Synapse alterations in autism: Review of animal model findings

Martina Zatkova^a, Jan Bakos^{a,b}, Julius Hodosy^{a,c,d}, Daniela Ostatnikova^a

Background. Recent research has produced an explosion of experimental data on the complex neurobiological mechanisms of developmental disorders including autism. Animal models are one approach to studying the phenotypic features and molecular basis of autism. In this review, we describe progress in understanding synaptogenesis and alterations to this process with special emphasis on the cell adhesion molecules and scaffolding proteins implicated in autism. Genetic mouse model experiments are discussed in relation to alterations to selected synaptic proteins and consequent behavioral deficits measured in animal experiments.

Methods. Pubmed databases were used to search for original and review articles on animal and human clinical studies on autism.

Results. The cell adhesion molecules, neurexin, neuroligin and the Shank family of proteins are important molecular targets associated with autism.

Conclusion. The heterogeneity of the autism spectrum of disorders limits interpretation of information acquired from any single animal model or animal test. We showed synapse-specific/ model-specific defects associated with a given genotype in these models. Characterization of mouse models with genetic variations may help study the mechanisms of autism in humans. However, it will be necessary to apply new analytic paradigms in using genetically modified mice for understanding autism etiology in humans. Further studies are needed to create animal models with mutations that match the molecular and neural bases of autism.

Key words: autism, synaptogenesis, cell adhesion molecules, scaffolding proteins, animal models

Received: May 21, 2015; Accepted with revision: December 4, 2015; Available online: January 5, 2016
<http://dx.doi.org/10.5507/bp.2015.066>

^a Institute of Physiology, Comenius University, Bratislava, Slovak Republic

^b Institute of Experimental Endocrinology, Bratislava, Slovak Republic

^c Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovak Republic

^d Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic

Corresponding author: Jan Bakos, e-mail: j.bakos@savba.sk

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with heterogeneous phenotype predominantly affecting males¹. According to the World Health Organization (WHO), there is a global median prevalence of autism spectrum disorder estimated at 62/10 000, that is one child in 160. The median rate of prevalence for Europe is 61.9/10 000 and for America is 65.5/10 000 (ref.²). Although still a matter of debate, the most common symptoms of autism include social and communication impairments and repetitive behavior. Currently, no single cause of ASD is known and pharmacological treatments for the core symptoms are not available¹.

Numerous animal models (lesion, transgenic, knockout, selective breeding, etc.) have been developed for a variety of psychiatric, neurodegenerative, and neurodevelopmental disorders. Animal models are often used to study maternal immune activation, genetic defects and environmental factors in autism too³⁻⁵. Mouse models for genetic defects include nonsyndromic autism associated mutations in single genes. The most common mutations in this context include cell adhesion molecules: neuroligins, neurexins and scaffolding proteins, i.e. Shank proteins. Neuroligins, neurexins and Shank proteins are

synaptic proteins important for the formation of neural circuits and brain development. The other group of models represents syndromic autism associated mutations in a single gene, for example fragile X syndrome and Rett syndrome. Genetic mouse models of fragile X syndrome and Rett syndrome are experimentally used to examine the effects of environmental stimulation on behavioral deficits⁶.

In autistic subjects, abnormalities of brain growth and connectivity are usually apparent after the first year of life^{7,8}. Early developmental stages are characterized by generation of new neurons, dendritic growth, synaptogenesis, neural circuit formation and experience-dependent remodeling⁹. Recent studies have suggested that early-life deprivation during a sensitive period can lead to commitments that are difficult to reverse at later ages¹⁰. The early years of development are crucial for the formation of neural circuits when there is a high predisposition to disruption. Thus, research on autism may benefit from transgenic mouse models and/or aversive stimuli at different stages of development. This review describes the most important mechanisms in synapse formation and synapse alterations in autism spectrum disorders, followed by a review of the results of experimental tests on animal models with mutations in neurexins, neuroligins and Shank genes.

