

# Synaptic Plasticity in Mouse Models of Autism Spectrum Disorders

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Analysis of synaptic plasticity together with behavioral and molecular studies have become a popular approach to model autism spectrum disorders in order to gain insight into the pathophysiological mechanisms and to find therapeutic targets. Abnormalities of specific types of synaptic plasticity have been revealed in numerous genetically modified mice that have molecular construct validity to human autism spectrum disorders. Constrained by the feasibility of technique, the common regions analyzed in most studies are hippocampus and visual cortex. The relevance of the synaptic defects in these regions to the behavioral abnormalities of autistic like behaviors is still a subject of debate. Because the exact regions or circuits responsible for the core features of autistic behaviors in humans are still poorly understood, investigation using region-specific conditional mutant mice may help to provide the insight into the neuroanatomical basis of autism in the future.

**Key Words:** Autism, Synaptic plasticity

## INTRODUCTION

Synaptic plasticity is frequently measured by analysis of long term potentiation (LTP) and long term depression (LTD) of synapses in different brain regions [1-3]. Historically, the best studied region for synaptic plasticity is the Schaffer collateral pathway in hippocampal CA1 region. The physiological and biochemical mechanisms underlying LTP and LTD have been extensively investigated [3,4]. Over the last decade, analysis of synaptic plasticity has become a popular technique to characterize animal models of neurodevelopmental disorders including autism spectrum disorders (ASD) [5,6]. Various abnormal findings in synaptic plasticity from different brain regions have been reported in mouse models with targeted mutations in genes implicated in ASD (Table 1). However, identifying and interpreting the defects in synaptic plasticity relevant to behavioral manifestations and disease pathophysiology of ASD remain a significant challenge. In this review, we will focus on reviewing the studies of synaptic plasticity in several prominent mouse models for neurodevelopmental disorders with pronounced autistic features and discussing the challenges and future directions in the field.

## ANGELMAN SYNDROME

Angelman syndrome (AS) is characterized by profound intellectual disability (ID), movement disorders, absence of speech, epilepsy, and autistic behaviors [7,8]. The molecular defects causing AS include maternal microdeletions on chromosome 15q11-q13 (60% of cases), point mutations in the maternal copy of the *UBE3A* gene (20%), paternal uniparental disomy (5%), and imprinting center defects (1%) [9]. Despite the presence of different molecular defects, it is a well-supported fact that the deficiency of maternal expression of the *UBE3A* gene in the brain is responsible for the key clinical features of AS [10,11]. To model human AS in mice, the first knock-out (KO) mouse that targeted exon 2 of *Ube3a* was reported in 1998 and recapitulated the major features of AS in maternal deficiency mice (*Ube3a* m-/-) [12]. Subsequently, *Ube3a* mutant mouse with a mutation in the last coding exon encoding the ubiquitin ligase domain and was reported [13]. In addition, mutant mice with a 1.6 Mb deletion from *Ube3a* to *Gabrb3* that is more similar to AS deletion patients were also reported [14]. However, the *Ube3a* exon 2 deletion mutant mice have been used more widely by investigators in the research community over the last 15 years.

Synaptic plasticity has been studied extensively in *Ube3a*

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**ABBREVIATIONS:** AS, Angelman syndrome; ASD, autism spectrum disorders; CaMKII, Ca<sup>2+</sup>/calmodulin-dependent protein kinase II; DHPG, dihydroxyphenylglycine; E-LTP, early phase LTP; FMRP, fragile X mental retardation protein; FXS, Fragile X syndrome; HFS, high frequency stimulation; ID, intellectual disability; KO, knock-out; LFS-LTD, low frequency stimulation LTD; L-LTP, late phase LTP; LTD, long term depression; LTP, long term potentiation; mEPSC, miniature excitatory postsynaptic current; mGluR, metabotropic glutamate receptor; mGluR-LTD, mGluR mediated LTD; NMDA, N-methyl-D-aspartate; PP-LFS, paired-pulse low frequency stimulation; PSD, postsynaptic density; RTT, Rett syndrome; TSC, tuberous sclerosis complex.

