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Shank mutant mice as an animal model of autism

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In this review, we focus on the role of the Shank family of proteins in autism. In recent years, autism research has been flourishing. With genetic, molecular, imaging and electrophysiological studies being supported by behavioural studies using animal models, there is real hope that we may soon understand the fundamental pathology of autism. There is also genuine potential to develop a molecular-level pharmacological treatment that may be able to deal with the most severe symptoms of autism, and clinical trials are already underway. The Shank family of proteins has been strongly implicated as a contributing factor in autism in certain individuals and sits at the core of the alleged autistic pathway. Here, we analyse studies that relate Shank to autism and discuss what light this sheds on the possible causes of autism.

1. Introduction

Research into autism or, more specifically, autism spectrum disorders (ASDs) began with its first description as a unique disorder by Leo Kanner in 1943 [1], before it was separately described in a report by Hans Asperger in 1944 [2,3]. Since this time, the clinical definition has been formalized and reformulated several times; most recently, in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [4], as well as in the tenth revision of the World Health Organisation's International Classification of Diseases (ICD-10) [5]. In DSM-5, ASD includes what was previously diagnosed separately as autistic disorder, Asperger's disorder, childhood disintegrative disorder and pervasive developmental disorder not otherwise specified. ASD is now defined by two broad types of impairment

— social communication and interaction, and
— restricted, repetitive patterns of behaviours, interests or activities.

Although these are broad categories, in practical terms, these symptoms tend to express themselves as characteristic signs that occur early on in development resulting in the missed milestones of language, attention and gestural behaviours of children to social/communicative cues and the onset of long-lasting repetitive interests (lights, lining toys up and spinning wheels) and lack of imaginative play before the age of three. There are also a number of other conditions that are often comorbid with ASD, including hyperactivity, obsessive compulsive disorder, anxiety, hypotonia, epilepsy, sleep disruption and gastrointestinal disorder [6,7].

As genome-sequencing technology is evolving, large amounts of data continue to reveal de novo and inherited mutations of certain genes from ASD patients [8–12]. These sequencing studies are providing promising clues for investigating the cause of and finding potential therapeutics for autism and ASDs. Indeed, many cell-signalling pathways are under investigation in relation to ASDs, especially the ones that have already been much studied for their synaptic functions [13–16]. Suspected brain regions also are widely diverse and range from the prefrontal cortex to cerebellum [17,18].

2. The synaptic theory of autism

In recent years, strong evidence seems to be converging on certain molecular pathways at the synapse, and this 'synaptic theory' of autism has been either implied or else explicitly stated by many groups already [10,19,20]. However, the evidence is not as simple as is sometimes suggested. Although many of the molecules implicated in autism fall into overlapping pathways, it is not yet clear how these interact to produce symptoms, nor precisely which mutations, or combinations of mutations, are necessary to cause autism or relate to ASD. These pathways are currently only partially understood at best and often involve multiple isoforms of proteins, molecules with overlapping functions, complex transcriptional and translational control, and precise timing. Finally, some molecules implicated in autism are not easily related to known synaptic pathways at all and so it remains to be determined whether they are indeed apart of the same signalling pathways. It is also likely that environmental factors play a significant role, as in other neurological conditions, for instance epilepsy [21]. Nevertheless, appropriate synaptic function is critical to development and ongoing learning and memory and so mutations in genes that are important in these signalling pathways that result in ASD point favourably towards this theory. The synaptic theory begins with two separate but converging streams of evidence: fragile X syndrome (FXS) and the mGluR theory [22], and neuroligins (NLGNs) and neurexins (NRXNs) [23]. FXS is a complex syndrome, often accompanied by autistic behaviour, that is caused by an insertion of CGG repeats in a single gene, encoding the fragile X mental retardation protein (FMRP1) [24]. FMRP is an inhibitor of protein translation that operates by binding RNA [25,26]. Most notably, FMRP knock-out (KO) mice have increased levels of protein synthesis and show enhanced mGluR-dependent long-term depression (mGluR-LTD) in the hippocampus [27] as well as impaired long-term potentiation (LTP) in the amygdala [28,29]. It has been shown that the synaptic-level symptoms in these mice are caused by increased sensitivity to mGluR5 signalling and extracellular signal-regulated kinase (ERK)-1/2 [30], and that this is related to a disrupted balance of Homer isoforms [31]. In addition to this, TSC1, a negative regulator of mammalian target of rapamycin (mTOR), which is upstream of FMRP, has been implicated in autistic symptoms through tuberous sclerosis (TSC) [32–34], and also tested in a mouse model [18]. TSC is a genetic disease and is caused by mutation of either of two genes, TSC1 or TSC2. ASD is common in TSC patients [35]. Deleting TSC1 has various effects on synaptic transmission including impaired hippocampal mGluR-LTD [36]. Mice with a heterozygous knockdown of the highly related TSC2 show electrophysiological defects that are remarkably rescued by crossing with FXS model mice, which seems to pinpoint the mGluR pathway as a fulcrum of autistic pathology [37].