Synapse formation

The formation of the nervous system requires multiple developmental events such as neuronal cell fate specification, cell migration, dendritic growth, axon guidance, synaptic target selection and synaptogenesis¹¹⁻¹³. All of these developmental steps have to be completed for both interconnections of microcircuits in the brain and peripheral innervations of a specific tissue. By mediating the information flow between neurons, synaptogenesis is the final step in the development of the nervous system that provides regulation of intercellular communications within the nervous system. After the completion of the process of axon guidance and target recognition, synapse formation proceeds. One of the first steps in synaptogenesis is the induction and adhesion of opposed presynaptic and postsynaptic domains. This is mediated by cell adhesion molecules which trigger the events that lead to the assembly and differentiation of pre- and postsynaptic specializations¹³. These molecules can have several different functions. They promote stability of neuronal connections by linking synaptic partners. Furthermore, the cell adhesion molecules regulate differentiation of pre- and postsynaptic specialization and they participate in modulation of synaptic structure and function¹⁴.

The second step in synaptogenesis is organization of pre synaptic and postsynaptic specializations by scaffolding proteins. These proteins are critical mediators in assembling synaptic proteins into domains specialized for neurotransmitter release and reception. Scaffolds form a link between the adhesion proteins, ion channels, neurotransmitter receptors, intracellular signaling cascades, and the actin cytoskeleton¹⁵.

Synaptogenesis includes regulation of growth by intercellular signaling pathways. Synaptic growth is regulated by anterograde and retrograde signaling molecules, including the wntless family proteins (Wnt) and the bone morphogenetic protein pathway. The Wnt signaling molecules are important for a broad range of processes, from neurogenesis to synaptic plasticity¹⁶. At the presynaptic site, Wnt regulates cytoskeleton dynamics through inhibition of glycogen synthase kinase-3 β (ref. ¹⁷⁻¹⁸). This cytoplasmic pathway component interacts with actin and the microtubules. Postsynaptically, Wnt signaling promotes the growth of the postsynaptic membrane by translocation of the cleaved receptor Frizzled into the nucleus¹⁹. The retrograde bone morphogenetic protein pathway includes transcription factors which target multiple genes, including Rac GTPase activator²⁰. This type of GTPase regulates the actin cytoskeleton and it is important for the growth of dendritic spines²¹. Impairment in the bone morphogenetic protein has been found in fragile X syndrome²². When growth signals are released, such as Wnts and bone morphogenetic proteins, they bind to the synaptic transmembrane receptors and are internalized as receptor-ligand signaling complexes. These complexes are transported within the endosomal system, which in synaptic development is critical for activity-dependent circuit refinement¹⁵.

Role of the cell adhesion molecules in synapse formation

Many cell adhesion molecules are localized at synaptic sites in neuronal axons and dendrites. These include

Fig. 1. Interaction of neuroligins and neuroligins in trans-synaptic complex. Neuroligins are located on the presynaptic terminal and neuroligins on the postsynaptic nerve terminal.

neuroligins and neuroligins. These molecules form the trans-synaptic adhesion complex molecules that connect presynaptic and postsynaptic neurons at synapses and mediate trans-synaptic signaling²³. Neuroligins and neuroligins interact with each other in a Ca²⁺ dependent manner (Fig. 1).

Neuroligins act predominantly on the presynaptic terminal in neuronal cells and play essential roles in the neurotransmission and differentiation of synapses²⁴. Each of three human neuroligin genes (neuroligin 1-3) generates transcripts under the control of two separate promoters for two neuroligin isoforms, the longer neuroligin- α form and the shorter neuroligin- β form (ref. ²⁵). Another important member of the neuroligin family of proteins includes contactin-associated protein-like 2 (CNTNAP2), which is involved in neuron-glia interactions and clustering K⁺ channels in myelinated axons²⁶.

Neuroligins are synaptic cell adhesion molecules that are enriched in postsynaptic membranes where they may recruit receptors, channels, and signal-transduction molecules²⁷. Neuroligins are encoded by four human genes (neuroligin 1-4), and like neuroligins, undergo alternative splicing²⁵. Interestingly, mammals express only three or four genes²⁸.

In the rodent brain, all three neuroligin (neuroligin 1-3) proteins are detected at low levels before birth and increase rapidly during the first three postnatal days. The plateau of these proteins culminates during the period when most synapses are formed and they remain at high levels during adulthood²⁹. The expression of neuroligin 1 and neuroligin 2 in the CNS is restricted to the excitatory and inhibitory synapses. Although neuroligin 3 is expressed mainly by neurons, a few studies suggest that neuroligin 3 can be expressed by the glial cells, astrocytes and Schwann cells in the developing embryo³⁰.