**Table 1.** Summary of synaptic plasticities with protocols

Animal model	Area	Type	Protocol	Effect	References
Ube3a m <sup>-</sup> /p <sup>+</sup>	CA1	LTP	2 HFS, interval 20 sec	↓	[12]
	CA1	LTP	2 HFS, interval 20 sec	↓	[15]
			3 sets of trains (10 min interval), one set (2 HFS, 20 sec interval)	NS	
	CA1	LTP	200 Hz (100 pulse), 3 trains, interval 2 min	↓	
	Visual cortex	LTP	2 HFS, interval 20 sec	↓, rescue	[16]
		LTP	40 Hz (40 pulse) trains, 3 times, interval 10 sec	↓	[17]
			2 HFS, interval 15 sec	NS	
		LTD	LFS	↓	
	CA1	LTP	2 HFS, interval 20 sec	↓, rescue	[23]
	CA1	LTP	2 HFS, interval 20 sec	↓, partial rescue	[22]
Fmr1 -/-	CA1	LTD	PP-LFS, DHPG (100 μM, 5 min)	↑	[46]
		LTD	LFS	NS	
	CA1	LTD	DHPG (50 μM, 5 min)	↑, rescue	[54]
	CA1	LTD	DHPG (50 μM, 5 min)	↑, rescue	[55]
	CA1	LTP	2 or 3 trains of 50 Hz (20 pulses), interval 10 sec	NS	[37]
	CA1	LTP	TBS (5 bursts)	NS	[40]
	CA1	LTP	TBS (5 bursts)	↓	[41]
	CA1	LTP	TBS (5 bursts)	↓	[42]
	Cingulate cortex, pyramidal	LTP	EPSC, 2 Hz (80 pulse)	↓	[43]
	Amygdala, pyramidal	LTP	EPSC, 2 Hz (80 pulse)	↓	
Fmr1 -/y (only male used)	Visual cortex	LTP	3 HFS, interval 5 min	↓, mGluR I	[44]
		L-LTP	3 trains of TBS (10 bursts)	NS	[38]
TSC2+/- (rat)	CA1	LTP	1 HFS	NS	[39]
		LTP	2 trains, 30 Hz (100 pulse), interval 20 sec	↓, mGluR I	[45]
TSC2+/-	CA1	LTP	TBS (10 bursts)	↓	[74]
		LTD	LFS	↓	
TSC2+/-	CA1	LTP	1 HFS	↑, L-LTP; E-LTP (NS)	[72]
		LTD	DHPG (50 μM, 5 min), PP-LFS	↓	[76]
TSC2 ΔRG	CA1	LTD	LFS	NS	
		LTP	1 HFS or 4 HFS, interval 5 min	NS	[131]
TSC1 -/- in glia	CA1	LTD	DHPG (50 μM, 10 min)	↓	
		LTP	4 HFS, interval 30 sec	↓	[78]
TSC1 -/-	CA1 pyramidal	LTD	DHPG (100 μM, 5 min), PP-LFS	↓	[77]
		LTD	LFS	NS	
Mecp2-null	CA1	LTP	2 HFS, interval 20 sec	↓	[95]
		LTD	LFS	↓	
Mecp2 <sup>lox-Stop/+</sup>	CA1	LTP	TBS (10 burst) or 1 HFS	↓	[24]
		LTP	1 HFS	↓	[97]
Mecp2 <sup>2308/Y</sup>	CA1	LTP	TBS (10 burst) or 2 HFS, interval 20 sec	↓	[96]
		LTP	In bicuculline, TBS (6~10 burst) at 2 times of test intensity	↓	
	Sensory cortex	LTP	3 trains of TBS (10 burst), interval 10 sec	↓	
		LTD	PP-LFS	↓	
MeCP2 <sup>Tg1</sup> overexpression	CA1	LTD	DHPG (50 μM, 10 min)	NS	
		LTP	2 HFS, interval 20 sec	↑	[89]
Tau-Mecp2 overexpression	CA1	LTP	2 HFS, interval 20 sec	↓	[90]
Shank1 -/-	CA1	LTP	1 HFS	NS	[106]
		L-LTP	4 HFS, interval 5 min	NS	
		LTD	LFS	NS	
Shank3 Δex4-9 <sup>B</sup> , +/-	CA1	LTP	4 HFS, interval 5 min or TBS (15 bursts)	↓	[107]
		LTD	LFS, PP-LFS	NS	
Shank3 Δex4-9 <sup>d</sup> , -/-	CA1	LTP	2 HFS, interval 15 sec	↓	[110]
Shank3 Δex21, +/-	CA1	L-LTP	4 HFS, interval 5 min	↓	[109]
		LTD	LFS or DHPG (100 μM, 5 min) or PP-LFS	↑	

