Review

Biological Psychiatry

Excitation/Inhibition Imbalance in Animal Models of Autism Spectrum Disorders

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ABSTRACT

Imbalances between excitation and inhibition in synaptic transmission and neural circuits have been implicated in autism spectrum disorders. Excitation and inhibition imbalances are frequently observed in animal models of autism spectrum disorders, and their correction normalizes key autistic-like phenotypes in these animals. These results suggest that excitation and inhibition imbalances may contribute to the development and maintenance of autism spectrum disorders and represent an important therapeutic target.

Keywords: Autism spectrum disorders, Circuit, Excitation/inhibition balance, Mouse models, Psychiatric disorders, Synapse

http://dx.doi.org/10.1016/j.biopsych.2016.05.011

A tight balance between excitation and inhibition (E/I balance) in synaptic inputs to a neuron and in neural circuits is important for normal brain development and function. Accordingly, disturbed E/I balances have been implicated in various brain disorders, including autism spectrum disorders (ASDs) (1–6). An early, illuminating review by Rubenstein and Merzenich (1) suggested the hypothesis that an increased E/I ratio in sensory, mnemonic, social, and emotional systems can cause ASDs. Since that time, a large body of clinical and neurobiological data has accumulated to support and refine this hypothesis.

ASDs represent neurodevelopmental disorders characterized by social deficits and repetitive behaviors and accompanying comorbidities, including intellectual disability, epilepsy, hyperactivity, and anxiety. ASDs are associated with heterogeneous genetic variations, and the number of ASD-associated genes has risen to approximately 800 (7). ASDs are now the subject of intense worldwide investigations that seek to identify key underlying mechanisms capable of accounting for a large portion of ASD-related genetic variations and thus can serve as important therapeutic targets.

This review summarizes results from animal models of ASD showing altered E/I balances. E/I balance is established and tightly regulated by a large number of factors, making it difficult to differentiate primary changes from secondary alterations in model animals, as was recently noted (3). Therefore, the emphasis is on those models that demonstrate rescue of ASD phenotypes using pharmacologic or cell type–specific gene-rescue approaches, or those models that use conditional gene-ablation approaches. Though valuable, other studies, including some that do not strongly support a causal relationship between the observed E/I imbalances and autistic-like phenotypes, are unavoidably less highlighted.

E/I IMBALANCE AND AUTISTIC-LIKE BEHAVIORS

Abnormal connectivity and neural integration (or temporal binding), manifesting as abnormal brain rhythms, have been suggested to underlie ASDs (8). Recent optogenetic studies have demonstrated that gamma-aminobutyric acidergic (GABAergic) interneurons expressing the calcium-buffering protein parvalbumin (PV) drive gamma rhythms and promote cortical circuit performance and cognitive flexibility (9,10). Importantly, a recent study has demonstrated that optogenetic stimulation of pyramidal neurons in the medial prefrontal cortex in mice induces social deficits associated with enhanced gamma oscillations, whereas coactivation of PV and pyramidal neurons does not induce social deficits (11). These results collectively suggest that an increased neocortical E/I ratio caused by malfunctions of PV-expressing interneurons induces excessive gamma oscillations and autistic-like behaviors.

FACTORS CONTRIBUTING TO E/I IMBALANCE

Neuronal E/I balance involves regulation at synaptic or circuit levels. Specific factors that contribute to synaptic E/I balance would include excitatory/inhibitory synapse development, synaptic transmission and plasticity, downstream signaling pathways, homeostatic synaptic plasticity, and intrinsic neuronal excitability (Table 1). At the circuit level, E/I balance involves local circuits such as the interplay between GABAergic interneurons and target pyramidal neurons, which would modulate long-range connections.

EXCITATORY SYNAPSE DEVELOPMENT

Cell adhesion molecules organize synapse development through transsynaptic adhesion and synaptic protein recruitment. Neuroligins and neurexins are prototypical members (6),