Role of the scaffolding proteins in synapse formation

As mentioned above, the interactions between the cell adhesion molecules and the scaffolding proteins are essential for functional synapse formation. Scaffolding proteins include a large group of mutually binding syn-

aptic proteins. In the developing brain, space and time regulation of expression of scaffolding proteins determine the morphology of neuronal processes and consequently synaptogenesis. A number of structurally related forms of scaffolding proteins have been identified. The most important group is the ankyrin related proteins. These proteins contain multiple domains for protein-protein interaction, including ankyrin repeats, and the SH3 domain. Particular members of this family are Shank proteins. Shank1-3 are synaptic scaffolding proteins that interact with a variety of membrane and cytoplasmic proteins. These proteins bind to neuroligin-neurexin complexes at postsynaptic excitatory glutamatergic synapses²⁶. Specific positioning of Shank proteins at the postsynaptic sites of excitatory synapses suggests a role for this protein family in the organization of cytoskeletal/signaling complexes at specialized cell junctions³¹. Moreover, the neuroligin-neurexin-Shank pathway is required during the stabilization phase of the synapse in response to neuronal activity³².

Formation of trans-synaptic neuroligin-neurexin complex is essential for neural circuit function resulting in transforming an assembly of synapses into fully functional units. Moreover, neuroligins and neurexins are involved in maintaining the excitatory/inhibitory balance within the nervous system. Mutations in the genes of cell-adhesion and scaffolding molecules might be important in the development of autism spectrum disorders.

Synaptic alterations in autism

Autism spectrum disorder is a pervasive neurodevelopmental disorder characterized by marked disruption in information processing and cognition. Many studies have found altered synaptic plasticity in the brains of affected individuals³³⁻³⁵. The early onset of ASD and relatively small pathological changes in the brain of patients with ASD led to the hypothesis that impaired synapse formation, neuronal connectivity and circuit stabilization may explain the pathogenesis of this disease^{7,36,37}.

Altered neuronal activity in ASD might be a reflection of disturbed synaptic integrity due to altered expression of cell adhesion and scaffolding molecules involved in synaptic development. Many of these molecules are associated with ASD, including neuroligins, neurexins, and Shank3²⁶. In particular, alterations in the genes encoding neuroligins 3 and 4, their binding partners neurexins 1 and 3, Shanks and CNTNAP2 are implicated in synaptic changes in autism³⁸. Many other genes encoding synaptic proteins involved in cell adhesion and scaffolding are associated with autism as can be seen from Table 1.

Alterations in genes encoding cell adhesion molecules and scaffolding proteins implicated in autism

Patients with mutations in the genes encoding neuroligins suffer from disruption in excitatory/inhibitory balance in terms of increasing the excitatory/inhibitory ratio, which in turn may disrupt the sensory, social and emotional systems⁴⁰. One type of neuroligin mutation that led to autism in a pair of Swedish twins was found to be R451 substitution in neuroligin 3. This mutation led to a 90% loss of total neuroligin 3 levels and also to a pre-

Table 1. List of major genes involved in cell adhesion and scaffolding associated with autism³⁹.

Gene	Gene product
NL3, NL4	Neuroligin 3, neuroligin 4
NRXN1	Neurexin 1, neurexin 3
SHANK3	SH3 and multiple ankyrin repeat domains 3
CNTN3	Contactin 3
CNTNAP2	Contactin-associated protein 2
CDH9,	Cadherin 9, cadherin 10
CDH10	
L1CAM	L1 cell adhesion molecule
NRCAM	Neuronal cell adhesion molecule
PCDH10	Protocadherin 10