Table 1. Continued

Animal model	Area	Type	Protocol	Effect	References
Shank2 $\Delta$ ex7, -/-	CA1	LTP 1 HFS LTD PP-LFS		$\uparrow$ NS	[112]
Shank2 $\Delta$ ex6-7, -/-	CA1	LTP 1 HFS or 4 TBS (10 bursts) LTD DHPG (100 $\mu$ M, 15 min), interval 10 sec		$\downarrow$ NS	[111]

$\downarrow$ , decrease;  $\uparrow$ , increase; HFS, 100 Hz, 100 pulses; LFS, low frequency stimulation, 1 Hz, 900 pulse; L-LTP, late-LTP; LTD, long-term depression; LTP, long-term potentiation; mGluR I, group I metabotropic glutamate receptor; NS, no significant difference; PP-LFS, paired pulse LFS, paired pulse (50 ms interval), 1 Hz, 900 or 1200 pulse; TBS, theta burst stimulation, 5 to 10 bursts (100 Hz, 4~5 pulse) interval 200 ms. All animals are mice except a note of "rat".

m-/p+ mice in different brain regions using different protocols (Table 1). In CA1, LTP is reduced in *Ube3a* m-/p+ mice using an induction protocol of two trains of high frequency stimulation (HFS) of 100 pulses at 100 Hz (Table 1) [12]. Interestingly, the reduced LTP in CA1 of *Ube3a* m-/p+ mice could be rescued if a stronger stimulation protocol, three sets of two trains of HFS, was applied [15]. This indicates that the role of *Ube3a* in synaptic plasticity is probably as a modulator for the expression of LTP and less likely to play an essential role in LTP induction. Unfortunately, LTD at the same synapses has not been investigated so far. In the same study, reduced Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) activity due to increased inhibitory autophosphorylation was observed in *Ube3a* m-/p+ mice [15]. In the subsequent rescue experiment, genetic reduction of inhibitory autophosphorylation of CaMKII rescued the LTP deficit as well as hippocampal-dependent learning as assessed by Morris water maze and fear conditioning in *Ube3a* m-/p+ mice [16]. This *in vivo* rescue indicates that a biochemical mechanism mediated by CaMKII activity underlies the impaired synaptic plasticity in *Ube3a*-deficient synapses. However, it remains unclear what is the molecular mechanism through which the deficiency of *Ube3a* contributes to the altered activity of CaMKII.

Synaptic plasticity was also investigated in visual cortex in *Ube3a* m-/p+ mice [17,18]. Both LTP and LTD are reduced in visual cortex in *Ube3a* m-/p+ mice (Table 1) [17]. Low frequency stimulation was used for *N*-methyl-D-aspartate (NMDA) receptor-dependent LTD (LFS-LTD) induction but group I metabotropic glutamate receptor (mGluR) mediated LTD (mGluR-LTD) was not analyzed. Interestingly, *in vitro* synaptic plasticity was affected by the *in vivo* visual experience of the mice. Deprivation of visual input achieved by a dark rearing environment could actually restore the impaired LTP and LTD in *Ube3a* m-/p+ mice [17]. This observation is intriguing because it indicates that the defective synaptic plasticity due to deficiency of *Ube3a* can be easily reversed in animals. On the other hand, it would be interesting to learn whether dark rearing may actually reverse abnormal behavioral phenotypes in *Ube3a* m-/p+ mice.

