CHAPTER NINE

Microglia in animal models of autism spectrum disorders

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Abstract

Various genetic and environmental factors have been suggested to cause autism spectrum disorders (ASDs). A variety of animal models of ASDs have been developed and used to investigate the mechanisms underlying the pathogenesis of ASDs. These animal models have contributed to clarifying that abnormalities in neuronal morphology and neurotransmission are responsible for the onset of ASDs. In recent years, researchers have started to focus not only on neurons but also on glial cells, particularly microglia. This is because microglial malfunction is strongly associated with structural and functional abnormalities of neurons, as well as the inflammation that is commonly observed both in the brains of patients with ASDs and in animal models of ASDs. In this chapter, we first introduce a list of commonly available animal models of ASDs and describe the validity of each model from the viewpoint of behaviors and neuroanatomy. We next detail the malfunction of microglia that has been reported in animal models of ASDs and discuss the roles of microglia in ASD pathogenesis. We will further propose possible therapeutic strategies to tackle ASDs by controlling microglial functions.

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1. Introduction

A variety of genetic and environmental factors have been suggested to cause autism spectrum disorders (ASDs).[1] However, studies using human autopsy brains from individuals with a history of ASD, when available, are not adequate to test whether genetic and environmental factors contribute to the development of ASDs. In order to elucidate the mechanisms underlying the pathogenesis of ASDs, it is essential to make use of appropriate animal models.

To date, a number of animal models of ASDs, which have been proposed to mimic the genetic and environmental factors of ASDs, have been developed and used for studying the mechanisms underlying ASD pathogenesis.[2] The surface validity of these ASD models has been evaluated primarily using behavioral tests that assess the main symptoms of ASDs. Tests such as the number of contacts with other mice in a three-chamber test (social interaction), the properties of ultrasonic vocalization (communication) and the length of grooming behavior or persistence in the same environment (repetitive or persistent behavior) are often used as an index.[2] In addition, whether model animals exhibit complications of ASDs, such as epilepsy, anxiety and neuroanatomical changes (decreased cerebellar Purkinje cell number, atrophy of the prefrontal cortex and hippocampus, increased spine density) is also taken into account.[2]

Common features found in the brains of ASD patients and in ASD animal models can be roughly classified into two phenotypes. The first phenotype includes morphological and functional abnormalities of synapses, which affects neural circuits, possibly triggering ASD-related behaviors. The second phenotype is brain inflammation, which is largely observed in the environmental factor-induced ASD models that mimic maternal immune activation (MIA) and infection. The expression levels of various inflammatory cytokines (tumor necrosis factor α [TNFα], interleukin [IL]-6, IL-18, etc.) tend to increase in the environmental factor-induced ASD models. Because of these pathological changes that are characterized by inflammation, the involvement of microglia, the brain-resident immune cells, in the pathogenesis of ASDs has been highlighted and enthusiastically investigated. As immune cells, microglia produce and release various inflammatory mediators such as IL-1β, TNFα, IL-10, TGF-β; additionally, they have phagocytic capabilities.

In the recent field of neuroscience, microglia are particularly recognized for their phagocytic ability. An increasing number of research studies have focused on activated microglia, which phagocytose dead cells and pathogens under inflammatory conditions caused by infection or trauma.[3] Morphologically activated microglia are often found in the autopsied brain tissue of patients who had ASD, and thus inflammation in ASD animal models has been targeted as a way to study the properties of activated microglia.[4] Furthermore, in recent years, it has been suggested that the phagocytic activity of microglia actively contributes not only to inflammation but also to normal brain development.[5] For example, it was shown that microglia participate in the maturation of functional neural circuits by pruning synapses that are less active in order to maintain more active synapses during development.[6] This discovery has led us to propose the hypothesis that a failure in microglial synaptic pruning causes ASDs.[7]

In this chapter, we first introduce the major animal models of ASDs. Second, we introduce the findings of studies that examined the involvement of microglia in ASDs and discuss the role of microglia in the pathogenesis of ASDs. Finally, we discuss the potential of microglial manipulation as a possible therapeutic strategy for ASDs.

2. Animal models of ASDs

2.1 Genetic animal models (Table 1)

Patients with mental development disorders such as Fragile X syndrome and Rett syndrome often manifest autistic behaviors (symptomatic autism). These diseases are attributed to mutations in a single gene; mice in which each gene is mutated have been used as ASD models. In addition, new ASD models that target other genes, such as *Neuroligin* and *Shank*, have been developed.

2.1.1 Fmr1 (fragile X mental retardation 1)

Fmr1, which is found on the X chromosome, is the gene responsible for Fragile X syndrome. Fmr1 protein (FMRP) regulates mRNA trafficking, dendritic maturation, and synaptic plasticity. When FMRP is not synthesized because of an increase in the number of codon repeats in *Fmr1*, normal brain development is affected. *Fmr1* knockout mice exhibit decreased sociality and persistent behavior.[8,9] In addition, neuroanatomical changes such as

Table 1 Genetically-induced animal models of ASDs.

Gene	Related disease; protein function	Rodent model of gene mutation	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Fmr1	Fragile X syndrome; mRNA trafficking, dendritic maturation, synaptic plasticity	*Fmr1* I304N knock in C57BL/6J mice	Persistence, seizure, hypoesthesia, increased anxiety	Enhanced LTD in hippocampal CA1	–	8
		Fmr1 knockout Sprague-Dawley rats	Decreased sociability, persistence	–	–	9
		Fmr1 knockout C57BL/6 mice	Decreased sociability, persistence	Enhanced LTD in hippocampus, increased spine density in hippocampal CA1	–	10
		Fmr1 knockout C57BL/6 mice	–	Immature spine and increased spine density in hippocampal CA1	Decreased PSD95 phagocytosis	11
Mecp2	Rett syndrome; transcription factor	$Mecp2^{308}$ mutation C57BL/6J mice	Decreased sociability, persistence, seizure, increased anxiety, motor dysfunction	–	–	12
		Viaat-$Mecp2^{-/y}$ C57BL/6J mice	Abnormal sociability, persistence, increased anxiety, motor dysfunction, enhanced prepulse inhibition	Inhibitory interneuron dysfunction, impaired LTP	–	13