ISSN: 0006-3223

Table 1. Mechanisms Underlying E/I Imbalances in Animal Models of ASD

E/I Imbalance Mechanisms	Examples of Animal Models of ASD
Excitatory Synapse Development	*Eif4ebp2* (12)
AMPARs	BTBR (14), *Emx1-Cre;Syngap1*$^{+/fl}$ (35), *Emx1-Cre;Syngap1*$^{+/lox\text{-}stop}$ (35), *Mecp2* (28,29), *Mecp2*^{Tg1} (31), *Shank3* duplication (27), *Shank3* (various exon deletions) (19–24), *Tau-Mecp2* (30), *Syngap1*$^{+/-}$ (34), *Ube3a* (15)
NMDARs	*Baiap2* (IRSp53) (53), BALB/c (46,47), BTBR (45), *Grid1* (GluD1) (44), *Grin1* (GluN1) (37), *Nlgn1* (38), Rats with low prosocial USVs (48), *Shank2* (exons 6–7) (39,40), *Shank2* (exon 7) (41), *Shank3* (exons 4–9) (18), *Shank3* (exon 21G) (23), *Shank3*$^{+/\Delta C}$ (43), *Tbr1*$^{+/-}$ (40,42), VPA rats and mice (49–52)
mGluRs	*Baiap2* (IRSp53) (53), BTBR (61–63), *Fmr1* (57–60,64,65), *Nlgn3* (88), *Shank2* (exons 6–7) (39)
Signaling Pathways	BTBR (82), *Cntnap2* (79), *Emx1-Cre;Tsc1* (71), *Fmr1* (76,78,81), *Nf1*$^{+/-}$ (77), *Nse-Cre;Pten* (69), *Pcp2/L7-Cre;Tsc1* (70), *Tsc2*$^{+/-}$ (72–74), *Ube3a* (15,75), *Shank3*$^{+/\Delta C}$ (43)
Inhibitory Synapse Development and Function	*D1-Cre;Nlgn3* (89), *Fmr1* (92–94,96–98), *Gabrb3* (90,91), *Nlgn2* (84,85), *Nlgn3* (86,87,89), *Nlgn3* R451C (86,87,89), *Ube3a* (101,102)
Interneurons	BTBR (95,106,119), *Cntnap2* (79,113), *Cntnap4* (80), *Dlx1/2;Scn1a*$^{+/fl}$ (118), *Dlx5/6-Cre;Tsc1* (110), *Fmr1* (108), *Gad2* (GAD65) (106), *Mecp2* (106,120), *Nkx2.1-Cre;Pten* (109), *Nlgn3* R451C (2), *Oxtr* (127), *Pvalb* (112), *PV-Cre;ErbB4* (123), *Pv-Cre;Mecp2* (107), *PV-RFP;Shank1* (111), *Scn1a*$^{+/-}$ (118), *Scn1a*$^{+/R1407X}$ (117), *Shank3* (exons 13–16) (106), *SST-Cre;Mecp2* (107), *Synl* (121,122), *Ube3a* (126), *Viaat-Cre;Mecp2* (120), VPA mice (2)
Glial Cells	*Gfap-Cre;ERT2;Mecp2*$^{lox\text{-}stop/y}$ (130), *Gfap-Cre;Pten* (129), *Glast-CreERT2;Glt1* (128), *Lysm-Cre;Mecp2*$^{lox\text{-}stop/y}$ (131)
Intrinsic Neuronal Excitability	*Nestin-Cre;Foxp1* (133), *Fmr1* (134), *Pv-Cre;ErbB4* (124,125), *Shank3* (exons 13–16) (135)
Homeostatic Synaptic Plasticity	*Fmr1* (140,141), *Mecp2* (137–139)
Temporal E/I Regulation	*CreERT2;MECP2* and *TG;Mecp2*$^{lox/y}$ (148), *CreERT2;Syngap1*$^{+/lox\text{-}stop}$ (146), *CreEsr1*;Mecp2*$^{lox\text{-}stop/y}$ (147), *CreEsr1*; Ube3a*$^{Stop/p}$ (149), *Fmr1* (144,145), *Nlgn3*$^{stop\text{-}tetO}$;*Pcp2*^{tTA} (88), VPA rats (144,145)