As the *UBE3A* gene is a brain-specific imprinted gene that is expressed primarily from the maternal chromosome, the *UBE3A* gene on the paternal chromosome is structurally intact but transcriptionally repressed [9,19]. Through a small molecule screening program, Huang et al. discovered that the silenced *Ube3a* from the paternal chromosome can be activated by topotecan, a topoisomerase inhibitor, *in vitro* and *in vivo* in mice [20]. This discovery

opens an exciting research avenue to explore the treatment of AS using pharmacological interventions and presumably through epigenetic modification [21]. The immediate question is whether postnatal treatment with topoisomerase inhibitors can rescue the synaptic plasticity and ultimately behavioral defects in *Ube3a* m-/p+ mice. Another approach of treatment is to use a virus-mediated gene delivery method in hippocampus *in vivo*. Restoring the expression of *Ube3a* could rescue the early phase of LTP impairment and cognitive deficits in *Ube3a* m-/p+ mice [22]. In yet another group, use of an ErbB inhibitor could rescue LTP in *Ube3a* m-/p+ [23]. These observations together suggest that impaired synaptic plasticity and behavioral abnormalities in *Ube3a* m-/p+ mice is reversible even during late development. A similar finding was also reported in *Mecp2* mutant mice [24,25]. However, it remains a subject of debate if the same phenomenon may exist in the treatment of human neurodevelopmental disorders.

A subset of AS patients meet the diagnostic criteria for ASD [26]. However, the behavioral studies of *Ube3a* m-/p+ mice have not revealed significant impairments in ASD-like behaviors although these mice have recapitulated the most salient features of intellectual disability and movement disorder seen in AS [27]. This result may indicate that other genes in the 15q11-q13 region may also contribute to the ASD in human AS. Alternatively, more extensive behavioral tests and comparisons between mutant mice with mutation only in *Ube3a* and a deletion from *Ube3a* to *Gabrb3* may be warranted [14].

## FRAGILE X SYNDROME

Fragile X syndrome (FXS) is one of the best studied disorders with intellectual disability ID and ASD both in humans and mouse models [28]. In addition to ID and ASD, FXS patients are characterized by seizures, macroorchidism, and dysmorphic facial features [29]. Molecularly, FXS is caused by the CGG triplet expansion in the 5' untranslated region of the *FMR1* gene on the X chromosome [30,31]. The expanded CGG repeat results in promoter methylation that represses the transcription of *FMR1* [32]. The *FMR1* gene encodes the fragile X mental retardation protein (FMRP), an RNA-binding protein which inhibits local protein translation stimulated by group I mGluR signaling [28,33,34]. To understand the pathogenesis of fragile X syndrome, *Fmr1* KO mutant mice were first developed in 1994 [35]. For almost two decades, *Fmr1* mutant mice have been extensively studied from many angles by numerous investigators [36]. The full review of the findings from

studying *Fmr1* mutant mice is beyond the scope of this review. Instead, we will focus on synaptic plasticity in *Fmr1* mutant mice.

The initial studies of synaptic plasticity in hippocampal CA1 region in *Fmr1* mutant mice did not reveal any impairment in LTP by a standard LTP induction protocol (Table 1) [37-40]. However when the stimulation for LTP induction was reduced to near the induction threshold level in subsequent studies, LTP in CA1 was found to be reduced in *Fmr1* KO [41,42]. Interestingly, LTP in brain regions including somatosensory cortex, anterior cingulate cortex, anterior piriform cortex and lateral amygdala was also found to be decreased [39,40,43]. Impaired LTP was also observed in visual cortex and basolateral amygdala, but notably they were mGluR dependent [44,45]. These observations indicate different region- or synapse-specific defects in *Fmr1* deficient mice. However, the most important finding from synaptic plasticity studies is the observation of enhanced mGluR-LTD in hippocampal CA1 region [46]. A similar phenomenon of enhanced LTD was observed in cerebellum [47]. The observation of enhanced LTD in hippocampal CA1 region led to a theory of aberrant mGluR signaling underlying the pathophysiology of FXS [48]. The central hypothesis of the mGluR theory is that loss of FMRP in the synapse leads to the up-regulation of the mGluR-mediated signaling pathway. The mGluR theory and the molecular mechanism underlying the enhanced mGluR-LTD have been tested extensively since it was proposed and these studies have validated the central hypothesis [46,49-51]. However, alterations of many signaling pathways and a long list of potential protein targets in synapses have been revealed in *Fmr1* mutant mice [28,52]. It is not entirely clear how the disruption of these different pathways can be integrated into a unifying mechanism responsible for the pathophysiology of FXS. Recent reports indicate an involvement of Homer proteins in the dysregulated mGluR signaling pathway [53]. Genetic reduction of mGluR5 or pharmacological inhibition of mGluR5 could rescue the abnormal behaviors in *Fmr1* mutant mice [54,55]. Similarly, genetic reduction of Homer1a in *Fmr1* KO could also improve behaviors, though this did not rescue mGluR-LTD in hippocampus [56]. These rescue experiments raise an interesting possibility for potential reversal of neurological impairments in human fragile X syndrome. The various synaptic defects found in different brain regions in *Fmr1* mutant mice raise an immediate question about the correlation between the defective synaptic plasticity and the abnormal behaviors for future investigation. For example, social behaviors are impaired in *Fmr1* KO mice which is consistent with autistic behaviors frequently seen in human fragile X syndrome patients [57-60]. These studies support *Fmr1* KO mice as a good model to dissect the pathophysiology and explore treatment strategies for ASD [28].

