular abnormalities and behavioral phenotypes remain elusive. The challenges encountered in modeling autism in mice demand a new analytic paradigm that integrates behavioral analysis with circuit-level analysis in genetically modified models with strong construct validity.

Keywords

autism mouse models; Mecp2; Fmr1; Ube3a; Pten; Shank3

Autism Spectrum Disorders (ASDs) are a group of conditions primarily characterized by impairments in social communication and engagement in restricted, repetitive behaviors (American Psychiatric Association, 2013). Common comorbidities include intellectual disability, epilepsy, anxiety, sleep disturbances, abnormal sensory processing, motor impairments, and gastrointestinal complaints (Argyropoulos et al., 2013). ASDs are heterogeneous in nature, as patients display a wide range of symptom severity and prognosis (Howlin et al., 2004; Lord et al., 2000), which is mirrored by hundreds of identified causal or potentially causal genetic variants (Persico and Napolioni, 2013; Willsey and State, 2015). Unfortunately, most genetic mutations are rare or private (i.e. observed only in a single family). Both the phenotypic and genetic heterogeneity present significant obstacles to understanding the disorders and attempts to associate phenotypic severity with genetic differences have had mixed results (Chang et al., 2015; Chaste et al., 2014). While genetics

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undoubtedly play a substantial role in ASD pathophysiology, the inexplicable phenotypic heterogeneity and incomplete concordance rates between monozygotic twins (Hallmayer et al., 2011), suggest that non-genetic factors may also contribute to the etiology.

A recent survey indicates that 1 in 68 children in the United States are diagnosed with ASDs, a drastic increase from previous estimates over the last few decades (Centers for Disease Control and Prevention, 2014). While there is considerable debate regarding the degree to which the increase in prevalence can be explained by broadened diagnostic criteria (King and Bearman 2009), increased awareness (Liu et al., 2010), or changes in environmental factors (Nevison, 2014), there is nevertheless an ever-increasing urgency to determine the underlying pathophysiology and develop safe, cost-effective interventions for ASDs to improve patient outcomes. Regardless of the source of the rising prevalence, it is an issue of great public health concern, as the lifetime cost of ASD-related care ranges from approximately $1.4 million to $2.2 million per individual (Buescher et al., 2014).

Studies in human clinical populations have been and continue to be critical for understanding the genetic and non-genetic contributions to ASDs (Willsey and State, 2015). However, animal models are needed determine the mechanisms leading to abnormal functioning. Although human brain imaging techniques have identified regions and circuits involved in the disorders (e.g. Karten and Hirsch, 2014), animal models provide opportunities for direct manipulation of these brain regions and circuits to test their precise functions. In current clinical practice, ASDs are defined by behavioral symptoms that are uniquely human and there is no singular neuropathological hallmark identified so far that is pathognomonic, so it is challenging to determine the validity of an animal model of autism. Nevertheless, recent successes in identifying genes implicated in ASDs have paved the way to explore the neurobiology underlying the disorders using animal models.

1. Genetic mutations implicated in ASDs

Substantial progress has been made to understand the genetic causes of ASDs. Genes implicated in syndromic forms of autism were first identified in the 1990s. Subsequent genomic copy number variant (CNV) analysis in the 2000s generated a list of rare but highly penetrant CNVs associated with ASDs. Pathogenic CNVs are estimated to account for ~10% of non-syndromic ASDs (Devlin and Scherer, 2012). Most recently, whole exome and whole genome sequencing techniques have been utilized to identify rare *de novo* and inherited sequence variants in hundreds of genes from ASD subjects. Despite the inability to establish a causal role for the majority of these sequence variants, a subset of new genes have emerged as strongly causal because *de novo* loss-of-function and likely-gene-disrupting mutations are found in multiple affected patients and are absent in a large number of controls (Table 1). In other cases, mutations that likely disrupt protein function are found in genes that are implicated in other neuropsychiatric disorders. Functional annotation of these genes immediately suggests the following molecular features: 1) neuronal ion channels and receptors; 2) synapse-related cytoskeleton and scaffolding proteins; 3) epigenetic and transcriptional regulators; 4) post-translational protein modifiers and regulators. An important question is whether or not the mutations in these genes share a common molecular

and/or circuit-level mechanism underlying the pathophysiology of ASDs. Modeling these mutations in animal models is essential to address this question.

2. What constitutes a valid animal model for ASDs?

Animal models of psychiatric disorders have classically been evaluated on three criteria, which were first applied to mouse models of depression: construct, face, and predictive validity (Willner, 1984). Ever since these criteria were articulated, they have been interpreted in a variety of ways (Belzung and Lemoine, 2011), making it worth elaborating on their precise meanings and their relationships to animal models of ASDs.

Construct validity, for our purposes, refers to the rationale behind the creation of the model and its ability to recapitulate the etiology of the disorder. For instance, a model of ASD with high construct validity mimics a genetic mutation that has been observed in affected human individuals, or at least has the same molecular consequences; this can perhaps more accurately be described as pathogenic validity (Belzung and Lemoine, 2011). The determination of construct validity is difficult for genes that have a complex structure at transcriptional level.

Face validity refers to the model's resemblance to the clinical features of the disorder, both in terms of behavioral symptoms and physiological biomarkers; this definition of face validity can be separated into ethological validity and biomarker validity (Belzung and Lemoine, 2011). Currently, ASD is diagnosed based solely on behavioral assessments, limiting discussion of face validity to the behavioral realm.

Finally, predictive validity refers to the model's ability to determine the effectiveness that interventions will have on a clinical population. A model of ASD with high predictive validity will respond (e.g. with a reduction in repetitive behaviors) to pharmaceuticals in similar manner to the patients they are intended to model. This is particularly challenging in autism models because there are no established treatment paradigms for the core symptoms in human autism clinics currently. Conceptually, interventions are not necessarily limited to drugs, but it is more difficult to assess the translational value of non-pharmacological therapies, such as environmental enrichment. Predictive validity can also refer to the penetrance of specific phenotypes, given a specific genetic mutation or other trigger (Belzung and Lemoine, 2011), but these data are seldom reported.

3. Overview of Monogenic Mouse Models of ASDs

By far the most common animals used to model ASDs are mice because of the well-established techniques to manipulate their genome and study brain function at several levels of analysis. As mammals, mice are genetically and biologically similar to humans, but their rapid reproduction and accelerated development allow for the testing of large numbers of animals at a relatively low cost. Various assays have been developed to test mice for behaviors that resemble the core features of autism (Silverman et al., 2010b), allowing researchers to determine the face validity of their models, but the translatability of these assays to humans is unknown.

Much of what we know about the underlying mechanism of ASDs comes from detailed analysis of mouse models of syndromic autism, for which a genetic cause is clearly defined. Unfortunately, while patients with these disorders frequently meet the diagnostic criteria for ASDs, autism is not always present and there are a variety of other symptoms related to abnormal brain function, making the results of these studies not necessarily generalizable to all ASDs. Nevertheless, careful review of common findings in these models contributes to a basic understanding of the pathophysiology contributing to autistic behaviors. The behavioral phenotypes in some of these ASD models have recently been reviewed in a separate paper (Bey and Jiang, 2014), so this review will focus on biochemical, cellular, and synaptic findings from select mouse models of ASDs induced by a mutation in a single gene. It should be noted that there are many models that we excluded, including inbred strains, chromosomal CNVs, and environmentally induced models, due to limited space and the fact that the biological mechanisms in these models are currently less understood. The models we selected to review are grouped by the reported function of the disrupted protein and the findings are summarized in Table 2.

3.1 Epigenetic and Transcriptional Regulator: *Mecp2* (Rett syndrome)

A number of genes classified as transcriptional or epigenetic regulators have been implicated in ASDs (Network and Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015). These genetic findings support a molecular mechanism involving transcriptional regulation underlying the pathogenesis of ASDs. One of the best characterized genes in this category is *MECP2*, a gene encoding a methylated DNA-binding protein (Amir et al., 1999; reviewed in Lyst and Bird, 2015). Although the protein was characterized as a transcriptional repressor when it was first identified (Nan et al., 1997), data from more recent studies indicate that MeCP2 acts as a global transcriptional regulator involved in both the suppression and activation of targeted genes (Chahrour et al., 2008; Ben-Shachar et al., 2009), as well as a regulator of RNA splicing (Young et al., 2005; Maunakea et al. 2013).

More than 10 distinct lines of *Mecp2* mutant mice have been produced. Mice with the deletion of exon 3 (*Mecp2*$^{tm1.1Jae}$; Chen et al., 2001), deletion of exons 3 and 4 (*Mecp2*$^{tm1.1Bird}$; Guy et al., 2001), and a 308× point mutation (*Mecp2*^{tm1Hzo}; Shahbazian et al., 2002) are among the best characterized. The complete loss of both MeCP2 protein isoforms were revealed in hemizygous males (*Mecp2*$^{-/y}$) of *Mecp2*$^{tm1.1Jae}$ and *Mecp2*$^{tm1.1Bird}$ mutants, whereas the 308× mutation in *Mecp2*^{tm1Hzo} mice introduces a premature stop codon that leads to truncation of the MeCP2 protein (Shahbazian et al., 2002). It should be noted that female heterozygous mice (*Mecp2*$^{-/+}$) are the model with best construct validity, as Rett syndrome primarily affects females and is lethal in males in most cases. However, most studies use hemizygous male mice because they develop more severe phenotypes. An important question that remains is why humans are more sensitive than rodents to *MeCP2* mutations.

Cellular and molecular abnormalities have been identified in *Mecp2* mutant mice that likely contribute to the ASD-like phenotypes. Both *Mecp2*$^{tm1.1Bird}$ and *Mecp2*$^{tm1.1Jae}$ mice have cortical neurons with decreased spine density (Belichenko et al., 2009), and *Mecp2*$^{tm1.1Bird}$

mice have decreased dendritic complexity (Fukuda et al., 2005). Similar results have been reported in *Mecp2*$^{tm1.1Jae}$ hippocampus (Smrt et al., 2007), but this varies across development: spine density is decreased at postnatal day seven but returns to wild type levels by postnatal day 15 (Chapleau et al., 2012). Moreover, adult *Mecp2*^{tm1Hzo} mice have normal spine density and dendritic complexity in both cortex and hippocampus (Moretti et al., 2006). More recently, it was demonstrated that neurons in somatosensory cortex of *Mecp2*$^{tm1.1Jae}$ mice are more stable than controls at postnatal day 25–26 (P25–P26), as assessed by *in vivo* two-photon imaging (Landi et al., 2011).