dominant endoplasmic reticulum retention of neuroligin 3 (ref.^{41,42}). In a large French family, a two-base pair deletion (1253delAG) was found that resulted in a premature stop codon in the sequence of the normal neuroligin 4 gene. Two members of the family suffered from autism, other members had non-specific X-linked mental retardation or pervasive developmental disorder⁴³. Rare copy number variations and/or point mutations in the neurexin gene have been reported in the context of autism pathology. Deletion of the neurexin 1 α promoter and exons 1-5 in a boy with autistic characteristics has been described in one study⁴⁴. In another study, a deletion that eliminated several neurexin 1 exons, including 1 α and 1 β , in two female siblings was found in one ASD family⁴⁵. Deletions of neurexin 3 were found in four ASD individuals. One was a *de novo* mutation, another one was inherited from a non-affected parents and the third was inherited from a father with subclinical autism⁴⁶. Rare non-synonymous variants of mutation of CNTNAP2, a member of the neurexin family⁴⁷, have been found in ASD patients. Several other studies have also found other polymorphisms of CNTNAP2 associated with ASD (ref.⁴⁸⁻⁵⁰).

Shank3 was the first gene from the Shank family reported to be associated with ASD. The Shank3 gene is located on chromosome 22q13.3. Three families with ASD were observed to carry alterations of 22q and/or the Shank3 gene⁵¹. In the first family, an individual carried a *de novo* deletion of 22q13. In the second family, two affected siblings carried also a *de novo* deletion, but they were heterozygous for an insertion of a guanine nucleotide in exon 21. In the last family, a terminal 22q deletion was identified in a girl affected autism who exhibited language delay. *De novo* deleterious mutations of Shank2 in ASD have also been identified⁵²⁻⁵⁴. Shank1 gene rare mutation has also been associated with ASD (ref.⁵⁵).

Genetic mouse models with respect to alterations in cell adhesion molecules and scaffolding proteins

Neurobiological studies using animal models are important for defining the core characteristics of ASD. Mutations in neurexins, neuroligins and Shank genes might have impact on the development of complex behaviors. Neuroligin 1 mutant mice targeting the first two

coding exons displayed a mild ASD-related phenotype⁵⁶. They showed increased repetitive, stereotyped grooming behavior. The mice displayed impaired spatial learning and memory in a Morris water maze test. Interestingly, Neuroligin 1 mutant mice displayed few deficits in social behavior. In only one of several tasks, Neuroligin 1 knockout (KO) mice interacted less with a caged adult target. In other social tasks, these mice showed normal interaction and approach to social targets and normal social recognition. They also showed normal anxiety-like behavior, locomotor activity, motor coordination/learning, auditory startle responses, and sensitivity to sensory stimuli. Neuroligin 1 KO mice showed altered sensitivity to pain and heat stimuli but no change in the perception of auditory stimuli⁵⁷.

Neuroligin 2 mutant mice targeting the first coding exon displayed abnormal communication with decreased number of pup ultrasonic vocalizations. These mice displayed marked increase in anxiety-like behavior, a decrease in pain sensitivity, exploration and a slight decrease in motor coordination. The mutant mice displayed delays in several developmental milestones (growth parameters, eye opening, teeth eruption and the acquisition of several reflexes). In contrast, social interactions, and social behavior appeared normal. Acoustic, tactile, olfactory sensory information processing and sensorimotor gating (the state-dependent regulation of transmission of sensory information to the motor system) were not affected^{58,59}.

Neuroligin 3 mutant mice, targeting exons 2-3 displayed behavioral phenotype reminiscent of the lead symptoms of ASD (ref.⁵⁶). These mice displayed reduced ultrasonic vocalization and a lack of social novelty preference. These observations might be related to the olfactory deficiency observed in the Neuroligin 3 mutants. The mice displayed enhanced motor activity in the open field, however anxiety-like behavior evaluated in the elevated plus maze test appeared normal. They also showed normal motor performance on the rotarod, and no gross performance deficits in a Morris water maze test. These mice displayed normal duration of time spent in social interaction, prepulse inhibition of the startle response (to access sensorimotor gating) and sucrose preference⁶⁰.