## TUBEROUS SCLEROSIS COMPLEX

Tuberous sclerosis complex (TSC) is a neurocutaneous condition with prominent neurobehavioral manifestations including seizures, ID, and autistic behaviors [61,62]. The neurobehavioral features are quite variable and range from mild to severe presentations in TSC patients [61]. TSC is caused by mutations in *TSC1* or *TSC2* genes that show a dominant inheritance pattern [63,64]. The proteins, hamartin encoded by *TSC1* and tuberin encoded by *TSC2* genes,

form a heterodimeric complex that functions as a negative regulator for the mTOR pathway [65-67]. Therefore, it has been hypothesized that loss of function mutations in *TSC1* or *TSC2* disinhibit mTOR signaling and lead to the up-regulation of the signaling pathway downstream of mTOR which promotes cell growth and proliferation [67,68].

Both homozygous *Tsc1* or *Tsc2* KO mice are embryonic lethal [69-71]. Heterozygotes of *Tsc1* or *Tsc2* mutation exhibit cognitive impairment and synaptic dysfunction in the absence of apparent neuroanatomical defects or seizures [72-75]. In *Tsc2*<sup>+/-</sup> rats (Eker rat), LTP and LFS-LTD was decreased in CA1 [74]. In *Tsc2*<sup>+/-</sup> mice, early phase LTP (E-LTP) in hippocampal CA1 is not affected but late phase LTP (L-LTP) was enhanced [72]. In *Tsc2*<sup>+/-</sup>, mGluR-LTD was decreased but LFS-LTD was intact [76]. The reduced mGluR-LTD in *Tsc2*<sup>+/-</sup> is opposite to what is seen in *Fmr1* KO mice although the mGluR-LTDs from both were insensitive to protein synthesis inhibitors [76]. As in *Tsc2*<sup>+/-</sup>, *Tsc1*<sup>+/-</sup> mutant mice showed a similar impairment in synaptic plasticity. In hippocampal CA1 pyramidal neurons with conditionally deleted *Tsc1*, mGluR-LTD was reduced but LFS-LTD was intact [77]. Interestingly, synaptic plasticity is also impaired when *Tsc1* was knocked out in non-neuronal cells. For instance, the E-LTP was reduced in *Tsc1* glia-specific conditional KOs [78]. A recent study on *Tsc1* deleted specifically in cerebellar Purkinje cells showed impaired social interaction, enhanced repetitive behaviors and abnormal ultrasonic vocalizations [79]. However, synaptic plasticity was not tested in cerebellum in this mouse model [79]. This observation raises a provocative question regarding the brain regions and circuits that are important for the pathophysiology of autistic behaviors because social interaction was significantly reduced both in *Tsc1*<sup>+/-</sup> and *Tsc2*<sup>+/-</sup> [73,79,80]. The advantage of TSC models over other ASD mouse models is that the signaling pathway involving dysregulation of mTOR is well defined in both *Tsc1* and *Tsc2* mutant mice.