The electrophysiological consequences of MeCP2 dysfunction have also been examined. Reduced long-term potentiation (LTP) and long-term depression (LTD) have been reported in hippocampal CA1 synapses in both *Mecp2*$^{tm1.1Bird}$ and *Mecp2*$^{tm1.1Jae}$ mice (Asaka et al., 2006), as well as *Mecp2*^{tm1Hzo} mice (Moretti et al., 2006). Recordings from sensory and motor cortex from *Mecp2*^{tm1Hzo} mice also revealed reduced LTP (Moretti et al., 2006). Whole-cell patch-clamp recordings from pyramidal neurons in brain slices of somatosensory cortex from *Mecp2*$^{tm1.1Jae}$ mice revealed reduced spontaneous activity due to a significant reduction in the amplitude of miniature excitatory postsynaptic currents (mEPSCs) and an insignificant increase in the amplitude of miniature inhibitory postsynaptic currents (mIPSCs; Dani et al., 2005). Similarly decreased frequency of mEPSCs and amplitude of evoked EPSCs have also been observed in cultured neurons from *Mecp2*$^{tm1.1Bird}$ mice and are associated with decreased numbers of glutamatergic synapses on individual neurons (Chao et al., 2007).

How the deficiency of MeCP2 results in abnormal functioning of neuronal synapses as well as ASD-like phenotypes is still not fully understood. Hundreds of genes are dysregulated in both directions in brains of *Mecp2* mutant mice. These genes are promising candidates to dissect the molecular mechanism underlying the deficiency of *Mecp2* in neurons or synapses. One such candidate is BDNF, a neurotrophic factor that is down-regulated in MeCP2-deficient neurons. Not only do decreases in BDNF expression correlate with symptom severity (Chang et al., 2006), but treatments that increase BDNF also improve symptoms (Ogier et al., 2007; Kline et al., 2010). Additionally, a non-pharmacological intervention – environmental enrichment, which improves synaptic and behavioral phenotypes in *Mecp2* mutant mice – is associated with increased expression of BDNF (Lonetti et al., 2010).

A number of research groups have manipulated the expression of MeCP2 in mouse brains temporally and spatially using various Cre lines to determine potential brain regions, cell types, and developmental time periods involved in Rett syndrome pathogenesis. Conditional knockout (KO) of *Mecp2* using either the brain-specific *Nestin-Cre* line in which the recombinase is activated during embryonic development or the forebrain-specific *CamK-Cre93* line in which the recombinase is activated postnatally recapitulate the phenotypes observed in the conventional *Mecp2* mutant mice (Chen et al., 2001). A subsequent study found that *Mecp2* deficiency in excitatory neurons of the forebrain using *CamKII-Cre93* mice also produce ASD-like features (Gemelli et al., 2006). However, other studies provide evidence that loss of MeCP2 specifically in GABAergic neurons (*Viaat-Cre*) is sufficient (Chao et al., 2010). Thus, loss of MeCP2 in a subset of either excitatory or inhibitory

neurons can lead to ASD-like features. It remains unclear whether the deficiency of MeCP2 in these excitatory or inhibitory neurons disrupts the same circuits. Using mice engineered with a stop codon surrounded by *loxP* sites crossed with a ubiquitously-expressed inducible Cre line (*CAG-Cre/Esr1*), researchers have provided evidence that Rett syndrome phenotypes can be induced in fully developed mice (McGraw et al., 2011) and rescued through gene reactivation (Guy et al., 2007; Robinson et al., 2012; Lang et al., 2014), suggesting that ASDs may arise from persistent abnormal neural functioning, rather than disrupted development. However, this hypothesis needs to be tested in other models of ASD, as the phenotypes reported in these studies are not necessarily generalizable.

3.2 Post-Transcriptional Protein Modifiers or Regulators: *Fmr1, Tsc1/2, Ube3a, and Pten*

3.2.1 *Fmr1* (Fragile X syndrome)—Fragile X syndrome (FXS), primarily affecting males, also has phenotypic overlap with ASDs and is caused by extended CGG trinucleotide repeats in the 5′ untranslated region of *FMR1* (Verkerk et al., 1991; Pieretti et al., 1991). Such mutations lead to hypermethylation of the *FMR1* promoter, which transcriptionally silences the fragile X mental retardation protein (FMRP), an RNA binding protein involved in suppressing activity-dependent translation of synaptic proteins, many of which have been independently implicated in ASDs (Darnell et al., 2011). Under normal conditions, FMRP is dephosphorylated after activation of metabotropic glutamate receptors (mGluRs), leading to de-repression of local translation (reviewed in Bassell and Warren, 2008). Extended CGG repeats in *Fmr1* in mice do not recapitulate the hypermethylation and transcriptional silencing of FMRP that is characteristic of human fragile X (Brouwer et al., 2007). However, researchers have mimicked the molecular consequences of the human mutation by deleting exon 5 (The Dutch-Belgian Fragile X Consortium, 1994) or exon 1 (Mientjes et al., 2006) of *Fmr1*. Almost all data related to fragile X syndrome mouse models in the literature have been generated from the exon 5 deletion mutant mice, but whereas both models have complete loss of FMRP expression, only the model produced by deleting exon 1 has no detectable *Fmr1* mRNA and is conducive to conditional knockout studies.

Many studies have reported differences in dendritic spines in *Fmr1* knockouts. The first study reported that the cortical neurons have increased numbers of spines in adult mice, most of which morphologically resemble immature spines (Comery et al., 1997). Since then, there have been conflicting reports regarding spine density in various brain regions and periods of development, but the immature morphology of spines is a relatively consistent finding (reviewed in He and Portera-Cailliau, 2013). Using *in vivo* two-photon imaging, researchers recently have found increased turnover in dendritic spines from *Fmr1* knockout mice in layer 2/3 neurons in the barrel cortex at P10–P12 (Cruz-Martin et al., 2010) and in layer 5 neurons in primary somatosensory cortex at P20–P30 (Pan et al., 2010). Together, these findings suggest that FMRP is required for the development of mature, stable dendritic spines.

In addition to dendritic spine morphology and stability, synaptic plasticity has been well characterized in FXS mouse models (reviewed in Chung et al., 2012; Sidorov et al., 2013). Initial studies reported no change in LTP (Godfraind et al., 1996; Paradee et al., 1999), but a significant enhancement in mGluR-dependent LTD in the hippocampus (Huber et al., 2002).

More recently, impaired LTP was reported in hippocampal CA1 synapses using threshold-level stimulation parameters, which are thought to more closely mimic *in vivo* conditions (Lauterborn et al., 2007; Lee et al., 2011). Furthermore, a transient decrease (at postnatal day 14) in the AMPA/NMDA receptor ratio and an increase in NMDA-dependent LTP in hippocampal neurons has been observed (Pilpel et al., 2009). Reduced LTP has also been reported in somatosensory cortical synapses (Li et al., 2002), and increased mGluR-dependent LTD has been reported in cerebellar Purkinje cells (Koekkoek et al., 2005). Therefore, like spine morphology, electrophysiological consequences of *Fmr1* knockout vary across brain regions and development, but increased mGluR-dependent LTD seems to be a relatively consistent finding.

The enhanced mGluR-mediated LTD observed in *Fmr1* knockouts (Huber et al., 2002) paved the way for the mGluR theory for FXS pathogenesis, which posits that FXS symptoms are caused by mGluR5 signaling leading to deregulated mRNA translation in the absence of FMRP (Bear et al., 2004). This theory has been supported by the effectiveness of genetic depletion of mGluR5 (Dölen et al., 2007) and mGluR5 negative allosteric modulators (e.g. CTEP; Michalon et al., 2012) in alleviating behavioral and synaptic abnormalities in *Fmr1* knockout mice. While neither of these studies included analysis of repetitive behaviors or social interactions, negative allosteric modulation of mGluR5 decreased repetitive behaviors and increased sociability of a different mouse model of ASD: the BTBR inbred strain (Silverman et al., 2012). However, the mGluR5 signaling pathway is not the only molecular cascade that results in FMRP de-repression and subsequent dysregulated translation. For instance, the BDNF/TrkB signaling pathway converges with the mGluR5 pathway to initiate translation (reviewed in Castrén and Castrén, 2014; see Figure 1). It remains to be determined whether dysregulation of the BDNF/TrkB signaling pathway, the mGluR5 pathway, or another pathway entirely contributes to the ASD-like behaviors in FXS mouse models. There is some evidence which suggests that social deficits in mice lacking FMRP are due to decreased neuroligin1 expression and can be rescued by overexpressing neuroligin1 (Dahlhaus and El-Husseini, 2010). Additionally, mice lacking FMRP exhibit elevated postsynaptic Shank1 levels, along with a number of other synaptic proteins (Schütt et al., 2009), but more work needs to be done to confirm these results and determine whether or how these changes may lead to changes in behavior.

Compared to other models, little work has been published utilizing *Fmr1* conditional knockout mice to determine the cell types and brain regions underlying the ASD-like phenotypes involved in FMRP dysfunction. One group reported that deletion of *Fmr1* in a subset of mature neurons in cortex and hippocampus (*Nse-Cre*) has no effect on ASD-like behaviors (Amiri et al., 2014), whereas previous work demonstrated that deletion of another ASD susceptibility gene (*Pten*; see section 3.2.3) using the same Cre line produces ASD-like phenotypes (Kwon et al., 2006a). This finding is somewhat surprising, given that PTEN expression is typically suppressed in FXS, indicating some mechanistic overlap between FXS and *PTEN*-related ASD (Hagerman et al., 2010). There is some evidence to suggest that, similar to *Mecp2* mutant mice, *Fmr1* knockout mice have deficits in GABAergic signaling ($GABA_B$ reviewed in Braat and Kooy, 2014; $GABA_A$ reviewed in Braat and Kooy, 2015), but so far this has not been directly linked to behavior. Clearly, more work

needs to be done to determine cell types and brain regions involved in the ASD-like features observed in FXS mouse models.