At the same time that the mGluR pathway of autism was being uncovered, other groups were investigating the role of two families of proteins that link trans-synaptically—neuroligins (NLGNs) and neurexins (NRXNs)—to facilitate synaptic transmission or synapse formation. Multiple groups have established links between different isoforms of NRXN or NLGN and ASD [10,38–45]. These experiments revealed roles for NRXNs and NLGNs in synapse formation, synapse constitution and the expression of some forms of synaptic plasticity. As ASD is a developmental delay disorder, one theory is that an imbalance in the levels of certain NLGNs might disrupt the equilibrium of excitatory and inhibitory synapses. Although all NLGNs are able to attach to the molecular scaffolding of excitatory synapses via postsynaptic density protein-95 (PSD-95), excitatory synapses tend to be dominated by NLGN1. By contrast, NLGN2 tends to locate preferentially to inhibitory synapses via an interaction with gephyrin [46]. A recent paper by Gkogkas *et al*. [47] provides a promising link between NLGN and the mGluR theory. The authors investigated a model of excessive protein synthesis by eukaryotic translation initiation factor 4E (eIF4E) overexpression or depression (knockout of eIF4E-binding protein 2) and found that it resulted in over-translation of NLGN, increased excitatory-to-inhibitory transmission ratio and autism-like behavioural symptoms. This suggests that maintaining the balance of NLGN1 and NLGN2 in turn preserves an appropriate excitatory-to-inhibitory ratio, and that this may be partially modulated by mGluR signalling. Also, NRXN4 is suggested to contribute to the aetiology of ASD. There are reports on an association between NRXN4 (CNTNAP2) and autism susceptibility, as disruption of contactin 4 (CNTN4) was discovered in human patient genomic analysis. Common genetic variation in the gene encoding NRXN4 on chromosome 7 was found in certain groups of ASD with strong affection to male patients rather than female [44,45].

3. The Shank family of proteins

The Shank proteins lie at the heart of this synaptic map of autistic pathology binding to mGluRs indirectly, via Homer, and to both *N*-methyl-D-aspartate receptors (NMDARs) and NLGNs indirectly, via guanylate kinase-associated protein (GKAP) and PSD-95. They also link to the actin cytoskeleton, providing a multivalent scaffold upon which to build the postsynaptic density (PSD; figure 1).

The Shank family of scaffolding proteins (also known as ProSAP, cortBP, SSTRIP, Synamon and Spank) consists of three major isoforms—Shank1, Shank2 and Shank3—all of which are present in the brain, though with very different patterns of expression. Shank1 is expressed throughout most of the brain, except the striatum, being particularly highly expressed in the cortex and the hippocampus. Shank2 and 3 are also present in the cortex and hippocampus. Shank2 is almost absent in the thalamus and striatum, while Shank3 seems to be dominantly expressed in those regions. In the cerebellum, Shank2 is restricted to Purkinje cells, while Shank3 is restricted to granule cells [48].

The Shank family of proteins has a number of domains in common that mediate binding to a variety of different interaction partners [49]. Beginning at the N-terminal, there is an ankyrin repeat domain present that mediates binding to α-fodrin, which links to the actin cytoskeleton and calpain/calmodulin-mediated Ca^{2+} signalling [50]. This is followed by a Src homology-3 (SH3) domain that mediates binding to glutamate receptor-interacting protein (GRIP), a seven PDZ domain-containing molecule that links α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the postsynaptic scaffold [48,51]. Next is a PDZ domain that binds many different molecules within the PSD, importantly including GKAP, which allows Shank to attach to PSD-95 and thereby to neuroligins [23,52] and NMDARs. The PDZ domain is followed by a proline-rich region which contains a binding site for Homer (linking Shank to the mGluR-PLC-IP_3 Ca^{2+}-signalling pathway) [53,54], and a site for cortactin, a molecule that is involved in actin polymerization [52,55,56].