Candidate mechanisms involved in causing E/I imbalances in some animal models of ASD. In some cases, more than one mechanism appears to apply to the same mouse model, possibly due to multiple effects of a single mutation or homeostatic interplay among different mechanisms. Additional studies may be needed to determine whether certain mechanisms listed here represent primary changes and, hence, fundamental pathogenic mechanisms. Heterozygosity and conditional gene deletion or re-expression are indicated; all other gene names without additional identifiers represent homozygosity (–/– or fl/fl) or X chromosomal/maternal deletion (*Mecp2*$^{-/y}$;*Ube3a*$^{m-/p+}$). Full names of the genes and their known functions are as follows: *Baiap2* (brain-specific angiogenesis inhibitor 1-associated protein 2; also known as IRSp53; excitatory postsynaptic adaptor and scaffolding protein); *Cntnap2/4* (contactin-associated protein-like 2/4; a member of the neurexin family of cell protein 2); *Eif4ebp2* (a member of the eukaryotic translation initiation factor 4E binding protein family; bind eIF4E and inhibit translation initiation); *ErbB4* (erb-b2 receptor tyrosine kinase 4; a receptor for neuregulins with tyrosine kinase activity); *Fmr1* (fragile X mental retardation 1; an RNA-binding protein that regulates messenger RNA trafficking); *Foxp1* (forkhead box P1; a transcription factor); *Gabrb3* (gamma-aminobutyric acid A receptor subunit beta 3; a GABA receptor subunit); *Gad2* (glutamic acid decarboxylase 2; also known as GAD65; a GABA-synthesizing enzyme); *Glt1* (solute carrier family 1 [glial high affinity glutamate transporter], member 2; also known as Sla1a2 or EAAT2, a glutamate transporter); *Grid1* (glutamate receptor, ionotropic, delta 1; also known as GluD1; a subunit of glutamate receptors); *Grin1* (glutamate ionotropic receptor NMDA type subunit 1; also known as GluN1; an NMDA receptor subunit); *Mecp2* (methyl CpG binding protein 2; a transcription factor that binds to methylated DNA); *Nf1* (neurofibromin 1; a negative regulator of ras signaling); *Nlgn1/2/3* (neuroligin 1/2/3; a synaptic cell adhesion molecule); *Oxtr* (oxytocin receptor); *Pten* (phosphatase and tensin homolog; a phosphatase for phosphoinositides); *Pvalb* (parvalbumin; a calcium ion-binding protein); *Scn1a* (sodium voltage-gated channel alpha subunit 1; a subunit of voltage-dependent sodium channels); *Shank1/2/3* (excitatory postsynaptic scaffolding proteins); *Syngap1* (synaptic Ras GTPase activating protein 1, excitatory postsynaptic scaffolding protein with GTPase-activating protein activity); *Synl* (synapsin I; a protein that associates with synaptic vesicles); *Tbr1* (T-box, brain 1; a transcription factor); *Tsc1/2* (tuberous sclerosis 1; a growth inhibitory protein); *Ube3a* (ubiquitin protein ligase E3A; an E3 ubiquitin-protein ligase).

AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; E/I, excitation/inhibition; GABA, gamma-aminobutyric acid; GTPase, guanosine triphosphatase; mGluR, metabotropic glutamate receptor; NMDAR, *N*-methyl-D-aspartate receptor; USV, ultrasonic vocalization; VPA, valproic acid.

and many additional molecules have recently been identified. Given their critical roles in synapse and circuit development, it is no wonder that neuroligin and neurexin genes have been among the first ASD-related genes identified in early autism studies (6). Contrary to initial expectations, however, neuroligin/neurexin knockout in mice did not induce significant changes in synapse number, except in a few specific brain regions; instead, it substantially modified synaptic functions (6), which may also contribute to impaired synaptic development in ASDs.

A recent study has shown that neuroligin expression can be altered indirectly. Knockout of 4E-BP2, known to inhibit eIF4E in the mechanistic target of rapamycin (mTOR) pathway in mice (*Eif4ebp2*, a member of the eukaryotic translation initiation factor 4E binding protein family), upregulates neuroligins (all four known isoforms), increases hippocampal synaptic E/I ratio, and induces autistic-like behaviors (12). Pharmacologic inhibition of eIF4E, or knockdown of neuroligin-1 (*Nlgn1*) but not neuroligin-2 (*Nlgn2*), which are excitatory and inhibitory synapse specific, respectively (6), normalizes the E/I ratio and rescues autistic-like behaviors.

AMPA RECEPTORS

Glutamatergic dysfunction involving alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA), and metabotropic glutamate (mGluR) receptors (AMPARs, NMDARs, and mGluRs) alters E/I balance. Supporting the role of AMPARs, social deficits in BTBR mice, an inbred mouse strain modeling ASD (13), are rescued by the AMPAR-activator ampakine (14). Ampakine also rescues impaired long-term potentiation (LTP) and long-term memory in Ube3a-deficient mice (*Ube3a*$^{m-/p+}$) that lack the maternal copy of an E3 ubiquitin ligase gene (15), a model of Angelman syndrome, characterized by intellectual disability,

absence of speech, seizure, ataxia, and frequent laughter and smiling (16).

Mice heterozygous for *Shank3* (exons 4–9 deletion), an excitatory postsynaptic scaffold (17), show reduced evoked AMPAR-mediated excitatory synaptic transmission, suppressed LTP, and impaired motor function (18). These phenotypes are rescued by insulin-like growth factor 1 (IGF-1) (19), known to activate AMPARs, but not NMDARs, through the PI3K pathway (20). Similar reductions in evoked AMPAR transmission are observed in other *Shank3*-mutant mice carrying different exon deletions (21–24), although two studies on *Shank3* mice report normal AMPAR transmission (25,26). Conversely, an increase in spontaneous AMPAR transmission is observed in mice carrying a *Shank3* duplication, and seizure and mania-like behaviors in these mice are corrected by the mood-stabilizing agent valproic acid (VPA) (27).