3.2.2 *Tsc1/Tsc2* (Tuberous sclerosis complex)—Tuberous sclerosis complex (TSC) is a disorder characterized by the formation of benign tumors, intellectual disability, epilepsy, infantile spasm, and ASD (Smalley et al., 1992). Heterozygous mutations in either *TSC1* or *TSC2* genes that encode the proteins hamartin and tuberin, respectively, are sufficient to cause TSC (van Slegtenhorst et al., 1997; European Chromosome 16 Tuberous Sclerosis Consortium, 1993). Hamartin and tuberin form a heterodimer that negatively regulates the mTORC1 (mammalian target of rapamycin complex 1) signaling cascade by inactivating the small G protein Rheb (Tee et al., 2003). The mTORC1 signaling cascade has been implicated in cell growth and proliferation (Fingar et al., 2002) and in synaptic plasticity (Hoeffer and Klann, 2010).

Homozygous deletion of *Tsc1* exons 6–8 (Kobayashi et al., 2001), *Tsc1* exons 17–18 (Kwiatkowski et al., 2002), *Tsc1* exons 5–7 (Wilson et al., 2005), *Tsc2* exon 2 (Onda et al., 1999), or *Tsc2* exons 2–5 (Kobayashi et al., 1999) in mice all result in embryonic lethality. However, heterozygous mice ($Tsc1^{+/-}$ or $Tsc2^{+/-}$) from these constructs recapitulate many of the phenotypes of TSC observed in humans, including ASD-like behaviors (reviewed in Bey and Jiang, 2014).

Depletion of *Tsc1* or *Tsc2* in cultured post-mitotic hippocampal neurons results in increased soma size, decreased dendritic spine density, and increased spine size (Tavazoie et al., 2005). However, loss of *Tsc1* in hippocampal neurons *in vivo* via viral delivery of Cre recombinase, soma size is increased but there is no significant change in spine density (Bateup et al., 2011). On the other hand, $Tsc2^{+/-}$ mice have normal spine density in cortical projection neurons at P19–P20, but increased spine density at P29–P30, suggesting a synaptic pruning defect (Tang et al., 2014). Thus, whereas the effects of *Tsc1/2* loss on spine density vary developmentally and across brain regions, soma size is consistently increased.

Decreased expression of *Tsc1* or *Tsc2* has drastic impacts on synaptic function as well, but the findings vary between *in vivo* and *in vitro* experiments, suggesting non-cell-autonomous and network effects. Organotypically cultured hippocampal neurons lacking *Tsc1* have higher amplitudes in mEPSCs and increased AMPA/NMDA current ratios (Tavazoie et al., 2005). However, loss of *Tsc1* in hippocampal CA1 neurons *in vivo* impairs mGluR-dependent LTD, decreases the inter-event-interval (a measure of frequency) in mEPSCs without changing mEPSC amplitude, and increases evoked AMPA and NMDA currents proportionally (Bateup et al., 2011). Mice haploinsufficient for *Tsc2* also have impaired mGluR-dependent LTD (Auerbach et al., 2011). Additionally, knockout of *Tsc1* in primary hippocampal cultures has no effect on mEPSC amplitude in glutamatergic neurons, but whole cell voltage clamp analysis of GABAergic neurons shows a decrease in evoked IPSC (eIPSC) and mIPSC amplitudes (Weston et al., 2014).

At the cellular level, loss-of-function mutations in *Tsc1* and *Tsc2* lead to hyperactivation of mTORC1, which is one of the major mechanisms for TSC pathogenesis (reviewed in Crino, 2013). This mechanism overlaps with other known genetic causes of ASDs. For example,

long-term potentiation initiated by BDNF is dependent on mTOR signaling (Tang et al., 2002). Additionally, as the mTOR pathway is involved in protein translation, it is hyperactivated in FXS (Sharma et al., 2010). However, *Tsc2*$^{+/-}$ mice show reduced protein synthesis, whereas *Fmr1*$^{-/y}$ mice have increased protein synthesis (Auerbach et al., 2011). One possible explanation for the different effects on protein synthesis is that Rheb has functions other than to negatively regulate mTORC1 activity, which is evident by the fact that inhibition of Rheb, but not inhibition of mTORC1, rescues the aberrant spine morphogenesis observed in *Tsc2*$^{+/-}$ neurons (Yasuda et al., 2014). More work needs to be done to determine whether ASD-like phenotypes in TSC mouse models are the result from disrupted mTORC1-dependent or mTORC1-independent signaling pathways.

Various conditional knockouts of *Tsc1* or *Tsc2* have been used to determine cell types and brain regions involved in the different components of TSC. Mice with *Tsc1* deficiency in cerebellar Purkinje cells by the expression of *L7-Cre* in either *Tsc1*$^{flox/+}$ or *Tsc1*$^{flox/flox}$ display ASD-like behaviors (Tsai et al., 2012). Moreover, conditional knockout of *Tsc1* in Purkinje cells leads to decreased cell numbers, increased soma size, increased spine density, and lower spontaneous spiking rates in these cells (Tsai et al., 2012). Another group crossed *Pcp2-Cre* mice with *Tsc2*$^{flox/-}$ mice, resulting in homozygous deletion of *Tsc2* in Purkinje cells and heterozygous deletion of *Tsc2* in all other cells, and report exacerbated ASD-like phenotypes (Reith et al., 2013). One may argue that the latter study doesn't make use of the high construct validity provided by the haploinsufficiency of the TSC mouse model. However, loss of heterozygosity (LOH) occurs in a cell-specific manner in TSC (Crino, 2013) and it is possible that LOH in Purkinje cells accounts for some of the ASD symptoms in human TSC patients, but more work needs to be done to confirm this hypothesis. More recently, a group knocked out *Tsc1* in all excitatory forebrain neurons (*CaMKII*α-*Cre*) and selectively in serotonergic neurons (*Slc6a4-Cre*) and each was sufficient to produce ASD-like behaviors (McMahon et al., 2014).

3.2.3 *Pten* (PTEN hamartoma tumor syndromes and non-syndromic ASDs)—

Mutations in phosphatase and tensin homolog (*PTEN*) were first identified in a number of patients with different conditions (reviewed in Eng, 2003), together called PTEN hamartoma tumor syndromes (PHTS). Although relatively rare, *PTEN* mutations are consistently identified in non-syndromic ASDs comorbid with significant macrocephaly (Butler et al., 2005; Buxbaum et al., 2007), a feature observed in approximately 20% of all ASD cases (reviewed in Fombonne et al., 1999; Courchesne et al., 2003; Courschesne et al., 2011).The Pten protein normally functions as a protein and lipid phosphatase that negatively regulates the Akt signaling pathway, particularly by dephosphorylating phosphatidylinositol (3, 4, 5)-triphosphate (PIP3; Maehama and Dixon, 1998). Tuberin, the protein product of *Tsc2*, is one target of the Akt signaling pathway (Potter et al., 2002), which is inhibited following phosphorylation by Akt (Inoki et al., 2002). Thus, the PI3K/Akt signaling pathway increases mTORC1 activity and Pten, like the *Tsc1* and *Tsc2* products, normally inhibits mTORC1 activity.

The first *Pten* knockout mouse models showed that homozygous deletion of exons 4 and 5 (Di Cristofano et al., 1998) or exons 3, 4, and 5 (Suzuki et al., 1998) results in embryonic lethality, whereas heterozygous deletions result in widespread tumorigenesis, resembling

PHTS. More recently, conditional *Pten* knockouts have been developed that more closely resemble PTEN-related ASDs. Although these models do not have good construct validity because humans with PTEN-related ASDs have germline missense mutations in *PTEN*, the various models provide insight into different brain regions and cell types involved in ASDs. Mice with *Pten* deleted selectively in a subset of mature neurons in cortex and hippocampus (*Nse-Cre*) show deficits in social interactions (Kwon et al., 2006a) and increased repetitive behaviors (Napoli et al., 2012). Similar behaviors have been observed in mice with *Pten* deleted in granule cells of the cerebellum and neurons in the dentate gyrus of the hippocampus (*Gfap-Cre*; Lugo et al., 2014). Another mouse model of PTEN-related ASD ($Pten^{m3m4}$) was created by knocking in missense mutations which decrease the amount of nuclear Pten (Tilot et al., 2014). Interestingly, only male mice with the missense mutations display increased social behavior, as opposed to the decreased social behavior observed in other mouse models of ASDs (Tilot et al., 2014). While these particular missense mutations have not yet been reported in humans, mutations that similarly affect the subcellular localization of PTEN have been observed in PTHS patients.

Detailed studies of the PTEN-related ASD mouse models have revealed several cellular structural abnormalities. Generally, neuronal cells lacking Pten have increased size, more abundant and ectopic axonal projections, increased numbers of presynaptic vesicles, increased numbers of dendritic spines, and larger postsynaptic densities (Kwon et al., 2006a; Fraser et al., 2008; Luikart et al., 2011a; Williams et al., 2015). However, one study suggests that overall numbers of dendritic spines are unchanged in neurons lacking Pten, but the spines are larger and there are a greater proportion of spines with mature morphology (Haws et al., 2014). The $Pten^{m3m4}$ mice, which have decreased nuclear Pten but normal levels of cytoplasmic Pten, have neurons with increased soma size but normal dendritic thickness (Tilot et al., 2014), indicating the importance of Pten in determining cellular morphology locally.

Not only do neurons lacking Pten have drastic morphological aberrations, but they also have significant changes in firing properties and synaptic plasticity. $Pten^{+/-}$ mice have decreased LTP and completely abolished NMDA-dependent LTD in hippocampal CA1 synapses (Wang et al., 2006). Moreover, *Pten* conditional knockouts (*GFAP-Cre*) also have decreased LTP in CA1 synapses (Fraser et al., 2008), whereas dentate granule cell synapses have impaired mGluR-dependent LTD (Takeuchi et al., 2013). Importantly, postnatal deletion of *Pten* with *CamKIIα-Cre* reproduced the deficits in LTP and LTD at CA1 synapses but had no effect on neuronal or dendritic morphology, thus indicating these phenotypes are not necessarily associated (Sperow et al., 2012).