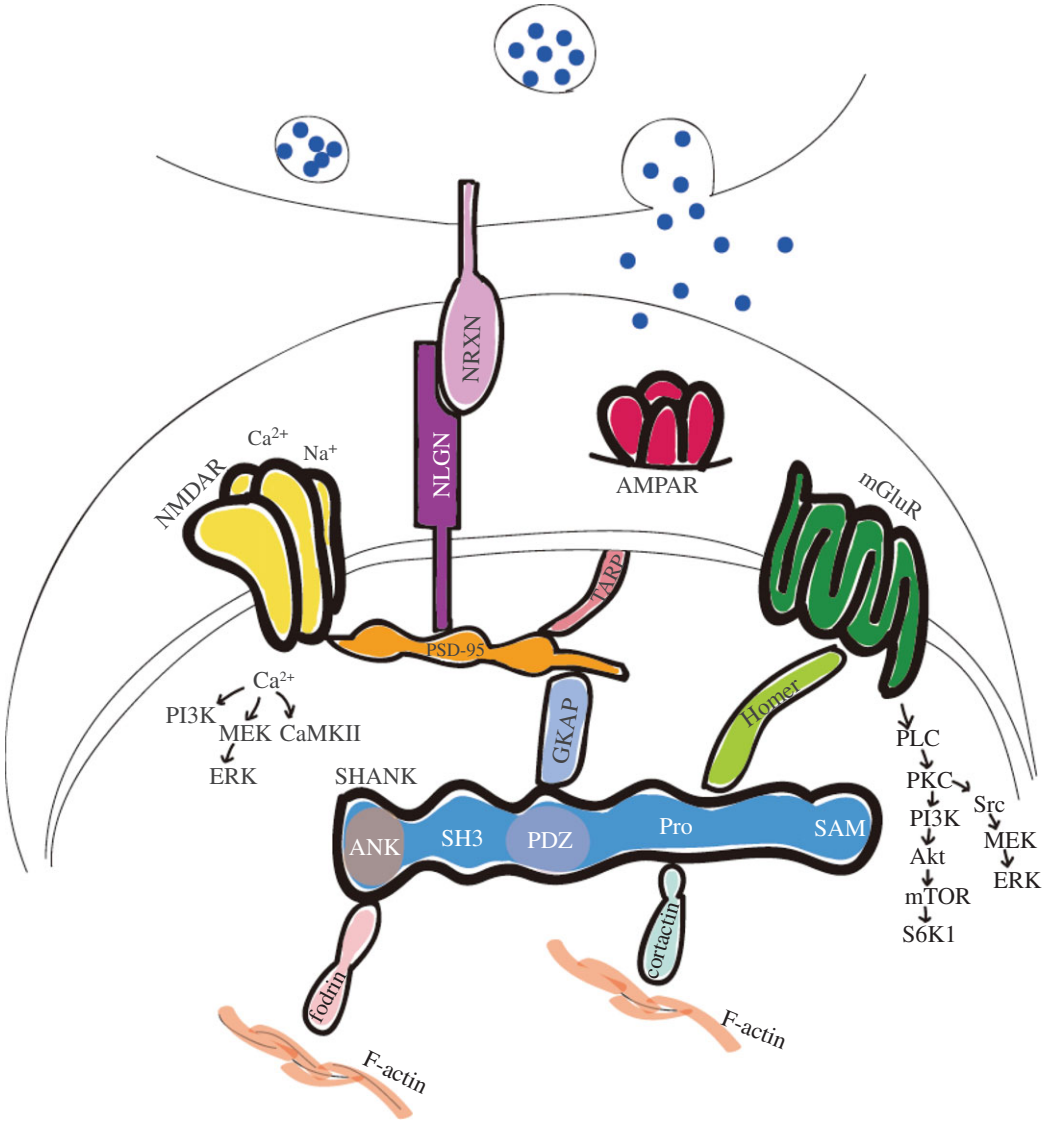

Figure 1. Schematic of Shank and interacting postsynaptic structures. Ank, ankyrin repeat domain; SH3, Src homology-3 domain; Pro, proline-rich-domain; SAM, sterile-alpha-motif domain; NLGN, neuroligin; NRXN, neurexin.

Lastly, at the C-terminal end, there is a sterile-alpha-motif (SAM) region that is involved in polymerization between Shank molecules, which may be regulated in a family member-specific manner by Zn^{2+} (figure 1; [57–59]).

Of the three Shank proteins, Shank3 was the first to be associated with autism. Initially, a link was established between Shank3 and 22q13.3 deletion syndrome (Phelan-McDermid syndrome), a form of mental retardation often presenting together with autistic traits [60–62]. Further analysis of autistic patients led to the discovery of a significant number of Shank3 mutations compared with the typically developing population, suggesting a specific role in autistic pathology [19]. Within a few years, Shank2 was also identified as a risk gene [9,63], and these genetic findings were soon followed by the generation and analysis of transgenic mouse models. Shank1 was also investigated in mice as it has homology with Shank2/3, though it is also of interest in itself [64–66] and later, genetic variants were discovered as risk factors in patients [11].

The creation of genetic constructs resembling different mutations for the Shank proteins, and their use in live mice and in cultured neurons, has been key to enhancing our knowledge of the role of Shank within the synapse and its contribution to autistic pathology.

4. Modelling Shank mutations in transgenic mice

(a) Shank1

Now that Shank1 mutations have been found in patients with autism, there is a greater impetus to properly understand the role of Shank1 and how it interacts with the other Shank family proteins. There is good reason to believe that Shank1 works differently to the other Shank proteins: for example, polymerization is not sensitive to the presence of Zn^{2+} ions, nor does it depend on the SAM domain for synaptic localization (the PDZ region being more important). In addition, it seems to be more important for synaptic maturation rather than synaptic formation [59].