		Mecp2$^{-/y}$ C57BL/6J mice	Increased anxiety, motor dysfunction	–	Decreased phagocytosis, improved behaviors by microglia replacement	14
		Mecp2$^{-/y}$ C57BL/6J mice	Motor dysfunction	–	No change in behaviors by microglia replacement	15
		Mecp2$^{-/y}$ *Cx3cr1*^{CreER}; *Mecp2*$^{fl/y}$ *Cx3cr1*^{CreER}; *Mecp2*$^{LSL/y}$ C57BL/6J mice	Motor dysfunction	–	Increased phagocytosis of the presynapse in the lateral geniculate nucleus in *Mecp2* full knockout	16
Tsc1/2	Tuberous sclerosis; inhibition of mTOR signaling	*Tsc1* knockout in cerebellum C57Bl/6J, BALB/c mice	Decreased sociability, persistence, motor dysfunction	Decreased density, abnormal morphology and decreased excitability of cerebellum Purkinje cell	–	17
		Tsc2 missense mutation C57BL/6J mice	Decreased sociability, persistence, impaired communication	–	–	18
Shank3	ASDs; scaffold protein at the postsynapse	*Shank3* knockout C57 mice	Decreased sociability, persistence	Abnormal morphology of striatum neuron, striatum hypertrophy, impaired synaptic transmission between cortex and striatum	–	19

Continued

Table 1 Genetically-induced animal models of ASDs.—cont'd

Gene	Related disease; protein function	Rodent model of gene mutation	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Pten	ASDs, cancer; inhibition of mTOR activation	*Pten* knockout in cortical and hippocampal neurons C57/BL6 mice	Decreased sociability, decreased prepulse inhibition, increased anxiety, seizure	Cortical and hippocampal hypertrophy, abnormal morphology of dentate granule cells	–	20
Cntnap2	CDFE syndrome; synaptic adhesion protein at the presynapse	*Cntnap2* knockout C57BL/6J mice	Decreased sociability, impaired communication, persistence, seizure	Impaired migration of cortical neurons, decreased inhibitory interneuron density	–	21
Scn1a	Dravet syndrome; codes for Nav1.1 (α subunit of sodium ion channel)	*Scn1a* hetero knockout C57BL/6J mice	Decreased sociability, persistence	Decreased number and dysfunction of inhibitory interneuron	–	22
15q11–13 (chromosome region)	ASDs, Prader-Willi syndrome, Angelman syndrome; multiple functions	patDp hetero knockout C57BL/6 mice	Decreased sociability, impaired communication, enhanced anxiety	Increased serotonin activity	–	23
		patDp hetero knockout C57BL/6 mice	Impaired communication, enhanced anxiety	–	Decreased Iba1 intensity in basolateral amygdala	24
		Ube3a duplication C57BL/6 mice	Decreased sociability, impaired communication, persistence	Impaired excitatory synaptic transmission	–	25

Nlgn3/4	ASDs, Asperger syndrome; anchor protein for Neurexin and PSD95	*NL3R451C* knock in 129S2 • SvPasCrl mice	Decreased sociability	–	–	26
		NL4 knockout C57BL/6 mice	Decreased sociability, impaired communication	Decreased volume of cerebellum and brain stem	–	27
Nrx1	ASDs, SCZ, epilepsy, Pitt-Hopkins syndrome; synaptic adhesion protein at the presynapse	*Nrx1a* knockout C57BL/6J mice	Decreased sociability	–	–	28
Shank	ASDs; scaffold protein at the postsynapse	*Shank1* knockout C57BL/6J mice	Decreased sociability, persistence	–	–	29
		Shank2 knockout C57BL/6J mice	Decreased sociability, impaired communication, enhanced anxiety	NMDA receptor dysfunction	–	30
Parvalbumin	ASDs, SCZ, bipolar disorder; calcium-binding protein of inhibitory interneurond	*Parvalbumin* knockout C57BL/6J mice	Decreased sociability, impaired communication, persistence, seizure, hypoesthesia	Dysfunction of inhibitory interneuron		31

Continued

Table 1 Genetically-induced animal models of ASDs.—cont'd

Gene	Related disease; protein function	Rodent model of gene mutation	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Cx3cr1	Psychiatric diseases; fractalkine receptor	*Cx3cr1* knockout C57BL/6 mice	Decreased sociability, persistence	Increased mEPSC frequency of hippocampal CA1 pyramidal cells, decreased functional connectivity between mPFC and hippocampus	–	32
BTBR-strain	–	BTBR mice	Decreased sociability, persistence	–		33
			–	Increased levels of IgG antibody, IL-1β, IL-33 and IL-18	Increased MHC II positive microglia	34

Abbreviations: ASD, autism spectrum disorders; CDFE, cortical dysplasia-focal epilepsy; IgG, immunoglobulin G; LTD, long-term depression; mEPSC, miniature excitatory post-synaptic currents; MHC, major histocompatibility; mPFC, medial prefrontal cortex; NMDA, *N*-methyl-D-aspartate; SCZ, schizophrenia.

enhanced long-term depression (LTD) and enhanced phosphorylation of transcriptional regulators in the hippocampus have been reported.[10] However, it has also been also reported that anxiety, a typical complication of ASDs, was reduced in this animal model.

2.1.2 Mecp2 (methyl-CpG binding protein 2)

Mecp2 is the gene responsible for Rett syndrome. Because *Mecp2* is found on the X chromosome and is a gene, coding transcription factor that binds to methylated DNA cytosine, mutations in *Mecp2* can cause abnormal expression of other genes. Heterozygous *Mecp2* deficient female mice (heterozygous male and homozygous are embryonically lethal) exhibit seizures along with decreased social behavior and persistent behavior.[12] Mice in which *Mecp2* is specifically knocked out in inhibitory interneurons exhibited abnormal social behavior and persistent behavior.[13] This study also showed that the activity of inhibitory interneurons was decreased, which resulted in a change in the balance between excitatory (E) and inhibitory (I) synapses, i.e., the synaptic E/I balance was tipped toward excitation in the hippocampus. These results suggest that ASD is caused by a disruption in the synaptic E/I balance. However, it has been reported that anxiety was reduced in the *Mecp2* mutated mice.

2.1.3 Tsc1/2 (tuberous sclerosis proteins1/2)

Tsc1/2 are the causative genes of tuberous sclerosis. Mutations in *Tsc1/2* activate mammalian target of rapamycin complex 1 (mTOR1) and promote cell growth and proliferation. The mutations also cause intellectual disability, seizures, and ASDs-like symptoms. Mice in which *Tsc1* is specifically knocked out in the cerebellum, and mice who have a missense mutation in *Tsc2* exhibit all the main symptoms of ASDs.[17,18] Based on the structural abnormalities of the cerebellum seen in patients with ASDs, the former report strongly suggests that the cerebellum is a brain region involved in the pathogenesis of ASDs.

2.1.4 Shank3 (SH3 and multiple ankyrin repeat domains 3)

Shank3 is located on chromosome 22 in the 22q13 region and is considered to be a risk gene for the development of ASDs. Shank3 is a post-synaptic scaffold protein that regulates synaptic functions and neurotransmissions. *Shank3b* knockout mice showed reduced social behavior and persistent behavior.[19] In addition, the striatum was enlarged and the excitatory synaptic transmission between the cortex and the striatum was impaired.