Pten is involved in the suppressing the Akt signaling pathway upstream of mTOR activity, which normally leads to dephosphorylation of FMRP and subsequent protein translation. However, there is some evidence that suggests Pten might be acting differently. In particular, deletion of *Pten* leads to decreased expression of mGluR5, increased expression of FMRP, and increased phosphorylation of FMRP (Lugo et al., 2014). These findings seem to oppose, rather than overlap with, findings from *Fmr1* knockout mice. More work needs to be done to determine the molecular consequences of *Pten* loss-of-function and how this contributes to ASD-like phenotypes.

3.2.4 *Ube3a* (Angelman syndrome and non-syndromic ASDs)—Patients diagnosed with Angelman syndrome (AS) frequently meet the diagnostic criteria for ASD (Peters et al., 2004). Angelman syndrome is caused by mutations in or lack of expression of the maternal copy of *UBE3A* (Kishino et al., 1997; Matsuura et al., 1997), the paternal copy of which is normally silenced in neurons (Albrecht et al., 1997; Rougeulle et al., 1997; Yamasaki et al., 2003). Maternally derived duplications and triplications of a genomic region which contains *UBE3A* have also been identified in patients with non-syndromic ASDs (Cook et al., 1997; Glessner et al., 2009; Hogart et al. 2010). The protein encoded by *UBE3A* is the ubiquitin-protein ligase (UBE3A), also known as E6AP ubiquitin-protein ligase (E6AP; Huibregtse et al., 1993). The ubiquitin-proteasome degradation system has been implicated in synapse function and plasticity (reviewed in Mabb and Ehlers, 2010).

Mouse models of AS that recapitulate the major features of the disorder, including some ASD-like phenotypes, have been created and studied extensively. The first *Ube3a* mutant mice were created by making a targeted deletion of coding exon 2 (Jiang et al., 1998) and another model was created by disrupting last two coding exons 15–16 (Miura et al., 2002), but only the model with exon 2 deletion has been extensively studied subsequently. The brains of the maternally deficient ($Ube3a^{m-/p+}$) mice have significantly low or no detectable Ube3a protein, conferring high construct validity. Another group created mice that express either one extra (1xTg) or two extra copies (2xTg) of *Ube3a*, with reasonable construct validity for patients with maternally derived duplications and triplications in the genomic region encompassing *UBE3A* (Smith et al., 2011).

Maternal deficiency of *Ube3a* affects dendritic and spine morphology. Specifically, $Ube3a^{m-/p+}$ mice have decreased spine density in hippocampal CA1 and cortical layer 3–5 pyramidal neurons (Dindot et al., 2008). In contrast, $Ube3a^{m-/p+}$ mice raised in darkness have similar spine densities in visual cortex, suggesting a deficit specifically in experience-dependent remodeling of synapses (Yashiro et al., 2009). Knockdown of Ube3a isoforms via *in utero* electroporation of shRNA revealed that Ube3a is also required for apical, but not basal, dendrite outgrowth (Miao et al., 2013). On the other hand, increased *Ube3a* gene dosage does not affect glutamatergic synapse number in layer 2/3 barrel cortex, as assessed by electron microscopy, immunofluorescence, and golgi staining (Smith et al., 2011).

Perhaps the best studied aspect of AS and other *Ube3a* mouse models are the electrophysiological properties of affected neurons. Reduced LTP has been observed in hippocampal CA synapses (Jiang et al., 1998; Weeber et al., 2003) as well as in visual cortex (Yashiro et al., 2009; Sato and Stryker, 2010) of AS model mice. Like dendritic spine density, LTP in visual cortex is not significantly different between wild-type and $Ube3a^{m-/p+}$ mice that were raised in darkness (Yashiro et al., 2009). In addition, reduced NMDA-dependent LTD has been reported in the visual cortex of $Ube3a^{m-/p+}$ mice raised in normal conditions (Yashiro et al., 2009), whereas enhanced mGluR-dependent LTD has been reported in hippocampal CA1 synapses (Pignatelli et al., 2014). A decreased AMPA/NMDA current ratio has also been reported in $Ube3a^{m-/p+}$ CA1 hippocampal neurons (Greer et al., 2010), but this ratio is unchanged in $Ube3a^{2xTg}$ barrel cortex (Smith et al., 2011). Whole-cell patch clamp recording revealed reduced frequency and amplitude of mEPSCs in the dorsomedial striatum of $Ube3a^{m-/p+}$ mice, but not in the dorsolateral

striatum (Hayrapetyan et al., 2013). Layer 2/3 pyramidal neurons in *Ube3a*^{2xTg} barrel cortex display reduced eEPSC amplitude, reduced sEPSC amplitude and frequency, reduced sIPSC amplitude, and reduced mEPSC amplitude and frequency, along with decreased release probability and synaptic glutamate concentration (Smith et al., 2011). In general, the mechanisms underlying the different impairments in synapses of different brain regions remains poorly understood.

Currently, no conditional knockouts of *Ube3a* have been reported, so there is limited knowledge about what cell types and brain regions are involved in the behavioral abnormalities in *Ube3a*$^{m-/p+}$ mice. However, using mice engineered with a stop codon surrounded by *loxP* sites that prevents maternal *Ube3a* expression crossed with the commonly used ubiquitously-expressed inducible Cre line (*CAG-Cre/Esr1*), it was recently demonstrated that Ube3a reinstatement during embryonic or early postnatal development can prevent the expression of some ASD-like phenotypes, but reinstatement in mice later in development is not effective (Silva-Santos et al., 2015). Interestingly, these results are in contrast with those reported in Rett syndrome mice (see section 3.1). This could be due to the different behaviors reported in these studies, or it could indicate that some ASD susceptibility genes have tightly-regulated functions during development whereas others are required during the entire lifespan. Again, these types of studies need to be investigated using other models of ASDs before making any conclusions about the developmental time course of ASD pathogenesis.

Numerous targets of Ube3a-dependent ubiquitination have been identified (Kumar et al., 1999; Mani et al., 2006; Mulherkar et al., 2009; Louria-Hayon et al., 2009; Zaaroor-Regev et al., 2010) in attempts to dissect the molecular mechanisms underlying Angelman syndrome. One target is activity-regulated cytoskeleton protein (Arc), which promotes the internalization of AMPA receptors, and is interesting considering the functional defect of AMPA receptor mediated synaptic transmission (Greer et al., 2010). More recent evidence suggests that Ube3a regulates Arc expression through estradiol-induced transcription rather than through ubiquitination (Kühnle et al., 2013). Recent findings also indicate that in *Drosophila* Ube3a acts as a cofactor for some MeCP2 functions (Kim et al., 2013) and *Ube3a*$^{m-/p+}$ mice demonstrate deficits in BDNF signaling (Cao et al., 2013). Additionally, *Ube3a*$^{m-/p+}$ mice have presynaptic vesicle cycling defects specifically in inhibitory interneurons (Wallace et al., 2012). These observations clearly demand further investigation on some very basic questions. For instance, is the dosage of Ube3a in excitatory or inhibitory neurons responsible for the profound neurobehavioral impairment? Moreover, is the dosage of Ube3a primarily affecting pre- or postsynaptic sites?

3.3 Synaptic Organizing and Scaffolding: Shanks, Neurexins/Neuroligins

3.3.1 *Shanks* (Phelan-McDermid syndrome and non-syndromic ASDs)—

Mutations in the SHANK/ProSAP family genes (*SHANK1–3*), particularly *SHANK2* and *SHANK3*, have been identified recently as pathogenic for non-syndromic ASDs (Durand et al., 2007; Berkel et al., 2010; Sato et al., 2012). In addition, loss of one copy of *SHANK3* in Phelan-McDermid syndrome is thought to contribute to the neurobehavioral features of the disorder, including ASD (Bonaglia et al., 2001; Wilson et al., 2003). The Shank proteins

function as scaffolds that organize postsynaptic densities (PSDs) in glutamatergic synapses and link receptors to cytoskeletal signaling molecules (Sheng and Kim 2000). Shank proteins have also been implicated in spinogenesis and synapse development. In particular, transfection of Shank3 is sufficient to induce dendritic spine formation in otherwise aspiny neurons (Roussignol et al., 2005). Another study provides evidence that Shank2 and Shank3 are involved in spine formation, whereas Shank1 is involved in synapse maturation and stability (Grabrucker et al., 2011).

Mouse models for each of the three Shank proteins have been created to better understand the role of these proteins *in vivo* and their contributions to ASDs. The first model was created by deleting exons 14–15 of *Shank1* (Hung et al., 2008) where homozygous deletion (*Shank1*$^{-/-}$) results in some ASD-like features (Silverman et al., 2011; Wöhr et al., 2011; Sungur et al, 2014). Two different lines of *Shank2* mutant mice have been reported, which involved deletion of exon 7 (Schmeisser et al., 2012) or exons 6–7 (Won et al., 2012) of the *Shank2a* isoform, corresponding to exon 17 and exons 16–17, respectively, of full length *Shank2* (Jiang and Ehlers, 2013). Both lines are assumed to have disrupted function of all *Shank2* isoforms and display stereotypy, diminished social interactions, and impaired vocalizations (Schmeisser et al., 2012; Won et al., 2012; Ey et al., 2013).

Ten different lines of *Shank3* mutant mice have been reported, but due to the transcriptional complexity of *Shank3* (Wang et al., 2014), none of the published lines disrupt all isoforms. Still, deletion of exons 4–7 (Peça et al., 2011), exons 4–9 (Bozdagi et al., 2010; Wang et al., 2011; Jaramillo et al., 2015), exon 9 (Lee et al., 2015), exon 11 (Schmeisser et al., 2012), exons 13–16 (Peça et al., 2011), or exon 21 (Kouser et al., 2013; Duffney et al., 2015), and an insertion mutation in exon 21 (Speed et al., 2015) all result in ASD-like behaviors to various degrees (reviewed in Bey and Jiang, 2014). Whereas humans with *SHANK3*-related ASDs are either haploinsufficient due to a large chromosomal deletion or have point mutations in one copy of their *SHANK* genes, many of the studies utilizing mouse models only report behavioral abnormalities in homozygous mutants. This, along with the fact that there currently is no model in which all *Shank3* isoforms are disrupted, limits the construct validity of these models.