Studies have been undertaken using Shank1 mutant mice ([64–66]; table 1). The mutation in these mice is a deletion of exons 14 and 15, which includes most of the PDZ region, and leads to a knockout of all detectable Shank1 protein in these animals. They present with an anxious phenotype, as measured by a light–dark test, and decreased movement in the open field. They have a deficit in motor learning and contextual fear conditioning, but are unaffected in spatial learning or working memory. They have no apparent repetitive behaviours and seem to display normal levels of social interaction, but there

Table 1. Synaptic and behavioural phenotype comparison on Shank1 mutant mice. n.d., not determined.

		synaptic morphology			synaptic physiology								
references	genotype	spine density	spine number	spine length	basal transmission	NMDA/AMPA ratio	NMDAR-LTP	NMDAR-LTD	GluN1	GluN2A	GluN2B	GluA1	GluA2
Hung *et al*. [64]	Shank1$^{-/-}$ Δ14–15	small decrease	n.d.	small decrease	decrease	no change	no change	no change	no change	n.d.	no change	no change	no change
Silverman *et al*. [65], Wöhr *et al*. [66]	Shank1$^{-/-}$ Δ14–15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

		behaviours			
		social behaviour	ultrasonic vocalization	repetitive behaviour	learning and memory	other behaviours
Hung *et al*. [64]	Shank1$^{-/-}$ Δ14–15	n.d.	n.d.	n.d.	impaired contextual fear memory, enhanced spatial learning	n.d.
Silverman *et al*. [65], Wöhr *et al*. [66]	Shank1$^{-/-}$ Δ14–15	reduced social sniffing by male in male–female interactions	reduced calls by males in male–female interactions	increased self-grooming	n.d.	mild anxiety-like behaviour, reduced scent-marking behaviour and reduced exploratory locomotion

appears to be a general deficit in social communicative behaviours, assessed by both ultrasonic vocalizations and urine-based communicative behaviours.

Although these papers do not give enough evidence alone for Shank1 KO mice as a model of autism, the discovery of deletions spanning the Shank1 gene (as well as neighbouring genes) in some males with ASDs further justifies continued research into the role of Shank1. It is interesting to note that in Shank1 mutant mice, there is a decrease in basal transmission and miniature excitatory postsynaptic current (mEPSC) frequency, as well as spine density, consistent with a decrease in the number of synapses, but no effect in synaptic plasticity was observed, despite testing both LTP and LTD [64].

(b) Shank2

Shank2 mutations are also found in human patients with ASD [63]. There have been pioneering studies using genetic constructs of Shank2 mimicking human Shank2 microdeletions (table 2). One group tested mice with a deletion of exons 6 and 7 (Shank2^{e6-7}) [67], while another group tested mice with a deletion of exon 7 only (Shank2^{e7}) [68]. Although the exons targeted by the two studies differ slightly, both target the PDZ region and should knock out all known Shank2 isoforms. This was confirmed in both studies by immunoblotting [67,68]. In both Shank2 mutant mice studies, there were marked alterations in behaviour and synaptic plasticity. However, there were perplexing differences in some of the findings pertaining to the synaptic physiology.

Looking into three behavioural categories in ASD, both sets of mice display impaired social interactions compared to wild-type (WT) littermates. Shank2^{e6-7} showed reduced interest toward a novel mouse in a home-cage social interaction assay. In a three-chamber test, Shank2^{e6-7} mice showed less preference to a novel animal over a novel object [67]. In a resident-intruder social interaction test, both male and female Shank2^{e7} could not maintain social contact with novel mice for as long as WT mice did [68].

Deficits in social communication were measured by checking altered ultrasonic vocalization (USVs) in both genotypes. USVs of adult Shank2^{e6-7} male mice call were less frequent when interacting with a novel WT female mouse. Adult Shank2^{e7} male mice made few USVs, but when they did they were not significantly different from WT mice. However, Shank2^{e7} female pups uttered significantly higher frequency USVs than WT. Other autistic symptoms were tested by measuring stereotypical behaviours, including grooming, jumping and digging. Shank2^{e6-7} mice spent more time grooming and jumping and less time digging. Shank2^{e7} mice also showed increased time grooming and had shorter bouts of digging. In addition, cognitive functioning tests were performed with Shank2^{e6-7} mice. They were shown to have impaired spatial learning in the Morris water maze test, with no change in object recognition memory, working memory or motor learning.

Broadly speaking, therefore, the two sets of mice display similar behavioural phenotypes.