2.1.5 Pten (phosphatase and tensin homolog deleted from chromosome 10)

Pten is a tumor suppressor gene that is considered to be a risk gene for development of ASDs. PTEN both negatively regulates the PIP3/Akt signaling pathway by dephosphorylating PIP3 and suppresses the activation of the downstream molecule, mTOR. *Pten*-mutated mice exhibit complication behaviors representative of those observed in patients with ASDs, including decreased social behavior, increased anxiety and epileptic seizures.[20] In addition, hypertrophy of the hippocampus and cortex, and abnormal morphologies of dentate granule cells, such as ectopic dendrites and increased spine density, were reported in these mice.

2.1.6 Cntnap2 (contactin-associated protein-like 2)

Cntnap2 is a member of the neurexin superfamily and is considered to be a risk gene for the development of ASDs. Cntnap2 is involved in synaptic junctions and is abundant in the cortical areas important for language development. *Cntnap2*-mutated mice exhibited decreased social behavior, persistent behavior and epileptic seizures.[21] These abnormalities have been suggested to be caused by both the impaired migration of cortical neurons and the decrease of inhibitory interneurons. Findings in these mice also suggest that there is a relationship between the breakdown of the E/I balance and the onset of ASDs.

2.1.7 Scn1a (sodium voltage-gated channel alpha subunit 1)

Scn1a is the causative gene of Dravet syndrome, an epilepsy syndrome in infants. *Scn1a* encodes Nav1.1, an α subunit of the sodium ion channel. *Scn1a* haploinsufficient mice exhibited impaired social behavior and persistent behavior.[22] This study further showed that administration of clonazepam, an antiepileptic drug, enhanced gamma-amino butyric acid (GABA) transmission and improved the social behavior of *Scn1a* haplosufficient mice. These results also indicate that the disruption of the E/I balance is involved in ASD pathogenesis.

2.1.8 15q11–13 duplicate

15q11–13 is the causative gene of two intellectual disorders, Prader-Willi syndrome and Angelman syndrome.[35] The duplication of this chromosomal region is most frequently observed in patients with ASDs.[35] 15q11–13 is the region where genomic imprinting occurs, and the phenotype differs depending on whether the genetic information is derived from the father or the mother.[35] Therefore, the 15q11–13 duplication is studied in mice

using either paternally- or maternally-derived chromosome-duplicated mice. In the paternally-derived chromosome-duplicated mice, decreased social behavior, abnormal communication and increased anxiety were observed, whereas in the maternally-derived chromosome-duplicated mice, no abnormal behavior was reported.[23] 15q11–13 contains *ubiquitin-protein ligase E3A* (*Ube3a*), which is a risk gene for the development of ASDs that is transcribed from a maternal allele. In mice with the triploid *Ube3a*, impaired excitatory synaptic transmission, decreased social behavior and persistent behavior were observed.[25]

2.2 Genetic animal models targeting genes which are not related to symptomatic autism (Table 1)

Genetic analysis of patients with ASDs identified more than 100 risk genes for ASDs. Many of them are related to brain development and function; synapse-related genes are particularly attracting attention.[36] Mutations in synapse-related genes cause abnormalities in the structure of the synaptic junction, resulting in impaired synaptic transmission. Epilepsy, a typical complication of ASDs, is caused by a synaptic E/I imbalance toward higher excitability, suggesting that a disruption of the synaptic E/I balance underlies the mechanism of ASD development.[37] In the following section, we describe abnormalities in synaptic transmissions and behaviors reported in the animal models of ASDs in which genes differing from those that are causative of symptomatic autism are mutated.

2.2.1 Nlgn3/4 (Neuroligin3/4)

Neuroligin is a transmembrane protein present at post-synapses that acts as an anchor between pre-synaptic neurexin and post-synaptic proteins, thus regulating the structure and function of synaptic junctions. Among the five members of the neurogligin family, *Nlgn3* and *Nlgn4* have been identified as risk genes for ASDs. *Nlgn* (R451C) knock-in mice, which were created by mimicking the mutations found in ASD patients, have been reported to exhibit impaired social behavior, while spatial learning was improved and anxiety was reduced.[26] In *Nlgn4* knockout mice, decreased social behavior and impaired communication were reported.[27]

2.2.2 Nrx1 (neurexin1)

Neurexin is a protein that binds to post-synaptic neuroligin as described above. Among *Nrx1–3*, mutations of *Nrx1*, especially the α isoform, are associated with ASDs. *NRX1α* knockout mice showed decreased social behavior.[28]

2.2.3 Shank (SH3 and multiple ankyrin repeat domains protein)

Shank is an excitatory post-synaptic scaffold protein. It binds to the *N*-methyl-D-aspartate (NMDA) receptor (NMDAR) and the metabotropic glutamate receptor (mGluR), and is involved in dendritic spine maturation. In both *Shank1* and *Shank2* knockout mice decreased social behavior and impaired communication were reported.[29,30] In *Shank2* knockout mice, administration of an mGluR5 positive allosteric modulator, which increases the function of NMDAR via mGluR5 activation, improved social behavior.[30] These results indicate that Shank2 knockout possibly decreased NMDAR accumulation to spines, which may contribute to the development of ASDs.

2.2.4 Parvalbumin

Functional deficits of inhibitory interneurons can cause the disruption of the synaptic E/I balance by inducing hyperexcitability of neural circuits. Parvalbumin is a calcium-binding protein which is expressed by some inhibitory interneurons. In mice with a heterozygous or homozygous parvalbumin deficiency, decreased sociality, persistent behavior and impaired communication have been reported.[31] It has also been suggested that these behavioral abnormalities were caused by functional impairment of inhibitory interneurons.

As described above, various genetic models have been established and used to elucidate the pathogenesis of ASDs. However, considering that only about 2% of patients with ASDs have mutations in these genes, they may affect the onset of ASDs by interacting with each other or with environmental factors.[38]

2.3 Animal models created by environmental manipulations (Table 2)

2.3.1 Valproic acid administration

Valproic acid (VPA) is a widely used antiepileptic drug that is also used to treat bipolar disorder, migraines and neuropathic pain. However, the inhibition of histone deacetylation by VPA may have teratogenic effects such as neural tube defects, cardiovascular malformations and delayed neurodevelopment. Prospective and retrospective studies have shown that exposure of pregnant mothers to VPA, especially during the first 3 months of pregnancy, may cause cognitive decline and increase risk of ASDs in their children. Based on these findings, attempts have been made to produce animal models of ASDs by exposing them to VPA. In rodents, offspring exposed to VPA during pregnancy exhibited decreased social behavior

and persistent behavior.[39,40] The VPA model mimics not only the behavioral phenotypes but also the neuroanatomical changes that are observed in patients with ASDs, such as a reduced volume of the cerebellum, frontal cortex and hippocampus, as well as abnormal levels of serotonin in the brain.[41–43]