Mice lacking the transcription regulator MeCP2, modeling Rett syndrome, which is characterized by loss of language and motor skills, show reduced spontaneous and evoked AMPAR transmission and excitatory synaptic connectivity (28,29). Conversely, mice with neuron-specific Mecp2 overexpression show increased spontaneous AMPAR transmission (30) that is in line with dose-dependent changes in spontaneous and evoked AMPAR transmission in autaptic hippocampal neurons from transgenic mice (31). Similar to *Shank3* mice, IGF-1 treatment of *Mecp2* mice partially rescues the reduced spontaneous excitatory transmission, spine density, and PSD-95 levels (an excitatory postsynaptic scaffold) (32).

Syngap1 is an excitatory postsynaptic guanosine triphosphatase-activating protein (Ras-GAP) implicated in intellectual disability and ASD (33). *Syngap1* heterozygous mice show increased AMPAR transmission, precocious spine development, and hyperactive circuits in the hippocampus, and reduced seizure threshold at ~postnatal day 14 (P14) but not at ~P7, P21, or P42 (34). *Syngap1* haploinsufficiency restricted to forebrain glutamatergic neurons (*Emx1-Cre*), but not GABAergic neurons (glutamic acid decarboxylase 2 [*Gad2-Cre*]), induces similar phenotypes (35). In addition, *Syngap1* re-expression in forebrain glutamatergic neurons (*Emx1-Cre;Syngap1*$^{+/\text{lox-stop}}$), but not in GABAergic neurons (*Gad2-Cre;Syngap1*$^{+/\text{lox-stop}}$), rescues cognitive and emotional deficits (35). Therefore, an early, excessive excitatory transmission in glutamatergic neurons impairs brain functions in Syngap1 mice.

NMDARs

Animal models of ASDs exhibit NMDAR dysfunction and behavioral abnormalities that respond to NMDAR-modulating reagents (36). Directly supporting the importance of NMDARs, mice with ~85% downregulation of the glutamate ionotropic 1 (GluN1) subunit of NMDARs (*Grin1*) show social deficits and repetitive behavior (37).

Nlgn1-mutant mice display NMDAR hypofunction and increased grooming responsive to the NMDAR agonist D-cycloserine (38). In addition, Shank2 mice (exons 6–7) show NMDAR hypofunction and social deficits rescued by D-cycloserine (39), or clioquinol, a zinc chelator that enhances NMDAR function through transsynaptic zinc delivery (40). Notably, other *Shank2*-mutant mice (exon 7) show enhanced NMDAR function (41), suggesting that different mutations in the same gene may cause NMDAR dysfunction in opposite directions. Mice heterozygous for Tbr1, encoding a transcription factor with targets that include the GluN2B subunit of NMDARs, show NMDAR hypofunction and social deficits responsive to D-cycloserine (42) and clioquinol (40).

Other *Shank3* mice (*Shank3*$^{+/\Delta C(\text{exon21})}$) show NMDAR hypofunction and social deficits responsive to inhibition of *cofilin* (43), a negative actin regulator. In addition, two different *Shank3* mouse lines (exons 4–9 and e21G) show similar NMDAR hypofunction (23,24,26), although rescue attempts were not made. Several other animal models, including *Grid1*, BTBR, and BALB/c mice, and rats with low play-related prosocial ultrasonic vocalizations, show autistic-like behaviors that are rescued by D-cycloserine or other NMDAR agonists (44–48), although NMDAR function remains to be investigated.

At the other end of the spectrum, excessive NMDAR function also appears to cause autistic-like behaviors. Rats prenatally exposed to VPA show increased NMDAR levels, enhanced NMDAR-dependent LTP, and hyperconnected local neocortical circuits (49,50). The autistic-like behaviors in VPA rodents are rescued by memantine (51,52). In addition, mice lacking IRSp53, or Baiap2, an abundant excitatory postsynaptic scaffold, show NMDAR hyperfunction and social and cognitive impairments responsive to memantine (53). These results collectively suggest that deviation of NMDAR function in either direction leads to autistic-like behaviors (36).

mGluRs

Metabotropic glutamate receptors have long been implicated in ASDs (54). A well-known example is mGluR5 hyperfunction in mice lacking FMRP (*Fmr1*$^{y/-}$), an RNA-binding protein implicated in fragile X syndrome (55,56). These mice show behavioral abnormalities that are rescued by mGluR5 antagonists (57–60). In addition, in BTBR mice, mGluR5 inhibition rescues social deficits and repetitive behaviors (61,62), as well as hippocampus-dependent memory (63).

The causal role of mGluR5 hyperfunction is further supported by genetic rescue of mGluR5 signaling. Suppressing exaggerated mGluR5 signaling in *Fmr1* mice by crossing them with mGluR5 heterozygous mice (*Grm5*$^{+/-}$) rescues disease-related synaptic, biochemical, and behavioral phenotypes (64). More recently, a genetic cross of *Fmr1* mice with *Tsc2* heterozygous mice (*Tsc2*$^{+/-}$), which display reduced mGluR5 signaling, rescued all disease-related phenotypes (65).