In electrophysiology, however, the two studies produced very different results [67,68]. The Shank2^{e7} mutant was reported to have a decrease in basal transmission of around 40%, while the Shank2^{e6-7} mutant was reported not to differ in this parameter. The Shank2^{e7} study also report enhanced NMDAR-LTP and no change in paired-pulse, low-frequency stimulation-induced LTD, concomitant with increased NMDAR-mediated synaptic transmission relative to AMPAR-mediated synaptic transmission. In contrast, the Shank2^{e6-7} mutation showed decreased NMDAR-EPSC/AMPAR-EPSC ratio, together with impaired NMDAR-LTP and NMDAR-LTD. In other words, the results of Schmeisser *et al*. [68] are consistent with increased NMDAR-mediated synaptic function, and the results of Won *et al*. [67] are consistent with decreased NMDAR-mediated synaptic function. The distinct findings of both enhanced and reduced NMDAR function in mice that display similar autistic-like behaviours suggest that maintaining normal levels of NMDAR function could be the critical factor.

(c) Shank3

There have been studies using mice in which exons 4–9 of Shank3 have been deleted (Shank3^{e4-9}), resulting in the loss of the longest two isoforms of Shank3 [69–71]. In fact, another study includes a similar deletion, which excised exon 4 to 7 (Shank3^{e4-7}), although the majority of the results from that study refer to a downstream deletion of exons 13–16, resulting in the knockout of many shorter isoforms not affected in the other studies [72]. In addition, two recent studies have used gene expression in cultured hippocampal neurons to look at the subcellular mechanisms of Shank3, and the effect of point mutations in the ankyrin repeats domain associated with autism ([14,73]; tables 3 and 4).

Deficits in social interaction were not clearly established by all groups. Bozdagi *et al*. [69] and Yang *et al*. [71] detected no difference in social preference between WT and Shank3^{e4-9} groups in a three-chamber task, Peça *et al*. [72] also produced a negative result with the Shank3^{e4-9} mice, using a protocol similar to Yang *et al*., although they did find that the Shank3 mice failed to show any preference for a novel mouse over an unfamiliar mouse. Additionally, Wang *et al*. [70] demonstrated a lack of social preference for a novel mouse over a non-social object.

In terms of impairments in social communication, analysis of USVs by Bozdagi *et al*. [69] shows fewer calls by Shank3 males in the presence of a female in oestrus, compared with WT mice. Meanwhile, Wang *et al*. [70] reported an increased number of calls by males in the presence of a female stranger, whereas females produced fewer calls in the presence of the stranger. Further analysis showed that the distribution of calls for Shank3 mice, both males and females, was skewed towards shorter calls, with fewer high-frequency calls, as compared with WTs.

There is a consistent phenotype of repetitive self-grooming in Shank3^{e4-9} mice. In addition, Wang *et al*. [70] showed repetitive head-poking behaviour in a hole-board test, and repetitive exploratory behaviour of a novel object that differed subtly from WT mice. Cognitive symptoms associated with autism were also investigated, with both Yang *et al*. [71] and Wang *et al*. [70] finding deficits in reversal learning, object recognition memory and motor learning, thereby comprising a complex behavioural phenotype.

Reduced basal AMPAR-mediated transmission (and normal NMDAR-mediated transmission) has been shown by Bozdagi *et al*. [69]. The negative result (no change in basal transmission) of Wang *et al*. [70] is likely due to the use of older mice (two to four months compared with four to six weeks in the other studies). Additional evidence is provided by the observation of decreased numbers of GluA1 puncta [69] and decreased levels

Table 2. Synaptic and behavioural phenotype comparison of Shank2 mutant mice. n.d., not determined.

		synaptic morphology			synaptic physiology								
references	genotype	spine density	spine number	spine length	basal transmission	NMDA/AMPA ratio	NMDAR-LTP	NMDAR-LTD	GluN1	GluN2A	GluN2B	GluA1	GluA2
Won *et al.* [67]	Shank2$^{-/-}$ Δ6–7	no change	no change	no change	no change	decrease	impaired	impaired	increase	no change	no change	n.d.	no change
Schmeisser *et al.* [68]	Shank2$^{-/-}$ Δ7	small decrease	small decrease	n.d.	decrease	increase	enhanced	no change	increase	no change	large increase	n.d.	no change

		behaviours				
		social behaviour	ultrasonic vocalization	repetitive behaviour	learning and memory	other behaviours
Won *et al.* [67]	Shank2$^{-/-}$ Δ6–7	less interest toward novel mice in three-chamber test and increased pup retrieval latency	fewer calls and longer latency for the first call by male in male–female interaction	repetitive jumping in male and decreased digging	impaired spatial learning, normal object recognition memory, normal working memory and normal motor learning	increased anxiety-like behaviour
Schmeisser *et al.* [68]	Shank2$^{-/-}$ Δ7	normal initiation of social interaction and impaired social interaction in three-chamber test	more calls by female pups. Fewer calls and longer latency in female–female interactions and longer latency for the first call by male in male–female interaction	increased self-grooming	normal working memory, normal novel object recognition memory	increased anxiety-like behaviour