Neuroligin 3 R451C knockin (KI) mice were also generated for illustrating the association of neuroligin 3 and ASD (ref.⁶¹). This mutation substitutes cysteine for arginine at residue 451 in Neuroligin 3, a gene located on the X-chromosome. This results in neuroligin 3 retention in the endoplasmic reticulum and a decreased delivery of the protein to the cell surface^{62,63}. Neuroligin 3 KI mice show impaired sociability and enhanced spatial learning and memory in a Morris water maze test^{42,64}. In the study of Chadman et al.⁶², juvenile reciprocal social interaction, adult social approach and cognitive abilities were normal in these mice but it was also found that these mice showed from minor developmental differences including slightly different rates of somatic growth, slower righting reflexes, faster homing reflexes in females and more vocalizations. Longer latencies to fall from the rotarod and less vertical activity in the open field in Neuroligin 3 KI mice were observed⁶².

Neuroligin 4 mutant mice also display ASD-related behaviors. Deficits in social interactions and ultrasonic communications were observed in these mice⁶⁵. Further, aggression, nest-building parameters, as well as self-grooming and circling as indicators of repetitive stereotypes were explored in these mutant mice⁶⁶. However, in another study, the authors failed to confirm the social or communication deficits. In the same study, anxiety-like behaviors, self-grooming, motor coordination and open field exploration did not differ across the genotypes. Measures of developmental milestones also remained unchanged⁶⁷.

Neurexin 1 α mutant mice represent mice lacking neurexin 1 α -encoded isoforms targeting the first coding exon yields. These mice display a mild ASD-related phenotype. The authors of the study Etherton et al.⁶⁸ observed a decrease in prepulse inhibition, an increase in grooming behaviors, impaired nest-building activity, and enhanced motor learning in these mice⁶⁸. In the same study, neurexin 1 α deficient mice did not exhibit any obvious changes in social behaviors, anxiety-like behaviors, locomotor activity or spatial learning. However, the authors Grayton et al.⁶⁹ observed an altered social approach, reduced social investigation and reduced locomotor activity in a novel environment⁶⁹. Males displayed increased aggressive behavior as well.

Neurexin 2 KO mice are generated by targeting exon 23, which is shared by Nrnx2 α and Nrnx2 β (ref.⁷⁰). These mice display behavioral abnormalities, characterized by deficits in social interaction and anxiety-like behavior⁷¹. Dachtler et al.⁷² reported also deficits in social memory, but prepulse inhibition and passive avoidance learning were normal.

Genetic mouse models of different Shank protein mutations were produced to investigate their contribution to autistic pathology. Shank1 mutant mice with a deletion of exons 14 and 15 resulted in the knockout of all detectable Shank1 proteins in these animals²². Shank1 mutant mice showed decreased movement in the open field test, anxiety-like behavior measured by a light/dark test and open field test. They showed a deficit in motor learning measured by rotarod, also a deficit in contextual fear conditioning and communicative behaviors by ultrasonic vocalizations. Interestingly, spatial learning and memory were enhanced⁷³⁻⁷⁵.

Two different lines of Shank2 mutant mice were generated. Won et al.⁷⁶ described exons 6-7 deletion mice and Schmeisser et al.⁷⁷ reported on exon 7 deletion mutant mice. Mutant mice with exons 6-7 deletion exhibited ASD-like behaviors including reduced social interaction, reduced social communication by ultrasonic vocalizations, and repetitive jumping. These mice displayed impaired spatial learning and memory in a Morris water maze test, but novel object recognition memory was normal. Impaired nesting behavior, hyperactivity in the open field test and anxiety-like behavior in an elevated plus maze were also reported⁷⁶. Animals with mutation targeting exon 7 also showed profound autistic-like behavioral alterations including repetitive grooming and abnormalities in vocal and social behaviors. Moreover, these mice showed anxiety-like behavior in the light/dark test, hyper-

activity in the open field test, but normal learning and memory in novel object recognition test⁷⁷.

Different lines of Shank3 mutant mice carrying different mutations in Shank3 gene have been reported. Mutant mice with exons 4-9 deletion (JAX 017890) displayed a mild ASD-related phenotype. These mice showed normal

sociability and social novelty, impairment in juvenile or male-female pair interactions, normal pup ultrasonic vocalizations with decreased adult ultrasonic vocalizations, an increase in self-grooming and inflexibility in the Morris water maze test. These mice showed slight hypoactivity in the open field test, but no enhanced anxiety-like behavior

Table 2. Behavioral changes resulting from alterations in cell adhesion molecules and scaffolding proteins in mouse autism model.