In general, VPA exposure is conducted at embryonic days 8–15 (E8–15), when the fetal tissue formation takes place; the risk of ASD development by VPA exposure is highest around E12. The dose of VPA used in animal models of ASDs is generally 600 mg/kg. However, it should be noted that clinical VPA doses range from approximately 3 to 55 mg/kg (plasma concentration 100 μg/mL), meaning the dose used in animal models is 10–20 times higher.[2] Few studies have mentioned the issue of different VPA doses between these animal models of ASDs and humans. The main argument to justify the differing doses would be that the pharmacokinetics of VPA is different between species: high bioavailability in humans and rapid clearance in rodents. Additionally, rodents receive a single dose whereas humans generally receive multiple doses. Some studies have exposed rodents to VPA postnatally; one such rat model showed impaired social behavior and motor ability after VPA exposure.[44]

2.3.2 Maternal immune activation

Epidemiological studies have shown that fever and severe infection during pregnancy increase the risk of ASDs in offspring. This is likely related to the immune response of mother; MIA has been shown to greatly affect central nervous system development in offspring.[59] Many MIA models have focused on the process of the inflammatory response rather than the effects of specific pathogens. In this article, we outline the lipopolysaccharide (LPS) model that mimics bacterial infection and the polyinosinic-polycytidylic acid (poly[I:C]) model that mimics viral infection.

2.3.2.1 LPS administration

LPS is a component of the outer membrane of the cell wall of Gram-negative bacteria. LPS elicits an immune response by activating toll-like receptor 4 (TLR4). It has been shown that in rodents, LPS exposure during pregnancy induces decreased social behavior and impaired communication in the offspring.[45,46]

2.3.2.2 Poly(I:C) administration

Poly(I:C) is a double-stranded RNA that elicits an immune response by activating toll-like receptor 3 (TLR3). When poly(I:C) is intraperitoneally administered to pregnant mice and rats, the offspring showed the main

symptoms of ASDs including decreased social behavior, impaired communication and persistent behavior.[48,49] In addition, behavioral abnormalities related to ASDs such as increased anxiety, learning disability, paresthesia and seizures have been observed in the poly(I:C) model. The poly(I:C) model mimics not only the behaviors observed in ASDs but also the neuroanatomical changes such as disordered cerebellar Purkinje cell location and decreased expression of inhibitory interneuron-related proteins.[50,51]

The MIA model is also used to study other psychiatric diseases such as schizophrenia; it has been controversial as to whether it is appropriate to consider it an "ASD model." Indeed, infection during pregnancy may be associated with various central nervous system disorders. However, it would be pointless to argue whether the MIA model mimics a specific disease. Rather, we should not narrow the usefulness of the MIA model and remember that it is important to study multiple aspects of behavioral changes in each disease by properly choosing animal models.

3. Animal models of ASD exhibiting possible microglia involvement

In the following section, we introduce studies that used animal models to examine the involvement of microglia in ASDs, specifically focusing on abnormalities in inflammatory responses and synaptic morphology and function.

3.1 Genetic animal models (Table 1)

3.1.1 Fmr1 knockout

Jawaid et al. conducted a study that focused on synaptic and microglial changes in *Fmr1* knockout mice.[11] At postnatal day 60 (P60), the spine density of CA1 pyramidal cells increased in *Fmr1* knockout mice compared to the control. They observed the spine morphology in these mice and found that mature spines decreased and immature spines increased, which suggests that spine maturation was impaired. The authors attributed the increased spine density in *Fmr1* knockout mice to a failure in microglial synaptic pruning. They measured postsynaptic density protein 95 (PSD95) phagocytosis by microglia in the hippocampus at P21, finding that microglial phagocytosis of PSD95 was significantly decreased in *Fmr1* knockout mice. Thus, it is possible that pyramidal cells in *Fmr1* knockout mice have abnormalities in synaptic plasticity and axonal terminal maturation, which may inhibit neuronal activity-dependent synaptic pruning by microglia.

3.1.2 Cx3cr1 knockout

CX3CR1 is a fractalkine receptor which is expressed specifically by microglia in the brain parenchyma. CX3CR1 signaling, which is activated by its ligand CX3CL1, regulates adult neurogenesis in the hippocampus, a phenomenon related to several psychiatric disease. Zhan et al. found that *Cx3cr1* knockout mice exhibited decreased social behavior in the three-chamber test and persistent behavior (grooming).[32] They recorded miniature excitatory post-synaptic currents (mEPSCs) from CA1 pyramidal cells in hippocampal acute slices at P15 and P40. While the frequency of mEPSCs decreased from P15 to P40 in wild-type mice, this decrease was not observed in *Cx3cr1* knockout mice. These results suggest that synaptic pruning by microglia was impaired in *Cx3cr1* knockout mice. Using local field potential recordings and functional magnetic resonance imaging analysis from multiple brain regions, they also found that *Cx3cr1* knockout mice had reduced functional connectivity between the frontal cortex and the hippocampus, as well as in the connection between the left and right hippocampus. Furthermore, it was shown that there was a positive correlation between the strength of the frontal cortex–hippocampus functional connectivity and the duration of social interaction. These findings suggest that these brain regions are important for social behavior.

3.1.3 BTBR mice

BTBR is a mouse strain which exhibits ASD-like behaviors such as reduced social behavior in the three-chamber test and persistent behavior in the water maze task.[33] Heo et al. compared BTBR mice with other mouse strains to test whether the inflammatory response is abnormal in BTBR mice.[34] They found that the levels of serum immunoglobin G (IgG) and IgE and the levels of IgG in the brain were increased in BTBR mice compared to B6 mice. In addition, they found that BTBR mice showed an increase in the expression levels of IL-33, IL-18 and IL-1β as well as an increase in the number of major histocompatibility (MHC) II positive microglia, which indicates that both microglial activation and inflammatory response were promoted in BTBR mice.

3.1.4 patDp/+ mice

In male mice having paternal duplication (patDp/+) of the mouse chromosome region corresponding to human chromosome 15q11–13, abnormal communication (increased ultrasonic vocalization) and increased anxiety in the open field test have been observed.[24] In this study, microglial

properties were examined in the amygdala, a brain region that regulates social behavior and anxiety. In the basolateral amygdala, the number of microglia did not change, but the expression levels of Iba1, a calcium-binding protein whose expression is increased in activated microglia, were decreased. Furthermore, administration of minocycline, which is widely used to inhibit microglial activation, from E17 to P21 did not alter the communication ability, but improved anxiety behavior in patDp/+ mice.