Table 3. Synaptic phenotype comparison of Shank3 mutant mice. n.d., not determined.

		synaptic morphology			synaptic physiology								
references	genotype	spine density	spine number	spine length	basal transmission	NMDA/AMPA ratio	NMDAR-LTP	NMDAR-LTD	GluN1	GluN2A	GluN2B	GluA1	GluA2
Bozdagi *et al.* [69]	Shank3$^{-/-}$ Δ4–9	n.d.	n.d.	n.d.	decrease	n.d.	impaired	no change	n.d.	n.d.	n.d.	reduced	n.d.
Wang *et al.* [70]	Shank3$^{+/-}$ Δ4–9	reduced (four weeks old), no change (10 weeks old)	no change	increase	no change	n.d.	impaired	n.d.	n.d.	reduced	no change	reduced	no change
Peça *et al.* [72]	Shank3$^{-/-}$ Δ4–7	n.d.	n.d.	n.d.	mild disruption in cortico-striatal synaptic transmission	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Peça *et al.* [72]	Shank3$^{-/-}$ Δ13–16	decrease	n.d.	no change	decreased cortico-striatal transmission								
Yang *et al.* [71]	Shank3$^{+/-}$ Δ4–9	n.d.	n.d.	n.d.	decrease	n.d.	impaired	no change	n.d.	n.d.	n.d.	n.d.	n.d.

Table 4. Behavioural phenotype comparison of Shank3 mutant mice. n.d., not determined.

		behaviours				
references	genotype	social behaviour	ultrasonic vocalization	repetitive behaviour	learning and memory	other behaviours
Bozdagi *et al.* [69]	Shank3$^{-/-}$ Δ4–9	less social sniffing by male in male–female interactions	fewer calls by male mice in male–female interactions	n.d.	n.d.	normal olfactory ability
Wang *et al.* [70]	Shank3$^{+/-}$ Δ4–9	impaired social affiliation	more calls by males and fewer calls by females	increased head pokes in hole-board test	impaired spatial memory, impaired novel object recognition and impaired social transmission of food preference	normal anxiety-related behaviour and mild motor dysfunction
Peça *et al.* [72]	Shank3$^{-/-}$ Δ4–7	normal initiation of social interaction and perturbed recognition of social novelty	n.d.	no self-injurious grooming	n.d.	no anxiety-like behaviour
Peça *et al.* [72]	Shank3$^{-/-}$ Δ13–16	preference for non-social object over social partner and impaired reciprocal interaction	n.d.	self-injurious grooming	normal spatial learning and memory	anxiety-like behaviour
Yang *et al.* [71]	Shank3$^{+/-}$ Δ4–9	less social sniffing and reduced male juvenile reciprocal social interactions	fewer calls by males in male–female interactions	increased self-grooming in males	normal spatial learning and memory, normal contextual and cued fear memory	increased self-grooming in males

of membrane GluA1 [70], suggesting that this may be the cause of the reduced synaptic strength in the Shank3 mutants.

Bozdagi *et al*. [69] also show a decreased paired-pulse ratio, increased mEPSC frequency and decreased mEPSC amplitude, all of which is consistent with weak synapses having a high presynaptic release probability. The idea that Shank3 is important in strengthening synapses on the postsynaptic side is supported by studies showing that overexpression of WT Shank3 increases the number of spines and decreases the number of immature filopodia in cultured hippocampal neurons as compared with expression of green fluorescent protein or compromised Shank3 [14].

Shank3^{e4-9} mice showed impaired NMDAR-LTP, while NMDAR-LTD and mGluR-LTD are intact. It is not clear whether this is a result of decreased levels of GluA1 making depolarization less likely, or whether it is a failure of expression, with synapses being unable to recruit sufficient GluA1 in the absence of Shank3. One clue might be that the ankyrin repeat domains of Shank proteins are capable of binding actin, via α-fodrin, and that this Shank–actin connection is broken by Ca^{2+}-mediated calpain signalling, which has been shown to be important in NMDAR-LTP [50,74–76]. This fits with a model in which Shank3 accumulates around the actin cytoskeleton, forming a platform for the specialized machinery of the PSD, such as neurotransmitter receptors and molecules involved in Ca^{2+} signalling.

Peça *et al*. make use of a Shank3 deletion of exons 13–16 in the PDZ region, resulting in the absence of all but the shortest two identified forms of Shank3 [72] consisting only of the proline-rich region and/or the SAM domain. The mice in this study display a comprehensive and strong autism-typical phenotype: impaired social interactions in a three-chamber task, as well as a lack of social novelty preference; repetitive behaviour, in the form of self-grooming to the extent of self-inflicted lesions; anxiety-related behaviour, as measured by an elevated zero maze, a light–dark test, and a lack of rearing in the open field. However, as neuronal-level tests were performed in striatal medium spiny neurons (MSNs), it should be considered that the other studies analysed results in hippocampal CA1 neurons.