Results obtained in studies related to glutamate receptor malfunctions should be interpreted with care because the three glutamate receptors can influence each other. For instance, it is well known that AMPARs are regulated by NMDARs and mGluRs. In addition, NMDARs and mGluRs can exert synergistic actions (66,67); social deficits in *Shank2* mice (exons 6–7), displaying NMDAR hypofunction, are rescued by indirectly stimulating NMDARs using the mGluR5 agonist CDPPB (39).

SIGNALING PATHWAYS

Signaling pathways downstream or upstream of synaptic receptors and channels can regulate E/I balance. The mTOR pathway, known to be activated by NMDARs, mGluRs, and receptor tyrosine kinases, has been implicated in fragile X syndrome and ASDs (68). For instance, the mTOR inhibitor rapamycin rescues autistic-like phenotypes in animal models with heightened mTOR signaling, including *Nse-Cre;Pten* (69), *Tsc1* (*Pcp2/L7-Cre;Tsc1*, *Emx1-Cre;Tsc1*) (70,71), *Tsc2* (*Tsc2*$^{+/-}$) (72–74), and *Ube3a* (75). In some studies, the most downstream proteins in the mTOR pathway are targeted for rescue; examples include eIF4E in *Eif4ebp2*-deficient mice (12), and S6K1 in *Fmr1* mice (76).

Actin-modulatory pathways, important for actin-rich excitatory synapses, have also been implicated. Ampakine, which rescues LTP and memory phenotypes in *Ube3a* mice, stabilizes synaptic actin filaments during LTP (15). In addition, pharmacologic or genetic modulation of the actin-regulatory proteins PAK and *cofilin* rescues autistic-like behaviors in Nf1 (neurofibromatosis type 1; *Nf1*$^{+/-}$) (77), *Shank3* (*Shank3*$^{+/\Delta C}$) (43), and *Fmr1* (78) mice.

Dopamine receptor agonists/antagonists and 5-hydroxytryptamine rescue autistic-like behaviors in *Cntnap2* (encoding contactin-associated protein-like 2) (79), *Cntnap4* (80), *Fmr1* (81), and BTBR (82) mice, implicating monoaminergic pathways. Although rescue mechanisms in these cases are generally unclear, the Ras-PI3K-Akt pathway and GluA1-dependent synaptic plasticity have been suggested for *Fmr1* mice (81).

INHIBITORY SYNAPSE DEVELOPMENT AND FUNCTION

GABAergic signaling is frequently altered in animal models of ASD (3,4,6), in line with many related human genetic variations (5,6,83). Inhibitory synaptic adhesion molecules are important regulators of GABAergic signaling. Virus-mediated deletion of inhibitory synapse-specific *Nlgn2* in the medial prefrontal cortex leads to delayed (6–7 postnatal weeks) decreases in inhibitory synapse density, miniature inhibitory postsynaptic current (IPSC) frequency and amplitude, and evoked IPSC input-output curves, effects that are accompanied by social and cognitive deficits (84). An earlier study also showed that *Nlgn2* deletion in mice suppresses GABAergic and glycinergic transmission through impaired assembly of postsynaptic receptor complexes at perisomatic inhibitory synapses (85).

Mice lacking *Nlgn3*, which is present at both excitatory and inhibitory synapses and implicated in ASDs (6), show minimal alterations in excitatory or inhibitory synapse density or function (86). However, recent studies have reported enhanced GABAergic transmission at cholecystokinin-positive basket cell synapses in the hippocampus (87) and impaired cerebellar mGluR-dependent synaptic long-term depression (88).

Intriguingly, *Nlgn3* knockin mice carrying a human mutation (*Nlgn3* R451C) show enhanced GABAergic transmission in the somatosensory cortex, together with increased frequency of spontaneous IPSCs and increased levels of the inhibitory synaptic proteins vesicular GABA transporter (VGAT) and gephyrin (86). In addition, the R451C knockin impairs GABAergic transmission in PV basket cell synapses, but enhances GABAergic transmission in cholecystokinin basket cell synapses in the hippocampus (87), suggesting that the same mutation induces distinct changes in GABAergic transmission in different brain regions and cell types.

In the striatum, *Nlgn3* deletion restricted to D1 medium spiny neurons (D1-MSNs), but not D2-MSNs, suppresses GABAergic transmission onto D1-MSNs (89). Importantly, re-expression of *Nlgn3*, or K^+ channel expression causing neuronal relaxation, in *Nlgn3*-deficient D1-MSNs rescues GABAergic transmission and rotarod performance (89), suggesting that abnormally excited D1-MSNs cause autistic-like phenotypes.