Although there are no published studies that have explored the role of the Shank proteins in different cell types and brain regions using conditional knockouts, there have been considerable efforts to characterize the cellular phenotypes that result from the loss of these proteins. Deletion of *Shank1* results in decreased spine density, shorter spines, and thinner PSDs in CA1 pyramidal neurons (Hung et al., 2008). However, both normal spine density (Won et al., 2012) and decreased spine density (Schmeisser et al., 2012) have been reported in hippocampal CA1 neurons from *Shank2* mutants. Moreover, PSD length and thickness are not affected in CA1 neurons from either *Shank2* line (Won et al., 2012; Schmeisser et al., 2012). Deletion of exons 13–16 of *Shank3* led to decreased spine density, increased dendritic complexity, and thinner PSDs in medium spiny neurons (MSNs) of the striatum (Peça et al., 2011). However, deletion of exon 11 (Schmeisser et al., 2012) or exon 21 (Kouser et al, 2013) of *Shank3* had no effect on either spine density nor on dendritic complexity in CA1 neurons. This may have been due to the age of the mice tested, as deletion of exons 4–9 of *Shank3* had an age-dependent effect on spine density and

morphology: CA1 neurons from 4 week old mice had decreased spine density and increased spine length, whereas CA1 neurons from 10 week old mice had normal spine density, but decreased spine length (Wang et al., 2011).

Accumulating evidence *in vivo* suggests that Shank proteins are required for proper synaptic function as well, but the findings are somewhat inconsistent. Extracellular recordings in the stratum radiatum of *Shank1*$^{-/-}$ mice revealed a significantly decreased excitatory synaptic strength, as assessed by the input-output relationship of field excitatory postsynaptic potentials, and recordings from CA1 hippocampal neurons in these mice revealed decreased mEPSC frequency (Hung et al, 2008). The two lines of *Shank2* mutant mice have distinct electrophysiological phenotypes, despite the two mutations having presumably the same effect on Shank2 protein expression. Deletion of *Shank2* exon 7 results in decreased excitatory synaptic strength, decreased mEPSC frequency, a decreased AMPA/NMDA current ratio, and increased LTP in the hippocampus (Schmeisser et al., 2012). However, deletion of *Shank2* exons 6–7 results in no significant changes in excitatory synaptic transmission, an increase in the AMPA/NMDA current ratio, and impaired LTP and NMDA-dependent LTD (Won et al., 2012).

The findings from *Shank3* mutants are also somewhat inconsistent, but many of the differences can be attributed to different isoforms disrupted due to the transcriptional complexity of the gene. Deletion of exon 9 leads to increased mIPSC frequency in the hippocampus and decreased mIPSC frequency in the prefrontal cortex (Lee et al., 2015). All three models which have exons 4–9 deleted have reduced LTP in the CA1 region of the hippocampus (Bozdagi et al., 2010; Wang et al., 2011; Jaramillo et al., 2015). Whereas the model produced by Bozdagi et al. has reduced excitatory synaptic strength, decreased mEPSC amplitude, increased mEPSC frequency, and a decreased paired-pulse ratio, the other models exhibit none of these phenotypes, but the model produced by Jaramillo et al. did have an increased AMPA/NMDA ratio in the striatum. Deletion of exons 13–16 had similar results to those observed by Wang et al. and Jaramillo et al. in the hippocampus, but resulted in decreased excitatory synaptic strength, reduced mEPSC frequency, and reduced mEPSC amplitude in MSNs of the dorsolateral striatum (Peça et al., 2011). On the other hand, deletion of exon 21 results in decreased LTP, an increased AMPA/NMDA current ratio, decreased mEPSC frequency, and reduced excitatory synaptic strength in the CA1 region of the hippocampus (Kouser et al., 2013) as well as an increased AMPA/NMDA current ratio in layer 5 prefrontal cortex pyramidal neurons (Duffney et al., 2015). The insertion mutation in exon 21 is similar to deletion of exon 21 in terms of hippocampal electrophysiology, but one difference is that LTD, and not LTP, is impaired (Speed et al., 2015).

The mechanism through which deletion of Shank proteins leads to synaptic dysfunction is likely due to impaired interactions between glutamate receptors and postsynaptic density proteins leading to impaired signaling in dendritic spines. For instance, rescue of NMDA receptor hypofunction with an actin stabilizer after knockdown of Shank3 in cultured rat cortical neurons suggests that some of the synaptic defects are due to disrupted cytoskeleton signaling (Duffney et al., 2013). Similarly, treating *Shank3* exon 21 deletion mice with an

actin stabilizer rescues both behavioral phenotypes and synaptic deficits (Duffney et al., 2015).

Moreover, changes in the densities of receptors and synaptic proteins have been reported *in vivo*. Mice lacking Shank1 have decreased expression of Homer and GKAP/SAPAP in hippocampal PSD fractions (Hung et al., 2008). Deletion of *Shank2* exon 7 results in significantly increased expression of NMDAR subunits in the hippocampus and significantly increased expression of both NMDAR and AMPAR subunits as well as Shank3 in the striatum (Schmeisser et al., 2012). Deletion of *Shank2* exons 6–7 also results in increased NMDAR subunit expression, but reduces NMDAR-associated signaling, as assessed by the proportion of phosphorylated CaMKII, ERK1/2, and p38 in whole brain lysates (Won et al., 2012).

Mice with different *Shank3* mutations have varying degrees of altered expression of receptors, synaptic proteins, and downstream signaling molecules. Deletion of exon 21 of *Shank3* results in increased mGluR5 expression in hippocampal synaptosome and PSD fractions (Kouser et al., 2013) as well as decreased Rac1/PAK signaling, increased activated cofilin, and decreased expression of F-actin in synapses (Duffney et al., 2015). Deletion of exons 4–9 of *Shank3* reduces Homer1b/c, GluA1, GluN2A, and SAPAP1 in hippocampal PSD fractions (Wang et al., 2011) as well as reduces Homer1b/c, PSD-95, GluA2, and GluA3 in striatal synaptosomes (Jaramillo et al., 2015). Finally, deletion of exons 13–16 results in reduced expression of SAPAP3, Homer, PSD-93, GluA2, GluN2A, and GluN2B in striatal PSD fractions (Peça et al., 2011) whereas deletion of exon 11 results in *increased* expression of GluN2B and Shank2 in striatal synaptosomal fractions (Schmeisser et al., 2012). It is currently unclear how these molecular changes lead to synaptic dysfunction and how synaptic dysfunction leads to changes in behavior.

3.3.2 *Neurexins/Neuroligins* (non-syndromic ASDs)—Mutations in the genes encoding the presynaptic cell-adhesion molecules, neurexins, and their postsynaptic binding partners, neuroligins, have been implicated in non-syndromic ASDs (Gauthier et al., 2011; Camacho-Garcia et al., 2013; Vaags et al., 2012; Steinberg et al., 2012; Jamain et al., 2003; Feng et al., 2006). Additionally, mutations in genes coding for other members of the neurexin superfamily, contactin associated protein-2 (*CNTNAP2*; Alarcón et al., 2008; Arking et al., 2008) and contactin associated protein-4 (*CNTNAP4*; Karayannis et al., 2014), have been identified in cases of ASD. Each of the three neurexin genes (*NRXN1, NRXN2, NRXN3*) contains two promoters, which generate a longer α-NRXN or a shorter β-NRXN, respectively (Tabuchi and Südhof, 2002). Moreover, the neurexins undergo extensive alternative splicing, resulting in thousands of different isoforms (Ullrich et al., 1995; Treutlein et al., 2014). On the other hand, the neuroligin genes (*NLGN1, NLGN2, NLGN3, NLGN4X, NLGN4Y*) contain one promoter and two alternatively spliced regions, allowing for up to four different isoforms per gene (Ichtchenko et al., 1996). Together, neurexins and neuroligins form trans-synaptic complexes that facilitate synapse formation and maturation, as well as excitatory and inhibitory transmission (reviewed in Bang and Owczarek, 2013).

A multitude of mice with mutations in the neurexins, neuroligins, and related genes have been generated to understand the roles of these cell adhesion molecules in normal

development and in ASDs. Mice lacking either *Nrxn1*α (Etherton et al., 2009; Grayton et al., 2013) or *Nrxn2*α (Dachtler et al., 2014) show some ASD-like features. Additionally, mice with a subset of forebrain neurons overexpressing of a dominant-negative form of *Nrxn1*β missing its cytoplasmic tail display ASD-like behaviors (Rabaneda et al., 2014). Mice lacking Cntnap2 (Peñagarikano et al., 2011) or *Cntnap4* (Karayannis et al., 2014) also have phenotypes which resemble ASD. Likewise, mice lacking *Nlgn1* (Blundell et al., 2010), Nlgn2 (Blundell et al., 2009; Wöhr et al., 2013), *Nlgn3* (Radyushkin et al., 2009; Rothwell et al., 2014), or *Nlgn4* (Jamain et al., 2008; El-Kordi et al., 2013; Ju et al., 2014) all have ASD-like behaviors to various degrees. Mice with a knock-in mutation of *NLGN3* (R451C) found in human populations have also been generated and recapitulate some ASD-like features (Tabuchi et al., 2007; Jaramillo et al., 2014; Rothwell et al., 2014).

In addition to the study that reported ASD-like behaviors in mice overexpressing a dominant-negative form of *Nrxn1*β in a subset of excitatory forebrain neurons (*CaMKII*α-*tTa*; Rabaneda et al., 2014), another study involved various conditional knockouts of *Nlgn3* to determine the cell types and brain regions involved in the behaviors (Rothwell et al., 2014). The authors of the latter study found that depletion of *Nlgn3* in the D1-MSNs (*D1-Cre*) of the nucleus accumbens (localized injection of *AAV-Cre*) was sufficient to drive repetitive motor routines on the rotarod test, whereas depletion of Nlgn3 in D2-MSNs (*A2a-Cre*) or in the dorsal striatum (localized injection of *AAV-Cre*) was not (Rothwell et al., 2014). Moreover, depletion of Nlgn3 in Purkinje cells (*P7-Cre*) or parvalbumin-positive interneurons (*PV-Cre*) did not affect motor performance, but increased and decreased activity in the open field, respectively (Rothwell et al., 2014).