Peça *et al*. showed that in the MSNs, basal transmission, mEPSC frequency and mEPSC amplitude are all decreased. This is accompanied by structural changes, including decreased spine density, increased dendritic length and more complex arborization, particularly closer to the cell body. Electron microscopy also revealed a decrease in both the length and the thickness of the PSD. In addition, there are molecular changes in the PSD, with decreases in the levels of structural proteins (SAPAP3, PSD93 and Homer) and glutamate receptors (GluA2, GluN2A and GluN2B).

So far, compared to other Shank proteins, more studies have been done with animals with mutation on Shank3. Behavioural phenotypes of those mutant mice fit the criteria of ASD. Synaptic function was altered in mutant mice as they were shown to have reduced basal transmission, impaired plasticity, etc. These discoveries are underscoring the possible aetiology of ASD.

5. Making progress in mouse models of autism

Just looking at mouse models of the three Shank proteins reveals some of the difficulties of investigating autism, with discrepancies within the same model (e.g. Bozdagi *et al*. and Yang *et al*.) and often large differences between different models (tables 3 and 4). When viewed broadly, these studies approach the task from four directions—electrophysiology, neuroanatomy, molecular biology and behaviour—reflecting the current climate of neuroscience research. However, when results in these four areas are inconsistent, it begs the question: what are the key deficits that are causative of autism?

We will not go into depth discussing behavioural testing of autistic mouse models—a comprehensive review is already available [77]. However, the validity of any behavioural test in mice must always be carefully considered in relation to human symptoms. Behavioural results can be conflicting and reproducibility across laboratories and different lines can be difficult to achieve. And these discrepancies are, to some extent, likely due to the laboratory environment and the genetic background of the transgenic mice [78,79]. For instance, can we claim the same phenotype for the Shank2 mice that show repetitive jumping behaviour in one line and repetitive self-grooming in another [67,68]?

There are a lot of unanswered questions in autism research; given the amount of research conducted, it is remarkable that the key location of the deficit in the brain is not yet pinned down. Of course, one assumes this may be because there is no one key brain region, but separate KO models restricted to the forebrain [80] and the cerebellum [18] both produce an autistic phenotype, suggesting that localized defects may be sufficient. Symptoms themselves show such a wide range in humans. This raises the very serious possibility that there are multiple pathologies capable of causing autistic symptoms, which may act along different pathways.

The genetic component of autism has long been known to be both strong and complex, and recent studies using the latest sequencing technology have revealed molecular overlap and showed clearly that there are strong risk genes for autism that are likely causative in at least some cases. The development of mouse models has created the possibility of strongly controlled gene-specific testing, which is an invaluable tool in unravelling the genetic contribution to autism. Nevertheless, from a clinical point of view, it is important that the environmental contribution should not be overlooked as, whether it be pre-conception, the fetal environment or early infancy, it is likely to play at very least a supplementary role.

In terms of deficits in the brain, the important question remains: which alterations, and where, are involved in autistic pathology? There are two roads of inquiry that have shown some success and much promise in finding the answer to this question: spatially and temporally controlled inducible transgenic lines, and rescue experiments. Cre-mediated inducible expression has been used to investigate other proteins implicated in autism, such as TSC1, SCN1A or SynGAP1 [18,80,81]. Remarkably, one of these papers induced autism-like symptoms in mice with a mutation limited to the cerebellum, while another caused a similar set of symptoms when the mutation was induced only in the forebrain. Can autism be caused by certain localized defects in humans or is autism a whole-brain deficit that occurs owing to a breakdown in communication between different areas of the brain? It certainly highlights the issue with the majority of electrophysiological experiments being performed in the hippocampus, where the procedures have been best established.

6. Rescuing autism phenotype

Rescue experiments can provide temporal resolution. In the case of Eif4ebp2 KO mice, in which Nlgn1 mRNA translation

is enhanced, the excitatory/inhibitory (E/I) ratio is increased. Reduction of NLGN1 levels using siRNA restored E/I balance and reversed deficits in social interaction behaviour [47]. Another example is the case of the Shank2 KO; it was shown that acute administration of either the NMDAR partial agonist D-cycloserine or an mGluR5-positive allosteric modulator (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide) could rescue the electrophysiological and social impairments in the KO mice [67]. This demonstrates convincingly that symptoms in these mice are not caused by a developmental defect, but are the result of an ongoing synaptic defect. Furthermore, this implicates both mGluR5 and NMDAR in the pathology of these particular mice. Indeed, Shank proteins, being able to connect NMDARs (via GKAP and PSD95) to mGluRs (via Homer), stand at the core of autistic pathology.