Alteration	Model	Behavioral test	Result	Ref.
↓ NL1	NL1 (-/-)	Social interaction Repetitive grooming Morris water maze Test of footshock Test of hotplate sensitivity	↓ social interaction ↑ stereotyped behavior ↓ spatial learning and memory ↓ sensitive to footshock ↑ sensitive to heat	57
↓ NL2	NL2 (-/-)	Open field Elevated plus maze test Light/dark test Social approach task Ultrasonic vocalization Rotarod Test of footshock Test of hotplate sensitivity	↓ motor activity ↓ vertical activity ↑ anxiety ↓ motor activity ↑ anxiety ↓ motor activity ↓ vocalization ↓ motor coordination ↓ rotarod learning ↓ pain sensitivity ↓ pain sensitivity	58,59
↓ NL3	NL3 (-/-)	Open field Elevated plus maze test Social novelty preference Buried food-finding test Ultrasonic vocalization Hole board test Fear conditioning test	↑ motor activity ↑ motor activity ↓ social novelty preference ↓ olfaction ↓ vocalization ↑ explored holes ↓ freezing response	60
↑ NL3	NL3 R451C knockin	Open field Elevated plus maze test Social interaction 3-chamber sociability Ultrasonic vocalization Rotarod Morris water maze Acoustic startle threshold	↑ motor activity ↓ vertical activity ↓ anxiety ↑ motor activity ↓ social interaction ↑ motor activity ↑ vocalization ↑ latencies to fall ↑ spatial learning and memory ↓ startle response	42, 62, 64
↓ NL4	NL4 (-/-)	Social interaction Resident-intruder test Ultrasonic vocalization Nest building Marble burying	↓ social interaction ↓ social aggression ↓ vocalization ↓ nest building ↑ stereotyped behavior	65, 66
↓ NRXN-1α	NRXN-1α (-/-)	Home cage task Light/dark test Social approach test Social investigation task Social novelty preference Nest Building Rotarod Repetitive Grooming Prepulse Inhibition Acoustic startle reactivity	↓ motor activity ↓ motor activity ↑ anxiety ↓ motor activity ↑ degree of social approach ↓ social investigation ↑ aggression ↑ social novelty preference ↓ nest building ↑ motor learning ↑ stereotyped behavior ↓ prepulse inhibition ↑ startle response	68, 69

Alteration	Model	Behavioral test	Result	Ref.
↓ NRXN-2α	NRXN-2α (-/-)	Home cage task Open field Elevated plus maze Light/dark test Emergence test Social investigation task Social novelty preference 3-chamber sociability Nest building Repetitive grooming Novel object exploration	↓ motor activity ↓ motor activity ↑ anxiety ↑ anxiety ↓ motor activity ↑ anxiety ↑ anxiety ↓ social investigation ↓ social memory ↓ sociability ↓ motor activity ↓ nest building ↑ stereotyped behavior ↓ novel object exploration	71, 72
↓ Shank1	Shank1 (-/-)	Open field Light/dark test Ultrasonic vocalization Scent marking Rotarod Eight-arm radial maze Fear conditioning test	↓ motor activity ↑ anxiety ↓ motor activity ↑ anxiety ↓ vocalization ↓ levels of scent marks ↓ motor coordination ↑ spatial learning ↓ long-term retention of information ↓ freezing response	73-75
↓ Shank2	Shank2 (-/-)	Open field Elevated plus maze Light/dark test Social interaction 3-chamber sociability Ultrasonic vocalization Nest building Repetitive grooming Repetitive jumping Morris water maze Novel object recognition	↑ motor activity ↑ anxiety ↑ anxiety ↓ social interaction ↑ latency to first contact ↓ conspecific recognition ↓ interest for social novelty ↓ vocalization ↓ nest building ↑ stereotyped behavior ↑ stereotyped behavior ↓ spatial learning and memory ↑ grooming	76, 77
↓, ↓ Shank3	Shank3 (-/-) Shank3 (+/-)	Novel home cage Open field Light/dark test Zero maze test Social interaction Social novelty preference Dyadic test Ultrasonic vocalizations Nest building Rotarod Repetitive grooming Hole-board test Marble burying Morris water maze Novel object recognition Test of hotplate sensitivity Foot-misplacement test Tests of vertical placement Delayed climbing down	↓ motor activity ↓ motor activity ↑ grooming ↓ rearing activity ↑ anxiety ↑ anxiety ↓ social interaction ↓ social novelty preference ↓ social interaction ↑ grooming ↑ sifting through bedding materials ↑ latency to first interaction ↓ / ↑ vocalization ↑ avoidance of inanimate objects ↓ motor coordination ↓ motor learning ↑ stereotyped behavior ↑ stereotyped behavior ↑ avoidance of inanimate objects ↓ spatial learning and memory ↓ preference for the novel object ↑ sensitivity to heat ↑ foot-faults ↑ motor abnormalities ↑ motor abnormalities	78-82