Unlike the other mouse models of ASDs, individual neurexin and neuroligin mutants do not have many reported changes in dendritic spine density, which is somewhat surprising, given that neurexins (Graf et al., 2004) and neuroligins (Scheiffele et al., 2000) induce synapse formation in cultured cells *in vitro* and that knockdown of neuroligins in cultured neurons reduces spine density (Chih et al., 2005). This suggests that there may be functional redundancy in the different proteins that can compensate for one another during development *in vivo*. However, even mice lacking either all three α-Nrxn proteins (Missler et al., 2003) or Nlgn1, Nlgn2, and Nlgn3 (Varoqueaux et al., 2006) have normal synapse numbers, suggesting that these molecules may not be required for synapse formation at all. A more recent study showed that knockdown of Nlgn1 *in vivo* reduces synaptic density when neighboring neurons still express Nlgn1 (Kwon et al., 2012). Whereas mice lacking *Nlgn2* have normal numbers of both excitatory and inhibitory synapses, they have decreased expression of VGAT, which represents an impairment in recruiting GABAergic synaptic vesicles to presynaptic terminals (Blundell et al., 2009). The *Nlgn3*^{R451C} mice also have normal numbers of both excitatory and inhibitory synapses, but have increased expression of VGAT (Tabuchi et al., 2007). These mice have increased dendritic complexity in the stratum radiatum, decreased synaptic terminal size, fewer vesicles per terminal, and decreased spine size (Etherton et al., 2011a). Additionally, the *Nlgn3*^{R451C} mice have increased dendritic spine turnover in pyramidal neurons in the anterior frontal cortex (Isshiki et al., 2014). Moreover, mice lacking *Cntnap4* have increased width of synaptic clefts and decreased PSD length in GABAergic synapses (Karayannis et al., 2014).

Although changes in cellular and spine morphology in neurexin and neuroligin mouse models of ASD are somewhat minor, synaptic function is drastically altered. Deletion of *Nrxn1*α leads to reduced mEPSC frequency and decreased excitatory synaptic strength in the CA1 region of the hippocampus (Etherton et al., 2009). Mice with the dominant-negative form of *Nrxn1*β have decreased mEPSC frequency and mIPSC frequncy in L5/6 pyramidal neurons from the somatosensory cortex (Rabaneda et al., 2014). Mice lacking *Cntnap4* have increased dopamine signaling through a presynaptic mechanism in the nucleus accumbens but fewer, smaller, and slower sIPSCs in PV-positive cells of the somatosensory cortex (Karayannis et al., 2014). Deletion of Nlgn1 results in a decrease in AMPAR EPSCs, NMDA EPSCs, and an increase in the AMPA/NMDA current ratio in the CA1 region of the hippocampus (Budreck et al., 2013) as well as impaired LTP at thalamic inputs to the amygdala (Jung et al., 2010). Impaired LTP has also been observed the hippocampi of Nlgn1 mutants *in vitro* (Blundell et al., 2010) and *in vivo* (Jedlicka et al., 2015). Mice lacking Nlgn4 have decreased network excitability in both excitatory and inhibitory circuits in layer 2/3 pyramidal cells, but the excitatory circuits are affected more greatly such that the excitation-inhibition ratio is decreased in the mutants (Delattre et al., 2013).

The electrophysiological consequences of the *Nlgn3*^{R451C} mutation in particular have been studied extensively. These mice have increased mIPSC frequency and eIPSC amplitude in layer 2/3 somatosensory cortex neurons (Tabuchi et al., 2007). However, they have decreased eIPSC amplitude in spiny neurons that receive input from PV-expressing basket cells in layer IV barrel cortex (Cellot and Cherubini, 2014). The R451C mutation also leads to an increased frequency of giant depolarizing potentials (GDPs) and mIPSCs in the CA3 region of the hippocampus starting during the first two weeks of postnatal life (Pizzarelli and Cherubini, 2013). These mice also exhibit increased excitatory transmission, increased LTP, and a decreased AMPA/NMDA current ratio in the CA1 region of the hippocampus (Etherton et al., 2011a). While most studies failed to find similar deficits in *Nlgn3* knockout mice, one study found that both the *Nlgn3*^{R451C} mice and *Nlgn3* knockout mice have increased GABAergic synaptic transmission (eIPSCs) in cholecystokinin (CCK) basket cells and impair tonic endocannabinoid (ECB) signaling (Földy et al., 2013). Mice lacking Nlgn3 also have decreased frequency of mIPSCs in D1-MSNs of the NAc while having normal frequency of mEPSCs, thereby increasing the excitatory/inhibitory current ratio (Rothwell et al., 2014).

Neuroligins interact with multiple postsynaptic proteins, including Shank3 (Meyer et al., 2004). Moreover, it was shown more recently that Shank3 expression mediates transsynaptic changes in synaptic proteins and that this function depends on the formation of neurexin/neuroligin complexes (Arons et al., 2012). Accordingly, neurexin and neuroligin mutants, like Shank mutants, exhibit changes in the expression of synaptic proteins. Specifically, mice lacking *Nrxn2*α have reduced Munc-18 expression in hippocampal lysates (Dachtler et al., 2014). Mice lacking *Nlgn1* have decreased expression of CSP, Liprin, Munc-18, Nxnα, and Nrxnβ, but increased expression of Nlgn3 and Synapsin1a in whole brain lysates (Blundell et al., 2010). As mentioned previously, Nlgn2 have decreased VGAT expression in hippocampus (Blundell et al., 2009), whereas *Nlgn3*^{R451C} mutants increased VGAT and Gephyrin expression in whole brain lysates (Tabuchi et al., 2007). The *Nlgn3*^{R451C} mutants

also have decreased expression of PSD95, SAP102, NR2A, and NR2B in hippocampal lysates (Etherton et al., 2011a). Analysis of synaptosome and PSD fractions from neurexin and neuroligin mutant mice could be performed to determine if the changes more similar to changes observed in Shank mutants.

4. Concluding Remarks

4.1 Convergent Molecular Pathways and Mechanisms

Although much remains to be investigated in various directions, it is tempting to generalize these findings on a number of levels. Etiologically, rare and private mutations clustered in select molecular classes appear to be a major driver for a genetic basis in a sub-set of ASD patients. At the molecular level, several pathways appear to emerge from analyzing the existing ASD mouse models. These include disruption of overlapping signaling pathways mediated by mGluR5, BDNF, and mTOR (see Figure 1 and Table 2), although the degree of evidence varies depending on the model.

Moreover, the available evidence strongly supports dysfunctional synapses as a component of autism pathophysiology. However, although there are widespread disruptions in synaptic function across the mouse models of ASD, the direction of change and magnitude of effect are inconsistent between different models, as well as between different types of synapses within any given model. Although there are many apparent differences between models, it is difficult to compare results that were reported in different brain regions or at different times during development. For instance, *Fmr1* knockout mice have decreased spine stability somatosensory cortex (Cruz-Martin et al., 2010), whereas *Mecp2*$^{tm1.1Jae}$ mice have increased spine stability in the same region (Landi et al., 2011), but the former study used mice that were postnatal day 10–12, whereas the latter study used mice that were postnatal day 25–26. Therefore, more side-by-side comparisons of different mouse models of ASDs would be valuable. One interesting discovery from genetics studies of ASDs is the frequent mutations in genes encoding epigenetic machinery. However, it is not immediately clear how deficiency of these proteins contributes to autism pathophysiology. As has been demonstrated in Rett syndrome mouse models, the dysfunction of synapses could still be the major mechanism. However, a mechanism that is independent from synaptic dysfunction may also be possible.

4.2 Missing Links and Challenges

There are some glaring missing links in our understanding of the pathophysiology of ASDs. Specifically, how the mutation of an individual gene, a disrupted molecular pathway, and dysfunctional synapses affect the circuitry and produce ASD-like behavioral manifestations is unclear. Understanding the circuitry underlying autism, both anatomically and functionally, is critical to the development of effective clinical intervention. Several competing hypotheses regarding the circuit-level mechanisms have been proposed in the human literature. One widely tested hypothesis is altered structural and functional brain connectivity (Belmonte et al., 2004; Geschwind and Levitt, 2007; Kana et al., 2014). Structural connectivity is the physical connections between different brain regions, while functional connectivity refers to the integrated relationship between spatially separated brain

regions. It is believed that structural connections within the brain give rise to functional network activity as measured by coherence or information flow. Neuroimaging investigations indicate that ASDs are associated with perturbed connectivity at both structural and functional levels (Minshew and Keller, 2010; Vissers et al., 2012); however, the exact nature and pattern of this aberrant neural connectivity remains uncertain due to inconsistent findings from neuroimaging studies in patients (Di Martino et al., 2014; Kana et al., 2014; Uddin et al., 2013). While early studies reported reduced functional connectivity (Just et al., 2004), recent investigations implicate hyper-connectivity in multiple brain regions and across neural circuits (Keown et al., 2013; Supekar et al., 2013). In addition to methodological and conceptual controversy, this uncertainty reflects the substantial molecular heterogeneity of human patients. Notably, these studies were conducted primarily in high-functioning ASD patients for whom the etiologies are mostly unknown. For these reasons, autism animal models offer a unique opportunity to test the functional connectivity hypothesis because of homogenous genetic defects. Using optogenetic tools, Gunaydin et al. have shown that the activity of ventral tegmental area (VTA) to nucleus accumbens (NAc) projections could encode and predict key features of social interaction in wild type mice (Gunaydin et al., 2014). Similarly, Felix-Ortix and Tye revealed the role of projections from the basolateral complex of the amygdala (BLA) to the ventral hippocampus (vHPC) in two different social interaction tests (Felix-Ortiz and Tye, 2014). Few reports have investigated the circuit-level mechanisms underlying autism-like behaviors in genetically modified autism models with strong construct validity. Therefore, the combination of optogenetics and CRISPR/Cas9 genome editing tools in ASD models is expected to produce significant insight into whether there are common circuits disrupted in ASD models with different genetic defects.