3.1.5 *Mecp2*

Derecki et al. tested the involvement of microglia in abnormal behaviors of male *Mecp2*$^{-/y}$ mice.[14] As mentioned in Section 2.1.2, increased social behavior, persistent behavior, increased anxiety and impaired motor ability have been confirmed in this mouse. Microglia were removed from the *Mecp2*$^{-/y}$ mice at P28 by γ-irradiation, followed by intravenous transplantation of bone marrow-derived wild-type microglia. The transplant improved dyskinesia and anxiety, and prolonged the lifetime of *Mecp2*$^{-/y}$ mice. The ex vivo capability for phagocytosis of dead cells by microglia isolated from the cortex of *Mecp2*$^{-/y}$ mice was reduced compared with wild-type. These results indicate that reduced phagocytosis by microglia in *Mecp2*$^{-/y}$ mice may lead to the development of Rett syndrome. On the contrary, another study reported that wild-type microglial transplantation neither prolonged the lifespan nor improved motor function in *Mecp2*$^{-/y}$ mice.[15]

Schafer et al. focused on neural circuit regulation by microglia in *Mecp2*$^{-/y}$ mice.[16] They found that in the lateral geniculate nucleus, phagocytosis of pre-synapses by microglia was significantly increased in *Mecp2*$^{-/y}$ mice at P56 when brain development was finished. However, when Mecp2 was gained or lost in a microglia-specific manner using *CX3CR1*^{CreER} mice, the abnormalities in synaptic phagocytosis and the decreased motor function in *Mecp2*$^{-/y}$ mice were not changed. These results suggest that the changes in microglial function caused by *Mecp2* knockout are not a direct cause of Rett syndrome.

3.2 Animal models using environmental manipulations to induce disease (Table 2)

Many studies have analyzed microglial morphology and function using animal models of ASDs that mimic MIA during pregnancy (for details and their references, please see Table 2). In the following, we introduce studies that verify ASD-like behaviors and microglial changes. Although not specifically

Table 2 Reagent-induced animal models of ASDs.

Reagent	Animal	Administration conditions (timing, dose, route)	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Valproic acid	C57BL/6Hsd (B6) mice	E13 600 mg/kg subcutaneous administration	Decreased sociability, impaired communication, persistence, decreased prepulse inhibition	Decreased power of gamma-wave	–	39
	Wistar rats	E12 600 mg/kg intraperitoneal administration	Decreased sociability, persistence, decreased prepulse inhibition, hypoesthesia, hyperesthesia	–	–	40
	Long Evans rats	E12 600 mg/kg intraperitoneal administration	–	Decreased cell number in cerebellum, decreased volume of cerebellum	–	41
	Sprague-Dawley rats	E12 500 mg/kg intraperitoneal administration	–	Decreased prefrontal cortex volume, hippocampal CA1, basolateral amygdala	–	42
	Wistar rats	E9 600 mg/kg subcutaneous administration	Decreased sociability	Decreased serotonin level in the hippocampus	–	43
	Long Evans rats	P6–12 150 mg/kg intraperitoneal administration	Decreased prepulse inhibition, hypoesthesia, motor dysfunction	–	–	44

Continued

Table 2 Reagent-induced animal models of ASDs.—cont'd

Reagent	Animal	Administration conditions (timing, dose, route)	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
LPS	CD1 mice	E9 0.008 mg/kg intraperitoneal administration	Decreased sociability, impaired communication, persistence, enhanced anxiety	Increased volume of the cortex and subventricular zone, increased ROS level in the subventricular zone	Increased microglial number in the frontal cortex	45
	Sprague-Dawley rats	E15 0.25 mg/kg intraperitoneal administration	Decreased sociability	Increased levels of TNFα, IL-6, and IL-1β		46
	C57BL/6 mice	E15 100 μg/kg intraperitoneal administration	Decreased sociability, impaired communication, persistence	Increased spine density of hippocampal granule cells	Decreased level of CX3CR1 mRNA	47
Poly(I:C)	C57BL/6J mice	E10, E12, E14 5 mg/kg intraperitoneal administration	Decreased sociability, impaired communication, persistence	Abnormal cortical formation		48
	C57BL/6 mice	E12 20 mg/kg intraperitoneal administration	Decreased sociability, impaired communication, persistence	Increased levels of TNFα, IL-6, IL-1β, and IL-17		49

	C57BL/6J mice	E12 20 mg/kg intraperitoneal administration	–	Decreased Purkinje cell density in the cerebellum	–	50
	C57BL/6N mice	E9 1 mg/kg intravenous administration	–	Decreased PV interneuron number in the dentate gyrus, decreased expression level of reelin in hippocampal CA1/3	–	51
	C57BL/6 mice	E15 5 mg/kg intraperitoneal administration	Decreased sociability	Increased level of IL-6 in the hippocampus	Increased Iba1 intensity in hippocampus, decreased gene expression related phagocytosis, decreased phagocytic capacity, improved behaviors by minocycline	52
	C57BL/6J mice	E12, E17 3 mg/kg, 5 mg/kg intraperitoneal administration	Decreased sociability, persistence, enhanced anxiety	Increased synaptic density in hippocampal CA3	Decreased synaptic phagocytosis in hippocampal CA3	7
IL-6	C57BL/6 mice	E12–P0 5 μg intraperitoneal administration	Decreased sociability, enhanced anxiety	Impaired migration of GABA expressing interneurons	Abnormal morphology of cortical plate	53

Continued

Table 2 Reagent-induced animal models of ASDs.—cont'd

Reagent	Animal	Administration conditions (timing, dose, route)	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Diesel exhaust particle	C57BL/6 mice	E2, E5, E8, E12, E16 50 μg oropharyngeal aspiration	–	–	Abnormal morphology of hippocampal microglia, increased interaction between microglia and neuron in parietal cortex	54
Diesel exhaust particle + maternal stress	C57BL/6 mice	Diesel exhaust particle: E2-E17 (6 times in total) 50 μg oropharyngeal aspiration maternal stress: E12–E17	Impaired cognitive function and memory, enhanced anxiety	Increased level of IL-1β, decreased level of IL-10	–	55
			Decreased sociability, impaired communication, enhanced anxiety, impaired cognitive function and memory	Increased IL-1β/IL-10 ratio, increased expression of TLR4 and caspase1	Abnormal morphology of cortical and hippocampal microglia	56
Ovalubumin	C57BL/6J mice	Before mating 10 μg intraperitoneal administration E9, E12, E17 aerosol	–	–	Changed microglial gene expression pattern	57
			Decreased sociability, persistence	–	–	58

Abbreviations: E, embryonic day; GABA, gamma-amino butyric acid; IL, interleukin; LPS, lipopolysaccharide; P, postnatal day; poly(I:C), polyinosinic-polycytidylic acid; ROS, reactive oxygen species; TLR4, toll-like receptor 4.

mentioned here, it should be noted that researchers have adopted a variety of drugs, dosages, administration routes and administration timings for use in these models, and that there are studies conducted using MIA animal models that have reported no microglial changes.