↑ (increased), ↓ (decreased), -/- (homozygote knockout), +/- (heterozygote knockout), NL (neurexin), NRXN (neurexin)

in the elevated plus maze or light/dark test. Impaired motor performance on the rotarod, impaired learning and memory in novel object recognition were also observed, however, acquisition of the Morris water maze test and fear conditioning were found to be normal^{78,79}.

Mutant mice with deletion of exons 4-9 (JAX 017442) were described by Wang and colleagues (2011) (ref.⁸⁰). These animals had impaired sociability, social novelty, social interaction in freely interacting pairs, abnormal ultrasonic vocalization, and increased self-grooming and stereotypic investigation of novel objects. Impaired motor performance on the rotarod and impaired learning and memory in the Morris water maze test as well as in novel object recognition test were also observed. However, their anxiety and locomotor activity were at normal levels.

Peça et al.⁸¹ generated mouse line targeting exons 4-7 and another targeting exon 13-16. Because of the more profound phenotypes in the latter, the authors of this study focused attention and further study on exon 13-16 of the mutant mice. These mice displayed alterations in social behavior including impaired sociability and preference for social novelty, as well as decreased pair interaction, and profound self-grooming. They also showed increased anxiety in the elevated zero maze and light/dark test, normal activity in the open field test, and normal motor performance on the rotarod. In the Morris water maze test, mice displayed normal acquisition and flexibility in reversal learning.

Kouser et al.⁸² targeted exon 21 of Shank3, reporting some ASD-related behaviors. These mice exhibited impaired social novelty in the three chamber test but normal social interaction or social learning during reciprocal social interaction with a juvenile mouse. Mice also showed impaired nest building, normal adult ultrasonic vocalizations, and increased self-grooming. In the Morris water maze test, impaired learning and memory was demonstrated. Anxiety-like behavior was manifested in the light/dark test but not in the elevated plus maze test and the open field test. They also reported a decreased activity in mice when initially presented to the open field and impaired motor performance on the rotarod. Table 2 summarizes the key behavioral changes in mouse autism model with respect to alterations in cell adhesion molecules and scaffolding proteins.

CONCLUSIONS

For effective use of animals in brain research it is essential to determine the most appropriate animal model. However, for autism, its heterogeneity makes it difficult to generalize the information acquired from any single animal model or test. In this review, we aimed to show the importance of changes in synapses or synaptogenesis which may be involved in the pathogenesis of ASD. We describes animal genetic models of ASD with respect to alterations in cell adhesion molecules and scaffolding proteins. We also showed synapse-specific or model-specific defects associated with a given genotype in these models. Characterization of mouse models carrying genetic

variations could help us study the mechanisms of autism development in humans more efficiently. However, it will be necessary to apply new analytic paradigms in using genetically modified mice for understanding autism etiology in humans. Further studies are needed to create animal models with mutations that match the molecular and neural bases of autism.

Acknowledgement: The work was supported by the project 2/0119/15 of the Grant Agency of the Ministry of Education, Science and Research and the Slovak Academy of Sciences (VEGA), and by the projects APVV-0253-10 and APVV-0254-11 of the Slovak Research and Development Agency.

Author contributions: MZ: literature search, manuscript writing; JB: concept, manuscript writing; JH: tables, text corrections; DO: proofreading, text corrections.

Conflict of interest statement: The authors state there are no conflicts of interest regarding the publication of this article.

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