## RETT SYNDROME

Rett syndrome (RTT) is a neurological disorder that primarily affects females and is caused by mutations in the *MeCP2* gene [81,82]. The clinical presentations of RTT are characterized by normal early neurodevelopment for the first 12~18 months followed by developmental regression [83]. The major symptoms of RTT include movement disorders, absence of speech, and repetitive hand movements [83]. MeCP2 protein generally is considered to suppress transcription by binding to methylated CpG DNA [84]. However, recent evidence suggests a role of bidirectional regulation with both repression and activation of transcription mediated by MeCP2 [85]. Several *Mecp2* mutant mice carrying slightly different mutations have been produced and characterized [24,86-88]. In addition, mutant mice with overexpression of *Mecp2* was also reported [89,90]. These mice are valuable models to ASD research because RTT is a prototype for syndromic ASD and because impairments in social behaviors were observed in both whole brain- and region specific *Mecp2* mutant mice [91-94].

In general, mice lacking the functional copy of *Mecp2* recapitulate the major features of RTT. In *Mecp2*-null mouse, synaptic plasticity was analyzed at two different ages because of the age-dependent regression in human RTT [86,95]. In male mice at a presymptomatic age (3~5 weeks

old), no difference in LTP at hippocampal CA1 region was found. However, at a symptomatic age (6~10 weeks old) LTP and LFS-LTD in CA1 was reduced. This indicates that the trajectory of impaired synaptic plasticity correlates well with the developmental phenotype changes as suggested in humans. In a model where *Mecp2* was truncated as in some human patients (*Mecp2*<sup>308Y</sup>), LTP and paired-pulse low frequency stimulation (PP-LFS) (Table 1) but not the group I mGluR agonist dihydroxyphenylglycine (DHPG)-LTD was reduced in hippocampal CA1 from male mice [87,96]. Similarly, LTP in primary motor cortex and sensory cortex was reduced [96]. More interestingly, the impaired LTP in CA1 and neurological phenotypes in *Mecp2*<sup>lox-Stop/+</sup> mice could be rescued by reintroduction of *Mecp2* by genetic manipulation in male or female mice [24,97]. At least in three different RTT models, decreased CA1 LTP was consistent.

Synaptic plasticity was also investigated in mice with overexpressed MeCP2 via BAC mediated transgenics (*MeCP2*<sup>Tg1</sup>) [89]. As predicted from the finding of reduced LTP in *Mecp2* deficiency mice, LTP in CA1 was *increased* in *MeCP2*<sup>Tg1</sup> [89]. However LTP in CA1 was *decreased* in a different animal model with the overexpression of *Mecp2* driven by Tau promoter in neurons (*Tau-Mecp2*) [90]. The explanation for this discrepancy is not apparent and additional investigation is warranted.

## SHANK FAMILY GENE CAUSING ASD

SHANK family proteins, SHANK1, SHANK2, and SHANK3, are scaffolding proteins enriched at the postsynaptic density (PSD) of excitatory synapses [98]. SHANK proteins share a similar protein domain structure that mediates protein-protein interaction at the PSD for synaptic function [98,99]. Molecular defects in *SHANK3* were first found in patients with ASD and ID [100]. Subsequently, genetic defects of *SHANK1* and *SHANK2* were also reported in ASD and ID [101-103]. Because of the existing knowledge of the function of SHANK family proteins at synapses, the discovery of mutations in *SHANK* family genes provide direct support for the notion that the pathogenesis of ASD may reside in the dysfunction of synapses [104,105]. Mutant mice for all *Shank* family genes have been reported [106-112]. *Shank1* mutant mice were first reported [106]. Surprisingly, the phenotype of *Shank1* deficiency mice was unexpectedly mild. No synaptic plasticity changes were detected in LTP and LFS-LTD in the *Shank1* KO even though mEPSC frequency and synaptic strength were decreased [106]. Because of the findings of both microdeletions of and point mutations in the *SHANK3* gene in human ASD [100,113], the interest to model *Shank3* mutations in mice has been intensified recently. This led to the simultaneous generation of multiple *Shank3* mutant mice by disrupting different portions of *Shank3* exons [107-110]. These mutations include deletion of exons 4-7 ( $\Delta ex4-7$ ) [108], exons 4-9 ( $\Delta ex4-9^j$ ) [110] and ( $\Delta ex4-9^b$ ) [107], exon 11 ( $\Delta ex11$ ) [112], exons 13-16 ( $\Delta ex13-16$ ) [108] and exon 21 ( $\Delta ex21$ ) [109]. We recently discovered that *Shank3* has an array of protein isoforms resulting from the combination of multiple intragenic promoters and extensive alternative splicing of coding exons [110]. Therefore, we concluded that different mutations in different exons resulted in the disruption of different *Shank3* isoforms but none of these mutant mice were *Shank3* complete knockouts. *Shank* proteins regulate the abundance and signaling of ionotropic and metabotropic