7. NMDARs and mGluRs as important players in autism

The functions of NMDARs and mGluRs are intricately linked, and though there is much detail to be deduced regarding their precise interactions, there have been several studies that suggest some degree of interdependency. For example, in early work it was found that activation of group I mGluRs is able to potentiate the excitation of neurons by NMDA [82,83] and that activation of NMDARs can potentiate the effects of group I mGluR action [84]. Therefore, NMDARs and group I mGluRs have the capacity to facilitate each other's actions, an effect that has been widely investigated. However, this positive interaction is not invariably the case because, occasionally, mGluR activation may be inhibitory of NMDAR function [85]. It is therefore important to define the precise roles of NMDARs and group I mGluRs in different experimental paradigms.

In terms of synaptic plasticity, global KO of mGlu5 receptors, the predominant group I mGluR expressed in the hippocampus, was found to result in a slightly smaller NMDAR-dependent LTP [86]. Interestingly, this effect appears to relate to a developmental consequence of the loss of mGlu5 receptors throughout life, as in another study the modest deficit in LTP in mGlu5 KO mice was replicated but it was not mimicked by acute pharmacological blockade of mGlu5 receptors [87]. A more recent study has identified mGluR/NMDAR interactions in a form of spike-timing-dependent LTP. In this paradigm, NMDAR-dependent LTP was impaired by blocking group I mGluRs, while pharmacological activation of mGluRs removed the postsynaptic spike frequency dependency of LTP-induction [88]. This suggests that, at least in some circumstances, NMDAR-LTP is dependent on mGluR activation.

In terms of LTD, it has been shown that both the synaptic activation of NMDARs and the synaptic activation of group I mGlu receptors can induce LTD. In both cases, the LTD is expressed, at least in part, by a reduction in the number of AMPARs expressed at synapses, but the signalling mechanisms involved are largely different [89]. In summary, both NMDARs and group 1 mGluRs are major players in LTP and LTD where their actions can be either interrelated or independent of one another.

Recent studies have addressed the role of Homer proteins in the interaction between group 1 mGluRs and NMDARs [79,84,85]. Working in the cerebellum, one set of authors demonstrate that precise timing of a postsynaptic action potential with presynaptic glutamate release can activate extrasynaptic mGluRs, and this induces a non-Ca^{2+}-dependent, millisecond time-scale enhancement of NMDAR currents, which is absent when long-form Homer is interfered with [84]. The short time scale suggests a physical link. Others have observed a role of Homer proteins in the inhibition of NMDARs by sustained mGluR activation [79,85]. It was suggested that expression of Homer1a results in competitive disruption of the Homer/Shank scaffold and this enables mGluRs to diffuse and bind to NMDARs to inhibit their function. Given more information, such conflicting results could even perhaps explain why one Shank2 KO study produced an increase in NMDAR signalling while the other produced a decrease in NMDAR-related signalling [62,63].

One promising road of inquiry might be mGluR-mediated homeostatic modulation of NMDARs; some of the linguistic symptoms of autism can even be seen as resulting from excessive weighting given to the first learning experience. It is likely that the ways in which mGluR/NMDAR function can influence behaviour are manifold, and if sufficiently high-resolution diagnostic tests were available, it may be possible one day to relate certain idiosyncrasies of any individual's autism with their specific pathology. These details are likely to be important, particularly when designing treatments, given that the electrophysiological phenotypes of the different mouse models sometimes differ dramatically. Either way, we determine that further research into the interactions between mGluR and NMDAR signalling has the potential to bear great fruit in the future of autism research.

8. Towards a unifying hypothesis for the cognitive alterations in autism spectrum disorder

As mentioned above, in addition to Shank proteins another prime focus of ASD research has been the neurexins (NRXNs) and neuroligins (NLGNs), for which ASD-associated mutations have been found in NRXN1–3 and NLGN1, NLGN3, NLGN4X and NLGN4Y. The Shank proteins bind neuroligins, suggesting that understanding the functional roles of this interaction may give insights into the underlying pathology of at least some forms of ASD. In this context, an intriguing recent study has shown that the overexpression of Shank3 results in alterations not just in postsynaptic function but also in presynaptic function and that this effect is mediated by the interaction between neuroligins and neurexins [73]. The introduction of ASD mutations in Shank3 (R87C, R375C, Q396R and InsG) impaired both pre- and postsynaptic function at glutamatergic synapses. These data point to a major underlying problem with the ability of excitatory synapses to coordinate their pre- and postsynaptic function.

In conclusion, the genetics of ASD have led to a focus on the glutamatergic synapse. Based on available evidence there does not appear to be a single causal deficit but rather various alterations in pre- and postsynaptic function, including changes in synaptic plasticity. Perhaps the most exciting results to emerge are the findings that, in mouse models, it is possible to reverse behavioural and physiological deficits with pharmacological treatments, indicating that the underlying cause may not

necessarily be an irreversible developmental abnormality but rather an ongoing synaptopathy. Thus, a greater understanding of the glutamatergic synapse in rodent models of autism should aid in the development of effective therapies for ASD.

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