4.3 Future Directions

Several critical questions remain poorly understood to understand the pathophysiology of ASDs. The molecular and circuit-level mechanisms underlying autism behaviors are mostly unknown. Moreover, the developmental origin of autistic behaviors has not been delineated. Numerous challenges have been encountered in modeling autism mutations in mice. First, recent genetics studies have identified mutations in a long list of genes implicated in ASDs. However, most of these mutations are rare and private. The full spectrum of clinical features associated with these genetic defects is not known, posing a significant challenge for experimental design and potential to overly generalize findings from individual models. Second, the lack of robust and strong behavioral assays for measuring behaviors that resemble human ASDs remains a significant obstacle to dissect the circuit-level mechanisms. Third, various effects on synapses in different brain regions and different stages of development makes it difficult to establish a link between synaptic dysfunction and behavioral impairments. These challenges thus demand a shift from the current analytic paradigm that combines behavior, synaptic electrophysiology, and biochemical approaches. We propose that an analytic paradigm that integrates the behavior and *in vivo* physiological recordings in multiple sites is a more productive approach to model autism in mice. Furthermore, manipulation of ASD candidate genes spatially and temporally will further delineate the circuits underlying ASDs. In the future, manipulation of the genomes in other species such as rat or non-human primate models may overcome the many limitations of

mouse models. A more complete understanding of the current genetic models of ASDs will allow researchers to study the role of non-genetic factors and their biological underpinnings. Ultimately, knowledge learned from animal models will lead to the development of effective clinical intervention that targets the specific molecular pathways and neural circuits.

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Highlights

- Disrupted synaptic function is common across mouse models of ASDs
- The synapses affected and the magnitude and direction of effects are inconsistent
- Overlapping molecular cascades include mGluR5, BDNF/TrkB, and mTOR signaling.
- The developmental origin and circuits underlying the disorders remain largely unknown

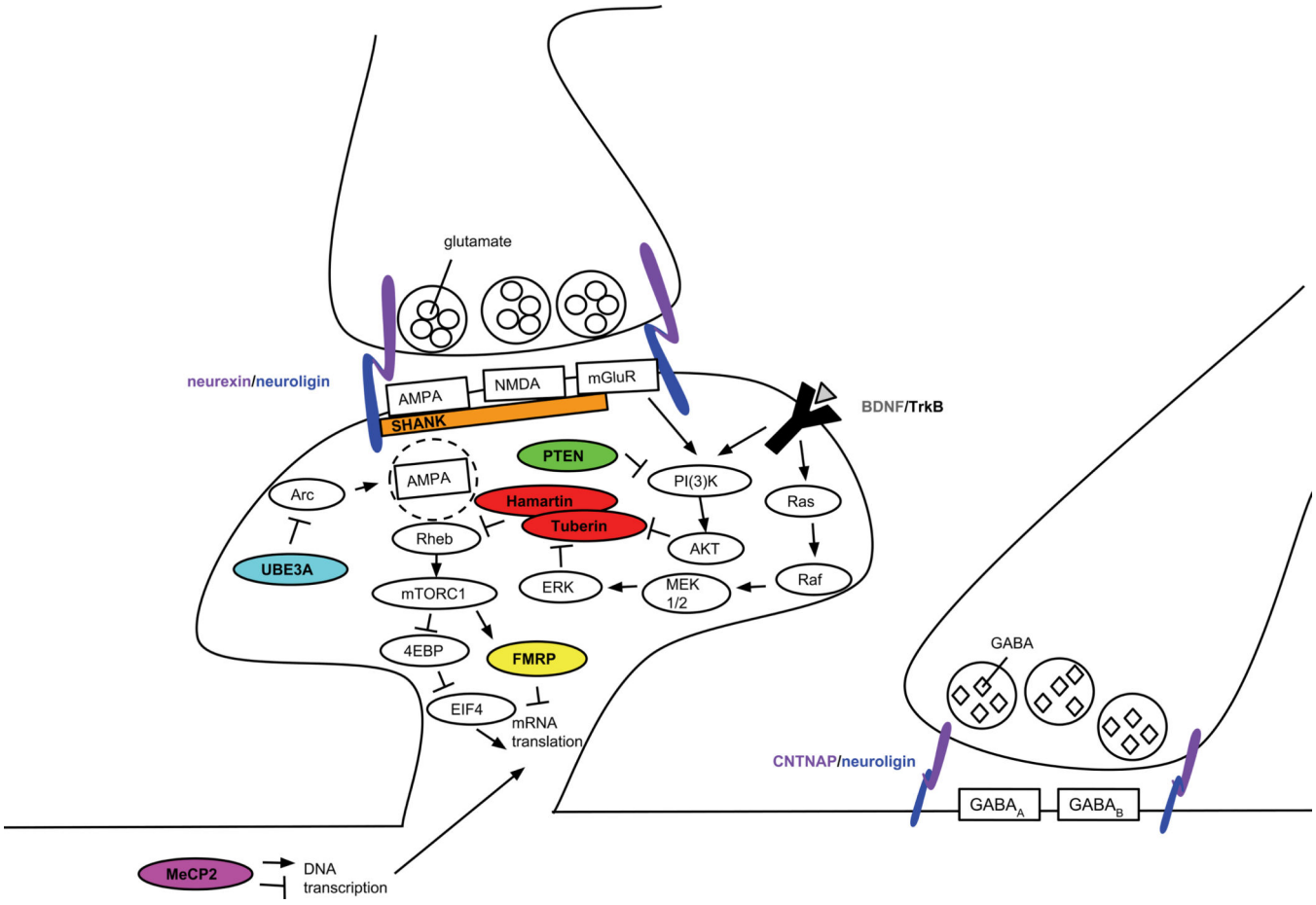

Figure. Monogenic mouse models of ASDs have disruptions in overlapping molecular pathways

The epigenetic and transcriptional regulator MeCP2 controls the expression of hundreds of different proteins, including BDNF. When BDNF binds to TrkB, its receptor, the resulting signaling pathways converge with pathways known to influence local protein synthesis. The RNA-binding protein FMRP is directly involved in suppressing the translation of mRNA, but other proteins implicated in ASDs, including hamartin and tuberin (the proteins encoded by *TSC1* and *TSC2*), as well as PTEN are upstream signaling molecules that converge on this pathway. The synaptic organizing proteins from the Shank and neurexin/neuroligin families influence these pathways indirectly by affecting the localization and function of glutamate receptors. Similarly, the ubiquitin protein ligase Ube3a normally suppresses the internalization of AMPA receptors, thereby affecting neuronal signaling and plasticity.

Table 1

List of genes with strong evidence for syndromic and non-syndromic ASDs

Syndromic ASDs		Non-Syndromic ASDs	
Gene Name	**Locus**	**Gene Name**	**Locus**
ADNP	20q13.13	ASH1L	1q22
ADSL	22q13	ASXL3	18q11
AHI1	6q23.3	CACNA1H	16p13.3
ALDH5A1	6p22	CACNA2D3	3p21.1
ANKRD11	16q24.3	CHD2	15q26
ARID1B	6q25.1	CHD8	14q11.2
ARX	Xp22.	CNTN4	3p26
ASXL3	18q11	CNTNAP2	7q35
CACNA1C	12p13.3	CUL3	2q36.2
CDKL5	Xp22	DEAF1	11p15.5
CHD2	15q26	DSCAM	21q22.2
CHD7	8q12.2	DYRK1A	21q22.13
CNTNAP2	7q35-q36	GABRB3	15q12
DHCR7	11q13	GRIN2B	12p12
DYRK1A	21q22.13	GRIP1	12q14.3
EHMT1	9q34.3	KATNAL2	18q21.1
FMR1	Xq27.3	KDM5B	1q32.1
HDAC4	2q37.3	KMT2A	11q23
KMT2A	11q23	KMT2C	7q36.1
MECP2	Xq28	MED13L	12q24.21
NIPBL	5p13.2	MET	7q31
PTEN	10q23.3	MSNP1AS	5p14.1
RAI1	17p11.2	MYT1L	2p25.3
SCN1A	2q24.3	NRXN1	2p16.3
SYNGAP1	6p21.3	POGZ	1q21.3
TSC1	9q34	PTCHD1	Xp22.11
TSC2	16p13.3	RELN	7q22
UBE3A	15q11.2	SCN2A	2q23
VPS13B	8q22.2	SETD5	3p25.3
		SHANK2	11q13.3
		SHANK3	22q13.3
		SUV420H1	11q13.2
		SYNGAP1	6p21.3
		TBR1	2q24

Table 2

Summary of Cellular, Molecular, and Electrophysiological Findings From Monogenic Mouse Models of ASDs