3.2.1 IL-6 administration

Smith et al. exposed groups of pregnant mice to either restraint stress or IL-6 administration from E12 to E19 and examined the properties of microglia in embryonic and adult offspring.[53] Although the number of microglia in the cortical plate did not change, the number of multivacuolated microglia increased in the offspring of pregnant mice exposed to restraint stress compared to the offspring of untreated mice. This morphological abnormality was also observed in the offspring of pregnant mice exposed to IL-6 administration; the increase in multivacuolated microglia was suppressed when and IL-6 antibody was administered simultaneously with exposure to restraint stress. Further, in the adult offspring, microglia became highly ramified when exposure to restraint stress or IL-6 administration occurred during pregnancy. These results indicate that IL-6 affects microglial properties.

Offspring from pregnant mice subjected to restraint stress showed increased anxiety in the elevated plus maze and decreased social behavior in the three-chamber test; however, these behavioral changes were not improved by IL-6 antibody administration. They also examined the migration of GABAergic neurons to the cortical plate and showed that both restraint stress and IL-6 administration inhibited this migration, a phenomenon which was not improved by IL-6 antibody administration. These results suggest that the relationship between restraint stress-induced ASD-like behaviors and morphological changes in microglia remains questionable.

3.2.2 LPS administration

Le Belle et al. administered LPS intraperitoneally (0.008 mg/kg) to CD-1 mice at E9 to induce MIA.[45] Offspring showed impaired communication (decreased duration of ultrasonic vocalization), decreased social behavior in the three-chamber test, increased anxiety in the elevated plus maze and increased persistent behavior (grooming). At P0, increased thickness of the cerebral cortex, increased number of microglia in the forebrain and increased proliferation of progenitor cells in the subventricular zone (SVZ) were observed. The authors also showed that the production of reactive oxygen species in the SVZ was increased in the LPS group at P3.

Further, simultaneous administration of LPS and apocynin, a nitrogen oxide (NOX) inhibitor, suppressed these structural changes and improved persistent behavior. However, NOX is expressed not only in microglia but also in neurons and astrocytes. Thus, it is unclear whether the LPS-induced neurological changes are a microglia-dependent phenomenon.

Another researcher induced MIA by administering LPS intraperitoneally (100 μg/kg) to pregnant C57BL/6 mice at E15.[47] In this protocol, offspring exhibited impaired communication (reduced duration of ultrasonic vocalization), decreased social behavior in the three-chamber test and persistent behavior in the marble burying test. The authors hypothesized that MIA caused synaptic abnormalities and investigated the morphology of hippocampal granule and pyramidal cells using Golgi staining. They found that the spine density of the granule cells in male mice was significantly increased after MIA. Next, they examined the mRNA expression levels of molecules involved in synaptic pruning. They found that the *Cx3cr1* mRNA level was decreased in male offspring from the LPS treated group. These results indicate that environmental factors can affect microglia-dependent synaptic pruning.

3.2.3 Poly(I:C) administration

Mattei et al. administered poly(I:C) intraperitoneally (5 mg/kg) to pregnant C57BL/6 mice at E15 to induce MIA.[52] Offspring exhibited decreased social behavior in the three-chamber test at P120. Administration of minocycline to MIA offspring from P70–P80 for 5 weeks improved social behavior. In the hippocampus of the MIA offspring, the intensity of Iba1 staining was increased (fluorescent immunostaining) and the IL-6 concentration was elevated (ELISA), suggesting microglial activation in response to MIA. Additionally, transcriptome analysis of hippocampal microglia revealed that MIA decreased the expression levels of phagocytosis-related genes such as *Cx3cr1*, *Itgam* and *Mertk*, among others. These changes were reversed by minocycline administration. Furthermore, hippocampal microglia isolated from the offspring of the MIA group showed a reduced capacity to phagocytose latex beads compared to the control group; this activity was also rescued by minocycline administration.

In our study, pregnant C57BL/6J mice were administered poly(I:C) at E12.5 and E17.5 (3 mg/kg on E12.5 and 1.5 mg/kg on E17.5) to induce MIA.[7] MIA offspring showed decreased social behavior in the three-chamber test, persistent behavior (grooming) and increased anxiety in the novelty suppressed feeding test at P60. We found no changes in microglial

morphology or density in the postnatal offspring. However, microglial phagocytosis of synapses in the hippocampal CA3 field was reduced at P18 in the offspring of the MIA group, resulting in surplus synaptic density. These results indicate that administration of poly(I:C) to pregnant mice suppresses the phagocytic activity of microglia and inhibits synaptic pruning in offspring, possibly inducing ASD-like behaviors.

3.2.4 Air pollution

To mimic exposure to air pollution, Bilbo and colleagues delivered diesel exhaust particles (DEP) to pregnant C57BL/6 mice by oropharyngeal aspiration at E2, E5, E8, E12 and E16.[54] In the hippocampal CA1 at E18, the number of immature microglia (round and stout morphology) was increased in offspring from pregnant mice exposed to DEP. This change was not observed in *Tlr4* KO mice, which suggests that DEP affects the brain structure via TLR4. At P30, the volume of microglia increased in the parietal cortex and the degree of contact between the cell bodies of microglia and neurons increased. These results suggest that microglia were activated by DEP, but it remains unclear how microglial functions were affected.

The authors have previously shown that DEP alone did not affect learning ability in the fear conditioning test or anxiety behavior assessed by elevated zero-maze in offspring at P60.[55] Next, they combined DEP with maternal stress (MS) by limiting bedding during pregnancy. These manipulations attenuated learning ability and increased anxiety in the offspring. In addition, abnormal communication (increased number of calls of ultrasonic vocalization) and decreased social behavior in the three-chamber social exploration test were confirmed in the offspring of the DEP + MS group.[56] The authors noted that the use of animal models with a single environmental stimulus could be too simplistic to assess the effects of the stimulus, suggesting that a single stimulus alone is not enough to elucidate the complex mechanisms underlying the development of disease, which is influenced by multiple environmental stimuli in the real world.

3.2.5 Ovalbumin administration

Vogel Ciernia et al. studied the mechanisms by which allergic asthma during pregnancy (maternal allergic asthma; MAA) increases the risk of ASD development in offspring.[57] First, ovalbumin (OVA) was intraperitoneally administered to C57BL/6J female mice before mating. Additional OVA was delivered by aerosol as secondary sensitization at E9, E12 and E17. In the male offspring of the MAA group, decreased social behavior in the

three-chamber test and increased persistent behavior in the marble burying test were observed, though persistent behavior (grooming time) was reduced.[58] The authors isolated microglia from the whole brains of P35 offspring and carried out RNA sequencing.[57] They found that the overlap between the genes for which expression levels were reduced in the MAA offspring and those reduced in the brains of humans with ASDs was high. In addition, DNA methylation patterns of the MAA offspring and those of poly(I:C) offspring were similar. Although the gene expression pattern in MAA offspring differs from that of other MIA models (LPS and IL-6) and does not overlap with ASD risk genes, these results suggest that the MAA model is a helpful tool to elucidate the role of microglia in the ASD brain.