glutamate receptors at excitatory synapses [105,114]. Accordingly, synaptic transmission and plasticity were examined in different brain regions in all *Shank3* mutant mice. Measurements of miniature excitatory postsynaptic current (mEPSC) frequency and amplitude, paired pulse ratio, input/output (I/O) curves, fiber volley, and population spikes indicated that synaptic transmission was reduced at hippocampal CA1 synapses of  $\Delta ex4-9^{B+/+}$  mice [107], but not in mice bearing  $\Delta ex4-9^{j-/-}$  [110],  $\Delta ex13-16^{-/-}$  [108], or  $\Delta ex21^{+/-}$  mutations [109]. The explanation for the difference between  $\Delta ex4-9^{B+/+}$  and  $\Delta ex4-9^{j-/-}$  is not immediately clear.

In striatum, the frequency of mEPSCs and amplitude of population spikes were significantly decreased in  $\Delta ex13-16^{-/-}$  mice, but only mildly affected in  $\Delta ex4-7^{-/-}$  mice [108]. Presynaptic responses measured by paired pulse ratio and input/output curves were not altered at corticostriatal synapses in  $\Delta ex13-16^{-/-}$  or  $\Delta ex4-7^{-/-}$  mice [108]. The different degree of synaptic transmission defects in mice with specific *Shank3* mutations supports the notion of an isoform-specific contribution to synaptic function. Moreover, the reduced NMDA receptor-mediated responses at cortical synapses of  $\Delta ex21^{+/-}$  [109] but not in the corticostriatal synapses of  $\Delta ex13-16^{-/-}$  mice [108] indicate distinct functions of *Shank3* at different synapses.

In terms of plasticity, hippocampal LTP was reduced at CA1 synapses of  $\Delta ex4-9^{j-/-}$ ,  $\Delta ex4-9^{B+/+}$ , and  $ex21^{+/-}$  mice [107,109,110]. In contrast, LFS-LTD was reduced in CA1 of  $\Delta ex21^{+/-}$  mice [109] but not in  $\Delta ex4-9^{B+/+}$  mice [107], suggesting an alteration in the set-point for bidirectional Hebbian synaptic plasticity [115]. mGluR-LTD induced by DHPG or PP-LFS was enhanced in CA1 hippocampus of  $\Delta ex21^{+/-}$  mice. However, a similar enhancement of mGluR-LTD was not evident in the  $\Delta ex4-9^{B+/+}$  mice induced by PP-LFS [107]. In addition, mGluR1/5 protein levels were not altered in  $\Delta ex21^{+/-}$  mice [109].

Collectively, these data support synaptic defects mediated by glutamate receptors in *Shank3* mutant mice that appear to be both synapse- and mutation-specific. It is not yet clear whether there are common core circuit defects in the various mutant mice, but the phenotypic heterogeneity itself appears consistent with the clinical heterogeneity of patients harboring *SHANK3* mutations. Since different mutations affect different isoforms of *Shank3*, some of the observed phenotypes may arise from isoform-specific effects on synaptic transmission.

Two mutant models for *Shank2* were reported recently [111,112]. Schmeisser et al. reported *Shank2* exon 7 deletion mutant mice (*Shank2*  $\Delta ex7$ ) in which LTP in hippocampal CA1 was increased but no change in LTD with PP-LFS was observed [112]. Reduced social interaction, increased stereotypical behavior, hyperactivity, and altered ultrasonic vocalization pattern were found in *Shank2*  $\Delta ex7^{-/-}$  mice. Won et al. generated *Shank2* mutant mice where exons 6-7 were deleted (*Shank2*  $\Delta ex6-7$ ) [111]. Both exon 7 and exon 6-7 deletion resulted in a frame shift mutation shortly after exon 7. Intriguingly, LTP in hippocampal CA1 was reduced in *Shank2*  $\Delta ex6-7$  mice and this is opposite to *Shank2*  $\Delta ex7$  mice [111]. In addition, the NMDA current and LFS-induced LTD were reduced in hippocampal CA1 region in *Shank2*  $\Delta ex7$  mice but DHPG-induced LTD was not affected. The behavioral profile of *Shank2*  $\Delta ex6-7$  mice is very similar to *Shank2*  $\Delta ex7$ . Interestingly, treatment with NMDA agonist current mediated signaling could rescue social interaction deficits [111]. The explanation for the