Altered GABA type A ($GABA_A$) receptor levels or function would directly affect E/I balance. A deficiency of the ASD-associated β3 subunit of the $GABA_A$ receptor (*Gabrb3*) in mice reduces $GABA_A$ receptor levels, enhances seizure susceptibility, and induces cognitive and motor deficits (90,91). Reduced levels of $GABA_A$ receptor subunits are also observed in *Fmr1* mice (92–94). Moreover, the $GABA_A$ receptor antagonist DMCM given to wild-type mice impairs social interaction (95).

Tonic GABAergic transmission, involving extrasynaptic $GABA_A$ receptors, appears to be altered in ASD model animals. *Fmr1* mice show reduced tonic but not phasic GABA currents in the subiculum (96), reduced phasic and tonic GABA currents in the amygdala (97), and increased phasic GABA currents due to enhanced GABA release in the striatum (tonic currents were not measured) (98), indicative of heterogeneous effects of *Fmr1* deletion. Importantly, the GABA agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP), a selective enhancer of tonic GABA currents, rescues hyperexcitability of principal neurons in the *Fmr1*-deficient amygdala (97). Therefore, deceased GABAergic signaling, in addition to enhanced mGluR signaling, may contribute to fragile X syndrome (3,99,100).

Decreased tonic inhibition is also observed in cerebellar granule cells of *Ube3a* mice, which have increased levels of GABA transporter 1, a substrate of UBE3A, and reduced levels of ambient GABA (101). These changes accompany abnormal Purkinje cell firing and cerebellar ataxia, both of which are rescued by THIP (101). In the hippocampus, however, UBE3A deficiency enhances neuregulin-ErbB4 (*Erb-B2* receptor tyrosine kinase 4) signaling and GABAergic output, and the resulting impairments in LTP in target pyramidal neurons and contextual fear memory are rescued by the ErbB inhibitor PD158780 (102), or the $GABA_A$ antagonist bicuculline (LTP rescue) (102). These results suggest that UBE3A deletion can cause region-specific changes in GABAergic signaling, which can interact with excitatory synaptic function.

INTERNEURONS

GABAergic interneurons are associated with various neurological and psychiatric disorders (3,4,103). Many ASD-associated genes are expressed in interneurons, and their mutations impair interneuronal development and input/output function, including dendritic synapse development and function, neuronal excitability and firing, nerve terminal development and function (i.e., GABA synthesis, packaging, and release), and inhibitory synapse formation with target neurons.

Several of these deficits are often caused by a single gene defect.

PV interneurons are critical regulators of gamma oscillations and are associated with various psychiatric disorders (9,10,104). Their functional importance is supported by the impaired multisensory integration (MSI) in the insular cortex, known to exhibit reduced connectivity in ASDs (105), observed in BTBR, *Gad2*(GAD65), *Mecp2,* and *Shank3* mice (106). Intriguingly, diazepam-treated adult BTBR mice show normalized MSI, whereas diazepam-treated control mice show impaired MSI (106), indicative of an optimum range of PV neuron inhibition. In addition, diazepam-treated young BTBR mice show normal MSI at adult stages (106), suggesting that early treatment has long-lasting effects. GABAergic interneurons other than PV such as somatostatin (SST), calretinin, and neuropeptide Y (NPY) are also important for ASDs. For instance, *Mecp2* deletion restricted to PV and SST interneurons leads to distinct phenotypes; motor, sensory, memory, and social deficits in PV-*Mecp2* knockout; and seizures and stereotypies in SST-*Mecp2* knockout (107).

One of the specific interneuronal defects observed in animal models is reduced cell density. PV interneuron density is decreased in mouse models, including *Fmr1* (108), VPA (2), *Nlgn3* R451C (2), and *Cntnap2* (79). Non-PV interneuronal density is also altered. *Cntnap2* mice show reduced calretinin and NPY interneuron counts, in addition to PV interneuronal reduction (79). Pten knockout restricted to cortical GABAergic interneuronal progenitors (*Nkx2.1-Cre;Pten*) preferentially decreases SST cell counts (relative to PV), leading to increases in PV-SST cell ratio and target-neuron inhibition (109). In addition, Tsc1 knockout in GABAergic interneuronal progenitors (*Dlx5/6-Cre;Tsc1*) decreases calretinin and NPY cell counts and seizure threshold (110).

Interneuronal input/output function can also be compromised. Deficiency of *Shank1*, highly expressed in PV cells, suppresses excitatory synaptic input and GABAergic output of PV interneurons, increasing E/I ratio in target neurons (111). Mice lacking PV (*Pvalb*) show altered short-term plasticity of excitatory cortical inputs to PV interneurons and social deficits and repetitive behavior (112). *Cntnap2* mice show suppressed perisomatic evoked IPSC input-output curve (113), in addition to reduced PV cell counts, defective neuronal migration, and epilepsy (79). However, CNTNAP2 also regulates dendrite and spine development and AMPAR trafficking in pyramidal neurons (114–116), suggesting that it regulates both excitatory and inhibitory synapses.