3.3 Possible animal models of ASD (Table 3)

In recent years, it has been found that mice with modified microglia-specific genes exhibit ASD-like behaviors and neurological changes related to ASD pathology.

3.3.1 *Trem2* knockout

Triggering receptor expressed on myeloid cells 2 (Trem2) is an innate immune receptor that belongs to the immunoglobulin superfamily and is expressed specifically by microglia in the brain. *Trem2* gene mutations have been reported to be involved in the development of various neurodegenerative diseases such as Nasu-Hakola disease, Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. *Trem2* knockout mice at P18–P20 showed increased mEPSC frequency in pyramidal cells and increased synaptic density in the hippocampal CA1.[60] The authors performed a synaptic phagocytosis assay in vivo using the neuron-microglia co-culture system, showing that *Trem2* knockout reduced synaptic phagocytosis by microglia. These findings are consistent with the mechanisms suggested as the possible cause of ASDs; in fact, P90 *Trem2* knockout mice showed ASD-like behaviors such as persistent behavior assessed by marble burying test, and increased grooming and decreased social behavior in the three-chamber test. Postmortem analysis in the brains of patients with ASDs has shown decreased expression of Trem2 protein.

3.3.2 *Atg7* knockout

Kim et al. focused on autophagy using mice in which *Atg*7, an autophagy-related gene specifically expressed by bone marrow as well as microglia, is knocked out.[61] *Atg*7 knockout mice exhibited decreased social behavior

Table 3 Possible animal models of ASDs.

Gene	Related disease; protein function	Rodent model of gene mutation	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Trem2	Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Nasu-Hakola disease; innate immune receptor belonging to immunoglobulin super family	*Trem2* knockout C57BL/6 mice	Decreased sociability, persistence	Increased mEPSC frequency and synaptic density of hippocampal CA1 pyramidal cells	Decreased synaptic phagocytosis	60
Atg7	Ubiquitin activation enzyme regulating autophagy	Bone marrow cell-specific *Atg7* knockout C57BL/6 mice	Decreased sociability, persistence	Increased spine density and synaptic protein expression in the sensory cortex	Increased PSD95 within microglia	61
Csf1	Hereditary diffuse leukoencephalopathy with spheroid; ligand of CSF1R; CSF1/CSF1R signaling is necessary for microglial survival and proliferation	Nestin expressing cell-specific *Csf1* knockout C57BL/6 mice	Decreased sociability, motor dysfunction	Decreased morphological complexity of Purkinje cells, increased synaptic density projecting to Purkinje cells, impaired migration of Purkinje cells	Decreased microglial density in the cerebellum	62

Abbreviation: mEPSC, miniature excitatory post-synaptic currents.

in the three-chamber test and persistent behavior in the marble burying test. Further, in *Atg7* knockout mice, spine density and expression levels of synapse-related proteins were increased in the sensory cortex, suggesting that synaptic phagocytosis by microglia was impaired; however, the level of PSD95 puncta contained in the microglia was increased. From these results, it was suggested that autophagy dysfunction impaired the digestion of synapses by microglia, leading to the inhibition of further synaptic phagocytosis.

3.3.3 CSF1-CSF1R signaling deficiency

Colony-stimulating factor 1 (CSF1) receptor (CSF1R) has two ligands, CSF1 and IL-34. It has been shown that signals mediated by CSF1R are involved in microglial differentiation and proliferation. Recently, an increasing number of studies have examined microglial functions by depleting microglia using CSF1R inhibitors. Kana et al. examined effects of nestin-positive cell-specific knockout of *Csf1*.[62] In these mice, IL-34 (CSF1R ligand) was expressed normally in the cortex and hippocampus, but was scarcely expressed in the cerebellum. Therefore, in nestin-positive cell-specific *Csf1* knockout mice, the microglial density was remarkably reduced in the cerebellum because CSF1 was knocked out in addition to the basically low level of IL-34. In addition, cerebellar Purkinje cells showed impaired migration as well as decreased dendritic length and complexity, and increased synaptic density near the cell body in these mice. These results suggest that microglia are required for normal development of Purkinje cells. The authors also revealed that nestin-positive cell-specific *Csf1* knockout mice exhibited decreased social behavior in the three-chamber test and impaired motor function.

3.4 Non-rodent animal models of ASDs (Table 4)

Many studies have used rodents to develop ASD models as they are widely available, relatively easy to handle, and can be genetically and pharmacologically manipulated. However, it is obviously difficult to study mental disorders using rodents that do not use language for communication. In addition, there are limitations to determining whether animal models successfully mimic human ASD phenotypes by merely using behavioral tests. For example, the three-chamber test used for the evaluation of sociability cannot completely eliminate the effects of memory; simultaneous performance of behavioral tests to verify memory loss is recommended. The most critical caveat in the use of rodent models is the anatomical differences in brain

Table 4 Non-rodent animal models of ASDs.

Animal	Reagent	Administration conditions (timing, dose, route)	Behavioral abnormalities	Neuroanatomical abnormalities	Microglial involvement	Reference
Marmoset	Valproic acid	GD60–66, 200 mg/kg, oral administration	Decreased sociability	–	–	63
			–	–	Abnormal morphology and density of cortical microglia	64
Pig	Reproductive and respiratory syndrome virus	GD76, 5×10^5 $TCID_{50}$, inoculation	Decreased sociability	–	No change (P27)	65
			–	Increased levels of TNFα, IL-1β, and GFAP in the hippocampus, decreased neuronal density in the dentate gyrus and subiculum	Increased level of MHC II in hippocampus (GD111)	66
			–	–	Increased level of MHC II (GD83, GD97), increased microglial density in amygdala (GD83)	67

Abbreviations: GD, gestational day; GFAP, glial fibrillary acidic protein; IL-1β, interleukin 1β; MHC, major histocompatibility complex; P, postnatal day; TCID, tissue culture infective dose; TNFα, tumor necrosis factor α.

structure between rodents and humans.[68–70] Furthermore, it has been recently reported that there are species-specific differences in microglial gene expression.[71] These issues make it difficult to apply the results of basic research to clinical therapeutics. This begs the question, what other species make suitable alternative options for the study of ASDs? In the next section, we will introduce studies in which the role of microglia was examined in ASD models using animals other than rodents.