Gene	Brain Regions, Cell Types, and Developmental Periods Implicated Through Conditional Mutants	Molecular Changes	Cellular and Spine Morphology	Electrophysiology
Mecp2	Excitatory forebrain neurons (*CamKII-Cre93*) GABAergic neurons (*Viaat-Cre*) Fully developed mice (*CAGCre/Esr1*)	↓BDNF, ↓RNA splicing	***Mecp2*$^{tm1.1Bird}$ CTX:** ↓spine density, ↓dendritic complexity ***Mecp2*$^{tm1.1Jae}$CTX:** ↓spine density ***Mecp2*$^{tm1.1Jae}$ CA1:** ↓cell size ***Mecp2*$^{tm1.1Jae}$ p7 CA1:** ↓spine density ***Mecp2*$^{tm1.1Jae}$ SCX**: ↑spine stability (p25–p26) ***Mecp2*$^{tm1.1Bird}$ PNC:** ↓glutamatergic synapses	***Mecp2*$^{tm1.1Bird}$ CA1:** ↓LTP, ↓NMDA LTD ***Mecp2*$^{tm1.1Bird}$ PNC:** ↓mEPSC freq, ↓eEPSC amp ***Mecp2*$^{tm1.1Jae}$ CA1:** ↓LTP, ↓NMDA LTD ***Mecp2*$^{tm1.1Jae}$ SCX:** ↓mEPSC amp ***Mecp2*^{tm1Hzo} CA1:** ↓LTP, ↓NMDA LTD ***Mecp2*^{tm1Hzo} SCX:** ↓LTP ***Mecp2*^{tm1Hzo} PMC:** ↓LTP
Fmr1	None reported (no behavioral phenotype in *Nse-Cre* mediated deletion)	↑mGluR5 signaling, ↑mRNA translation, ↓NLGN1, ↑Shank1	**SCX:** ↑spine density, ↓spine stability (p10–p12), ↓mature spines **CA1:** ↓mature spines	**SCX:** ↓LTP **CA1**: ↓LTP, ↑mGluR LTD **p14 CA1:** ↓AMPA/NMDA, ↑NMDA LTP **PC:** ↑mGluR LTD
Tsc1/Tsc2	Purkinje cells (*L7-Cre* and *Pcp2-Cre*) Excitatory forebrain neurons (*CaMKIIα-Cre*) Serotonergic neurons (*Slc6a4-Cre*)	↑mTORC1 signaling, ↑activated Rheb	**Tsc1/Tsc2 PNC KD:** ↓spine density, ↑spine size, ↑soma size **Tsc1$^{fl/fl}$ + viral Cre CA1:** ↑soma size **Tsc2$^{+/-}$ CTX**: ↑spine density, ↑spine stability	**Tsc1 OT KD:** ↑mEPSC amp, ↑AMPA/NMDA **Tsc1$^{fl/fl}$ + viral Cre CA1:** ↓mGluR LTD, ↓mEPSC freq, ↑AMPA, ↑NMDA **Tsc1$^{fl/fl}$ + viral Cre PNC GABAergic:** ↓eIPSC amp, ↓mIPSC amp **Tsc2$^{+/-}$ CA1:** ↓mGluR LTD
Pten	Cortical and hippocampal neurons (*Nse-Cre*) Cerebellar granule cells and DG hippocampal neurons (*Gfap-Cre*)	↑Akt signaling, ↑mTORC1 signaling, ↓mGluR5, ↑FMRP, ↑p-FMRP	**DG:** ↑cell size, ↑axonal processes, ↑ectopic projections, ↑presynaptic vesicles, ↑spine density, ↑spine size, ↑mature spines **CTX:** ↑cell size, ↑presynaptic vesicles, ↑PSD size **CGC:** ↑cell size, ↑presynaptic vesicles, ↑spine density **Pten^{m3m4}:** ↑cell size	**Pten$^{+/-}$CA1:** ↓LTP, ↓NMDA LTD **Pten$^{fl/fl}$ + *CamKIIα-Cre* CA1:** ↓LTP, ↓NMDA LTD **Pten$^{fl/fl}$ + *GFAP-Cre* CA1:** ↓NMDA LTD **Pten$^{fl/fl}$ + *Nse-Cre* DG:** ↓mGluR LTD
Ube3a	Embryonic/early postnatal development (*CAG-Cre/Esr1*)	**m−/p+**: ↑Arc, ↓Mecp2 function, ↓BDNF signaling **2xTg:** ↓Arc	**m−/p+ CA1:** ↓spine density **m−/p+ CTX:** ↓apical dendrite length **m−/p+ PVC:** ↓spine density	**m−/p+ CA1:** ↓LTP, ↑mGluR LTD, ↓AMPA/NMDA **m−/p+ PVC:** ↓LTP, ↓NMDA LTD **m−/p+ DMS:** ↓mEPSC amp, ↓mEPSC freq **2xTg BC:** ↓eEPSC amp, ↓sEPSC amp, ↓sEPSC freq, ↓sIPSC amp, ↓mEPSC amp, ↓mEPSC freq
Shank1	None reported	**HS:** ↓Homer, ↓SAPAP	**CA1:** ↓spine density, ↓spine length, ↓PSD thickness	**CA1:**↓excitatory synaptic strength, ↓mEPSC freq
Shank2	None reported	**Δe7 HS:** ↑NMDA **Δe7 SS:** ↑AMPA, ↑Shank3 **Δe6–7 WB:** ↑NMDA, ↓NMDA signaling	**Δe7 CA1:** ↓spine density	**Δe7 CA1:** ↓excitatory synaptic strength, ↓mEPSC frequency, ↓AMPA/NMDA, ↑LTP

Gene	Brain Regions, Cell Types, and Developmental Periods Implicated Through Conditional Mutants	Molecular Changes	Cellular and Spine Morphology	Electrophysiology
				Δe6–7 CA1: ↑AMPA/NMDA, ↓LTP, ↓NMDA LTD
Shank3	None reported	**Δe4–9^{J} HPSD:** ↓Homer1b/c, ↓GluA1, ↓GluN2A, ↓SAPAP1 **Δe4–9^{P} SS:** ↓Homer1b/c, ↓PSD-95, ↓GluA2, ↓GluA3 **Δe11 SS:** ↑Shank2, ↑GluN2B **Δe13–16 SPSD:** ↓SAPAP3, ↓Homer, ↓PSD-93, ↓GluA2, ↓GluN2A, ↓GluN2B **Δe21 HS:** ↑mGluR5 **Δe21 PFC:** ↓Rac1/PAK signaling, ↑active cofilin, ↓F-actin	**Δe13–16 DLS:** ↓spine density, ↑dendritic complexity, ↓PSD thickness **Δe4–9^{J} 4 week CA1:**↓spine density, ↑spine length **Δe4–9^{J} 10 week CA1:** ↓spine length	**Δe9 CA1:** ↑mIPSC freq **Δe9 PFC:** ↓mIPSC freq **Δe4–9^{J} CA1:** ↓LTP **Δe4–9^{P} CA1:** ↓LTP **Δe4–9^{P} DLS:** ↑AMPA/NMDA **Δe4–9^{B} CA1:** ↓LTP, ↓excitatory synaptic strength, ↓mEPSC amp, ↑mEPSC freq **Δe13–16 DLS:** ↓excitatory synaptic strength, ↓mEPSC freq, ↓mEPSC amp **Δe21 CA1:** ↓LTP, ↓excitatory synaptic strength, ↑AMPA/NMDA, ↓mEPSC freq **Δe21 PFC:** ↑AMPA/NMDA **21insG CA1: :** ↓LTD, ↓excitatory synaptic strength, ↑AMPA/NMDA, ↓mEPSC freq
Nrxn family	Dominant negative effect in forebrain excitatory neurons of fully developed mice (*CaMKIIα-tTa*)	**ΔNrxn2α HL:** ↓Munc18-1	**ΔCntnap4 GABAergic synapses:** ↓PSD length, ↑cleft width	**ΔNrxn1α CA1:** ↑mEPSC freq, ↓excitatory synaptic strength **DN-Nrxn1β SCX:** ↓mEPSC freq, ↓mIPSC freq **ΔCntnap4 NAc:** ↑DA release **ΔCntnap4 SCX:** ↓sIPSC freq, ↓sIPSC amp, ↓sIPSC kinetics
Nlgn family	D1-MSNs (*D1-Cre*) of the NAc (localized injection of *AAV-Cre*)	**ΔNlgn2 WB:** ↓CSP, ↓Liprin, ↓Munc-18, ↓Nrxnα, ↓Nrxnβ, ↑Nlgn3, ↑Synapsin1a **ΔNlgn2 CA1:** ↓VGAT **R451C Nlgn3 WB:** ↑VGAT, ↑Gephyrin **R451C Nlgn3 HL:** ↓PSD95, ↓SAP102, ↓NR2A, ↓NR2B	**sparse Nlgn2 KD CTX**: ↓synaptic density **R451C Nlgn3 KI CA1:** ↑dendritic complexity, ↓synaptic terminal size, ↓synaptic vesicles, ↓spine size **R451C Nlgn3 KI AFC**: ↑spine turnover	**ΔNlgn1 CA1:** ↓LTP, ↓AMPA EPSCs, ↓NMDA EPSCs, ↑AMPA/NMDA **ΔNlgn1 AMG TI:** ↓LTP **ΔNlgn4:** ↓excitatory network excitability, ↓inhibitory network excitability, ↓E/I **R451C Nlgn3 KI SCX:** ↑mIPSC freq, ↑eIPSC amp, **R451C Nlgn3 KI BC:** ↓eIPSC amp **R451C Nlgn3 KI CA3:** ↑GDPs freq, ↑mIPSC freq **R451C Nlgn3 KI CA1:** ↑LTP, ↓AMPA/NMDA **R451C Nlgn3 KI CCK:** ↑eIPSC amp, ↓tonic ECB **ΔNlgn3 CCK:** ↑eIPSC amp, ↓tonic ECB **ΔNlgn3 D1-MSNs NAc:** ↓mIPSC freq, ↑E/I

Legend:
↓, decreased;
↑, increased;
Δ, deletion;
Δ4–9^{B}, Shank3 model created by Bozdagi et al., 2010;
Δ4–9^{J}, Shank3 model created by Wang et al., 2011;
Δ4–9^{P}, Shank3 model created by Jaramillo et al., 2015;
AAV, adeno-associated virus
AFC, anterior frontal cortex;
AMG TI, thalamic inputs to the amygdala;
amp, amplitude;
AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid;

AMPA/NMDA, ratio of AMPA-induced to NMDA-induced current
BC, barrel cortex;
BDNF, brain-derived neurotrophic factor;
CA1, region of the hippocampus;
CA3, region of the hippocampus;
CCK, cholecystokinin basket cells
CGC, cerebellar granule cells;
CTX, cortex;
DA, dopamine;
DG, dentage gyrus;
DLS, dorsolateral striatum;
DMS, dorsomedial striatum;
ECB, endocannabinoid
eEPSC, evoked excitatory postsynaptic current;
eIPSC, evoked inhibitory postsynaptic current;
E/I, excitation-inhibition ratio;
GDPs, giant depolarizing potentials
freq, frequency;
HL, hippocampal lysate;
HPSD, hippocampal postsynaptic density fraction;
HS, hippocampal synaptosomal fraction;
KD, knockdown;
KI, knock-in;
LTD, long-term depression;

NMDA LTD, NMDA-dependent LTD

mGluR5 LTD, mGluR5-dependent LTD

LTP, long-term potentiation;
mEPSC, miniature excitatory postsynaptic current;
mGluR5, metabotropic glutamate receptor;
mIPSC, miniature inhibitory postsynaptic current;
NAc, nucleus accumbens;
NMDA, N-methyl-D-asparate;
OT, organotypic;
p7, postnatal day 7;
p14, postnatal day 14;
PC, Purkinje cells;
PMC, primary motor cortex;
PNC, primary neuron culture;
PVC, primary visual cortex;
sIPSC, spontaneous inhibitory postsynaptic current
SCX, somatosensory cortex;
SPSD, striatal postsynaptic density fraction;
SS, striatal synaptosomal fraction;
WB, whole brain;