Impaired interneuronal firing would suppress GABAergic signaling. Mice heterozygous for the voltage-gated sodium channel Nav1.1 (*Scn1a*$^{+/-}$), a model for Dravet syndrome characterized by intractable seizure and social and cognitive deficits, show limited action potential firing and GABAergic output, together with social and spatial and fear memory deficits (117,118). These features are recapitulated by Nav1.1 knockout restricted to forebrain GABAergic interneurons (*Dlx1/2-Cre;Scn1a*$^{+/-}$) and are rescued by the $GABA_A$ receptor agonist clonazepam (118). Similarly, BTBR mice show reduced hippocampal spontaneous IPSC frequency and social and cognitive deficits responsive to clonazepam (95), similar to the improved social avoidance observed in diazepam-treated BTBR mice (119).

Limited nerve terminal development and function would affect GABAergic signaling. Mice lacking *Mecp2* in GABAergic interneurons (*Viaat-Cre;Mecp2*) show reduced quantal GABA content and messenger RNA levels for GABA-synthesizing GAD65 and GAD67 (120). Mice lacking the synaptic vesicle protein synapsin I (*SynI*), implicated in ASD and epilepsy, show epileptic propensity, and neurons from these mice re-expressing a disease-related mutant SynI display reduced readily releasable pool of GABA-containing vesicles and stronger short-term depression (121), which may involve abnormally activated eEF2K/eEF2 signaling (122). Conditional deletion of ErbB4 in PV neurons causes reduced neuregulin-dependent GABA release in the prefrontal cortex and cognitive deficits that are rescued by diazepam (123), results in line with the increased seizure susceptibility observed in these mice (124,125). *Ube3a* mice show reduced GABAergic output in the visual cortex due to reduced defective presynaptic vesicle cycling prominent at P80 but not at P25 (126).

Dysfunctional interneurons would fail to properly develop inhibitory synapses with target neurons. A deficiency of CNTNAP4, highly expressed in developing interneurons, in mice causes reduced PV interneuronal output through limited inhibitory synapse maturation, as evidenced by widened synaptic cleft gap, and enhanced startle responses rescued by the $GABA_A$ receptor agonist Indiplon (80). In addition, mice lacking the oxytocin receptor (*Oxtr*) show decreased hippocampal inhibitory presynaptic density and increased seizure susceptibility, together with social and learning defects responsive to prosocial neuropeptides, oxytocin and vasopressin (127), although rescue mechanisms downstream of receptor activation remain unclear.

GLIAL CELLS

Glial cell dysfunctions can disturb neuronal E/I balance. Supporting astrocytic contribution, astrocyte-specific deletion of GLT1 (*Glast-CreERT2;Glt1*), a glutamate transporter expressed in neurons and glia, induces increased excitatory transmission, seizure susceptibility, and repetitive behavior that is responsive to memantine (128). Mice lacking PTEN in astrocytes (*Gfap-Cre;Pten*) show abnormal excitatory synapse structure and reduced excitatory transmission and LTP (129). In addition, astrocyte-specific re-expression of *Mecp2* in *Mecp2*-deficient mice restores disease-related phenotypes, including premature lethality, abnormal respiration, hypoactivity, and reduced dendritic complexity [a noncell-autonomous effect (130)]. Nonastrocytic glial cells such as microglia and oligodendrocytes are also important. For instance, microglia-specific re-expression of MeCP2 in Mecp2 mice (*Lysm-Cre;* *Mecp2*$^{lox\text{-}stop/y}$) ameliorates disease-related phenotypes (131).

INTRINSIC NEURONAL EXCITABILITY

Neuronal excitability acts together with synaptic E/I balance to modulate neuronal firing. Rats prenatally exposed to VPA show reduced neuronal excitability in addition to enhanced NMDAR function and NMDAR-dependent LTP (50,132). Interestingly, both enhanced NMDAR function and reduced excitability peak around birth and are progressively and concurrently corrected to normal levels within ~3 weeks (132),

suggesting that neuronal excitability compensates for NMDAR function. Similarly, enhanced excitatory transmission and reduced excitability are observed in brain-specific *Foxp1*-deficient mice (*Nestin-Cre;Foxp1*), which display social impairments and repetitive behavior (133).

An important regulator of intrinsic excitability is dendritic ion channels. *Fmr1* mice show reduced expression and impaired function of dendritic h- and BK_{Ca} (big potassium) channels in cortical pyramidal neurons, associated with dendritic hyperexcitability and sensory hypersensitivity that are corrected by the BK_{Ca} channel activator, BMS-191011 (134). Recently, reduced h-channel function has been reported in *Shank3*-deficient mouse and human neurons (135).