3.4.1 Marmoset

Marmosets are New World monkeys that have attracted attention as primate model animals for the study of mental disorders. Their attractiveness comes from the fact that they have higher social cognitive functions and that they can be genetically manipulated. Yasue et al. orally administered VPA to pregnant marmosets 60–66 days after they conceived.[63] The authors used third-party reciprocal/non-reciprocal exchange to examine the social cognitive abilities of marmosets. In this behavioral test, marmosets watch two actors changing food and whether the marmosets receive food from the two actors was examined. In the control group, in non-reciprocal situations, the probability of receiving food from the actor who delivered the food increased, whereas in the VPA group, there was no difference in the preference of the actors, suggesting that social skills were impaired due to VPA exposure. The authors provided three interpretations of this result: the VPA group (1) might have impaired individual discrimination, (2) might not be able to distinguish between reciprocal and non-reciprocal behavior, and (3) might not have negative emotions for non-reciprocal behavior. Future studies are needed to verify that marmosets can distinguish between reciprocal and non-reciprocal situations.

Using a similar VPA exposure protocol, Sanagi et al. examined the morphology of microglia in young, adolescent and adult offspring.[64] At each stage of development, microglia in the VPA offspring group showed thin and fragmented processes. A decrease in the density of microglia and a decrease in the primary process number were also confirmed in the VPA offspring group. It is unclear whether these morphological changes represent changes in microglial function. Additional analysis of synaptic structures and neuronal functions in the VPA-treated marmoset brain may provide further insight.

3.4.2 Pig

Antonson et al. used a pig MIA model.[65–67] Reproductive and respiratory syndrome virus (PRRSV) was administered intranasally to pregnant pigs at

gestational day (GD) 76. PRRSV is a single-stranded RNA virus that causes interstitial pneumonia by infecting alveolar macrophages. P27 piglets exhibited decreased social behavior in the three-chamber test.[65] Microglial analysis was also performed, but the inflammatory responses of microglia, including MHC II expression and the response to LPS (determined by the level of released cytokines), were not significantly different from control animals. A subsequent report from the same research group showed that TNFα, IL-β and glial fibrillary acidic protein levels were significantly increased in the hippocampus of fetuses at GD111, whereas MHC II levels were significantly reduced.[66] In addition, the density of neurons was significantly reduced in the dentate gyrus and hippocampal subiculum. In recent research by this group, microglia were analyzed at earlier time points, GD83 and GD97, and the percentage of microglia expressing MHC II was increased in the MIA offspring group.[67] In addition, immunostaining of tissue sections was performed, showing no change in microglial density in the hippocampus. In contrast, the microglial density was significantly increased at GD83 in the amygdala. This series of studies using a pig model of MIA suggests that transient changes in microglial properties caused by MIA could affect neuronal function and induce abnormal social behaviors in offspring.

4. Treatment of ASDs by controlling microglial functions

As mentioned above, the relationship between abnormalities in microglial functions and the pathogenesis of ASDs has been enthusiastically investigated. Besides being highlighted as a key player for elucidating the mechanism of ASD onset, microglia are beginning to attract attention as a new target for the treatment of ASDs. Microglia regulate neural circuit formation and plasticity, so strategies to modulate microglial functions can be used to repair neural circuits that have been degenerated during the development of ASDs. Possible methods to manipulate microglial functions for ASD treatments include drug-mediated activation and inactivation (or even spatiotemporal depletion) of microglia, microglial transplantation and non-invasive methods such as exercise and sensory stimulations. We have previously described the possible efficacy of drugs, cell transplantation and exercise on microglial manipulation in ASD treatments; interested readers can find further details of this by referring to our article.[72] In this section, we discuss whether non-invasive sensory stimulations are useful for the manipulation of microglial functions.

Tsai and colleagues have found that gamma-wave sensory stimulation modulates microglial functions. Iaccarino et al. gave a 40 Hz flickering to Alzheimer's disease model mice (5xFAD mice).[73] They found that microglia in the visual cortex exhibited cell body hypertrophy and processes shortening. Further, increased Aβ phagocytosis by microglia and decreased Aβ plaques in the brain were confirmed. In contrast, the visual stimulus did not affect the amount of synaptophysin, a possible target of synaptic phagocytosis by microglia, which suggests that whether 40 Hz stimulation enhances the phagocytic capacity of microglia is target-dependent. Next, the authors provided 40 Hz sound stimuli to the 5xFAD mice and were able to reproduce the microglial morphological changes and reduction of Aβ plaques not only in the auditory cortex but also in the hippocampus.[74] In addition, the sound stimuli improved cognitive and learning performance in 5xFAD mice. When visual and sound stimuli were simultaneously applied at 40 Hz to 5xFAD mice, microglial activation and accumulation around Aβ plaques were observed even in the medial prefrontal cortex. In their subsequent report, CK-p25 mice, an animal model of neurodegenerative disease, were treated with 40 Hz visual stimuli and microglia were collected from the primary visual cortex (V1) for RNA sequence analysis.[75] The 40 Hz visual stimuli reduced the expression levels of genes related to immune response and MHC I-dependent antigen presentation in CK-p25 mice. Furthermore, the increases in microglial density and hypertrophy in the V1, hippocampal CA1, sensory cortex and cingulate cortex were also suppressed. These results suggest that 40 Hz visual stimuli may normalize activated microglia. As a next step, it would be interesting to examine whether 40 Hz sensory stimuli would modulate the pathogenesis of various diseases, including ASDs. As an example of a possible effect on ASDs, we may expect that 40 Hz stimuli would suppress microglial activation while promoting the phagocytosis of excess synapses by microglia.

5. Conclusion

In this chapter, we first introduced various animal models of ASDs, specifically focusing on the changes in microglial properties, and discussed the role of microglia in the development of ASDs. The phenotypes commonly observed in the brains of patients with ASDs and in animal models of ASDs include morphological and functional abnormalities in synapses and accelerated inflammation. Thus, it is likely that microglia are responsible for neural circuit formation and inflammatory responses that contribute to

the pathogenesis of ASDs. However, histological analysis of brain slices alone falls short of clearly revealing whether morphological and functional changes in microglia are the cause or result of ASDs. For this purpose, it is necessary to examine microglial properties at multiple time points, such as before and after ASD-like behaviors appear. Additionally, the role of other cell types, including neurons and astrocytes, must also be considered. It should be also noted that we should always pay attention to the differences between humans and animals, including monkeys. Indeed, when we use non-human primate models of ASDs, it is possible that we are confronted with higher order differences that make the interpretation of data more complex compared to rodent models. Thus, in addition to comparing the results from different animal models, the development and use of sophisticated and well-validated in vitro system to understand the cellular and molecular dynamics underlying the pathogenesis of ASDs should be pursued.

Finally, we mentioned the possibility of treating ASDs by controlling the function of microglia (also see Ref. 72). In particular, the use of non-invasive methods such as exercise and sensory stimuli would be a major breakthrough. Further clarifying how these stimuli affect microglial dynamics and ASD pathogenesis in animal models will forge a new path for the treatment of ASDs.

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