apparent discrepancy in synaptic plasticity but similar behavioral profile in mice with two very similar mutations is not immediately clear and further investigation is warranted. However, available data strongly support that both *Shank2* and *Shank3* mutant mice are valid ASD models to dissect the pathophysiology.

## FUTURE DIRECTIONS AND CHALLENGES

It is clear that abnormalities in synaptic plasticity vary significantly among different animal models of ASD. For instance, *Tsc2* mutations in rat and mice have opposite effects on LTP [72,74]. *Shank1* mutant mice do not show plasticity defects using standard protocols unlike *Shank2* and *Shank3* mutants (Table 1). In *Shank2* mutants, LTP impairment is in opposite directions in two different lines of mutant mice despite similar mutations and behavioral profiles [111,112]. However, as an example of convergence, LTP in three different lines of *Shank3* mutants from three different groups was decreased consistently but the LTD defects are significantly different [107,109,110]. On the other hand, it is difficult to correlate the abnormal plasticity with the corresponding behavioral manifestations in each model.

Currently, most synaptic plasticity experiments were performed in the hippocampus while the deficiency of targeted genes was in the whole brain. Therefore, it is difficult to establish causality between brain region and abnormal behaviors studied in the models. Because the neuro-anatomical basis for autism is still poorly understood, an unbiased survey for synaptic plasticity in other different brain areas may provide more informative data about the pathophysiology of autism.

In human brain, the superior temporal sulcus region, the fusiform gyrus and amygdala are considered important for social interaction and gaze behaviors [116]. However, gaze behavior in mice, which are nocturnal, is difficult to monitor technically. A neural circuit involving amygdala could be important region to study in ASD mouse model. The study of amygdala and related circuits such as medial prefrontal cortex in autism mouse models is within reach [117,118].

For stereotypical behaviors, cortical-striatal circuits are hypothesized to be important in ASD [119-121]. Significant repetitive behaviors measured by increase in self-grooming and inflexibility in the reversal phase of the Morris water maze are frequently reported in ASD mouse models [107,108]. The synaptic plasticity in cortical-striatal circuit activity is less well characterized in these most ASD models [122].

For the aspect of communication/ language impairment in humans, ultrasonic vocalization (USV) recording in mice has become a popular approach despite ongoing debate about the value of the USV relevant to human communication [123,124]. Abnormal USV measurements have been reported in numerous ASD mouse models [14,108-112,125-128]. These observations support the value of USV recording because of easy quantification and detailed numerical analysis. However, several challenges remain. First, what is the ethological meaning of USV in mice? Second, what is the circuit in rodent brain responsible for USVs [129,130]. More investigation are clearly warranted in future.

The studies of synaptic plasticity and behaviors in these high profile mouse models with defined genetic defects have produced many interesting findings but also raise numerous challenging questions. First, what is the implication of

variable, or opposite in some cases, synaptic plasticity related to understanding the pathophysiology of ASD and other comorbidities? Second, what is the molecular mechanism underlying the different synaptic plasticity between different brain regions? Third, can impaired synaptic plasticity in a particular brain region predict abnormal behaviors? Fourth, which is a more reliable biomarker, the synaptic plasticity or behavioral defects, to use for future drug screening? Future investigations may focus on 1) generation of brain region-, cell type-, or circuit-specific targeted gene KO, 2) *in vivo* physiology or circuit analysis, and 3) development of new and sensitive behavioral tests. Despite these challenges, we have reasonable confidence that studying these and other new ASD models will lead to better understanding the pathophysiology of ASD and ultimately lead to the development of new treatments.

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