Some intrinsic excitability mechanisms seem to overlap with ASD mechanisms. Neuregulin-ErbB4 signaling, an aforementioned regulator of interneuronal GABAergic output, increases the intrinsic excitability of PV interneurons by inhibiting the voltage-dependent potassium channel, $K_V1.1$ (124,125).

HOMEOSTATIC SYNAPTIC PLASTICITY

Homeostatic plasticity works at the level of neuronal synapses in addition to intrinsic neuronal excitability (136). *Mecp2* mice show impaired upward excitatory synaptic scaling in visual cortical neurons (137) and downward scaling in hippocampal neurons (138,139). *Fmr1* mice show blocked upward excitatory synaptic scaling in the hippocampus (140), and upward inhibitory synaptic scaling in the amygdala (141). In addition, GKAP/DLGAP1/SAPAP1, a postsynaptic scaffold implicated in ASD, regulates hippocampal excitatory synaptic scaling in a bidirectional manner (142).

TEMPORAL E/I REGULATION

Synaptic and circuit E/I balances are established and fine-tuned during brain development and through sensory experience. In immature brains, GABA acts as an excitatory neurotransmitter because of the prevailing high intracellular chloride concentration ($[Cl^-]_i$) (143). The high $[Cl^-]_i$ built up by the chloride importer, NKCC1, is gradually diminished by the chloride exporter KCC2, shifting GABA action from depolarization to hyperpolarization. The depolarizing GABA action is transiently inhibited during birth by maternal oxytocin, but is abnormally suppressed in *Fmr1* mice and VPA rats (144). Elegantly, maternal pretreatment with the NKCC1 blocker bumetanide before delivery normalizes disease phenotypes in both models (P15) (144). In addition, bumetanide rescues impaired ultrasonic vocalization in pups (P4) (144) and social deficits in adult offspring (2.5/4.5 months) (145), suggesting that an early E/I imbalance has long-lasting effects (143).

Another example of temporal E/I imbalance is *Syngap1* heterozygous mice. In these mice, induction of a *Syngap1* mutation in adult mice (>8 weeks) does not alter synaptic function, but restoration of *Syngap1* expression in newborn Syngap1-mutant mice at P1 improves cognitive and memory functions (34,146), suggesting again that an early E/I imbalance has long-lasting effects.

However, there are cases in which delayed restoration rescues abnormal phenotypes. For instance, re-expression of *Nlgn-3* in *Nlgn3*-deficient mice at P30 restores mGluR1α expression and ectopic synapse formation in the cerebellum (88). In addition, re-expression of *Mecp2* in adult *Mecp2* mice (~12–17 weeks) restores LTP and disease-related phenotypes (147), and, conversely, genetic or antisense-mediated Mecp2 suppression in adult *Mecp2*-overexpressing mice (~7–8 to 11–12 weeks) rescues major phenotypes (148). Moreover, re-expression of maternal *Ube3a* in *Ube3a* mice at 3, 6, and 12 weeks differentially rescue disease-related phenotypes (149), collectively suggesting that ASD-related mutations have distinct time course of phenotype development and reversibility.

PERSPECTIVES

To minimize the difficulty of differentiating primary and secondary changes in animal models of ASD, we have sought in this review to highlight studies that attempted pharmacologic rescue and conditional knockout/rescue experiments.

However, care should be taken in interpreting these results because the observed rescues may merely represent apparent phenotypic alleviation rather than fundamental correction of key pathogenic mechanisms. In addition, the phenotypes observed in conditional knockout mice, although clearer than those from conventional knockouts, may not represent the consequences of the intricate interplay among different cell types that occurs in real pathological conditions.

Adding to this complexity, a substantial portion of the primary pathological changes likely occurs during embryonic or early postnatal periods and exerts long-lasting effects. Therefore, the rescue results obtained using adult animals may not necessarily provide insights into early and primary changes. We thus need to identify key mechanisms that contribute to the initiation, development, and maintenance of autistic-like phenotypes along the temporal axis. These efforts would need to involve genetic manipulations and pharmacologic interventions at different time points, and observation of their short- and long-term consequences.

Pathogenic mechanisms underlying E/I imbalance in ASDs are more complex than might have been expected. Recent studies have even begun to show that the same gene mutation leads to distinct synaptic E/I imbalances in different synapses, cell types, and brain regions at different time points. Collectively, these findings highlight the importance of pursuing detailed and integrative analyses of E/I imbalances in future studies of animal models of ASD.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the Institute for Basic Science Grant No. IBS-R002-D1 (to EK). All authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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Received Feb 17, 2016; revised May 2, 2016; accepted May 16, 2016.

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