CHAPTER NINE

Microglia as possible therapeutic targets for autism spectrum disorders

Megumi Andoh, Yuji Ikegaya, Ryuta Koyama*
Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan
*Corresponding author: e-mail address: rkoyama@mol.f.u-tokyo.ac.jp

Contents

1. Introduction 224
2. Therapeutic targets for ASD treatment and possible involvement of microglia 226
   2.1 Drug therapy 226
   2.2 Cell-transplantation-based therapy 232
3. Synaptic deficits in ASDs 233
4. Physical exercise, possibly a potent therapeutic strategy for ASDs 236
5. Concluding remarks 238
Acknowledgment 238
References 239

Abstract

Malfunctions of the nervous and immune systems are now recognized to be fundamental causes of autism spectrum disorders (ASDs). Studies have suggested that the brain's resident immune cells, microglia are possible key players in ASDs. Specifically, deficits in synaptic pruning by microglia may underlie the pathogenesis of ASDs, in which excess synapses are occasionally reported. This idea has driven researchers to investigate causal links between microglial dysfunction and ASDs. In this review, we first introduce the characteristics of microglia in ASD brains and discuss their possible roles in the pathogenesis of ASDs. We also refer to immunomodulatory agents that could be potentially used as symptomatic therapies for ASDs in light of their ability to modify microglial functions. Finally, we will mention a possible strategy to radically cure some of the symptoms reported in ASDs through reorganizing neural circuits *via* microglia-dependent synaptic pruning.

Progress in Molecular Biology and Translational Science, Volume 167
ISSN 1877-1173
https://doi.org/10.1016/bs.pmbts.2019.06.012

1. Introduction

Some decades ago, it was revealed that the synaptic density in the human brain is drastically increased during childhood and decreased during adolescence to the adult level.[1] The decrease in synaptic density has been called synaptic pruning or synapse elimination and has been suggested to be crucial for the normal development of brain functions.[2,3] Accumulating studies have shown that microglia, the brain-resident immune cells, are the executors of synaptic pruning.[2,4] Deficits in synaptic pruning are suggested to perturb refinement of neural circuits, leading to abnormal development of higher-order brain functions such as cognition and social communication, which are the main symptoms of autism spectrum disorders (ASDs), one of the neurodevelopmental diseases.[3,5] Therefore, many researchers have focused on the relationship between ASDs and microglia.

Neuropathological analysis using postmortem brains of ASD patients shows that microglial number is increased in that population and that microglia exhibit the activated form (enlarged cell body and thick process) in multiple regions, including the frontal lobes.[6,7] PET/CT scan analysis also shows that microglia are activated in ASD frontal lobes and cerebellum.[8] Moreover, in the ASD prefrontal cortex (PFC), the microglia-neuron distance is reduced, and microglial processes enclose the neuronal cell body, which suggests that the interaction between microglia and neurons is facilitated in ASD brains.[9] It should be noted, however, that the likely activated status of microglia in ASD brains is reported from studies that analyzed postmortem specimens or individuals who had been already diagnosed with ASDs; whether microglia are activated or even inactivated during the development of ASDs remains to be elucidated.

Recently, the complement molecules C1q and C3, which stimulate the microglial complement receptor CR to prune synapses,[4] have been suggested to be related to ASDs. In the serum of ASD patients, the levels of C1q and C-reactive protein, which promote the inflammatory response through binding to C1q, are elevated.[10,11] An increased concentration of complement molecules in ASDs may enhance microglia-dependent synaptic pruning. It is also possible that excess complements mask the differences in complement deposition levels on each synapse, which may result in the disruption of complement-targeted specific synaptic pruning.

Almost all of the reports from human studies have been based on samples from patients already diagnosed with ASDs or on postmortem brain

specimens. To investigate microglial contributions to the development of ASDs in detail, animal models of ASDs have been widely used. In maternal immune activation (MIA) models, which mimic maternal infection during pregnancy as a risk factor for ASDs, microglial density and proinflammatory cytokine levels are increased in the cerebral cortex and the hippocampus.[12,13] Neurons also exhibit unusual morphologies in these models. For example, hippocampal granule cells have more spines.[14] Furthermore, the expression levels of CX3CR1, a molecule suggested in neuron-microglia interactions, were decreased in the hippocampus, suggesting that MIA perturbs microglia-dependent synaptic pruning.[14] Fmr1 KO mice, in which the risk gene for fragile X syndrome is depleted, were also used as a model of ASD and exhibited impaired microglial phagocytosis of postsynaptic protein PSD95.[15] In addition to these primary ASD models, the BTBR mouse strain, which is used as an idiopathic ASD model, has more activated microglia with higher levels of MHC-II compared to the C57BL/6J mouse strain.[16] It has also been revealed that hippocampal pyramidal cells show increased spontaneous excitatory postsynaptic currents (EPSCs) in BTBR mice, suggesting deficits in synaptic pruning.[17]

Some reports have shown that deficits in microglia-dependent synaptic pruning cause ASD-like features. Inhibition of microglial autophagy decreases microglial synaptic phagocytosis, leading to increased spine density and impaired social interaction.[18,19] Mice lacking CX3CR1, which is expressed by microglia and regulates synaptic pruning, exhibited decreased functional connectivity in ASD-related brain regions, impaired social interaction, and increased repetitive behaviors.[20] More recently, TREM2, which is a risk gene of Alzheimer's disease, has been suggested to be necessary for developmental synaptic pruning, and TREM2 KO mice exhibit reduced functional connectivity in the brain, impaired social interaction and increased repetitive behavior.[21]

The male-to-female ratio of ASD morbidity is approximately 4 to 1 in humans; thus, it is believed that there are relationships between sexes and ASDs. Microglia have attracted attention as a factor that causes sexual differences in ASDs. A genome-wide association study (GWAS) has revealed that genes expressing at higher levels in prenatal human males are also upregulated in postmortem brains of ASD patients and, importantly, these genes include microglial markers.[22] Sex differences in microglial properties are now being investigated energetically. One such study has shown that expression levels of the lysosomal protein CD68 are higher in female mice during development when surplus synapses are eliminated, suggesting that

phagocytic capacity differs between sexes.[23] This finding may suggest an excess of synapses underlying the elevated ASD morbidity rate in males.

Astrocytes are the most abundant glia cells in the brain. They contact adjacent synapses and form the tripartite synapse, modulating synaptogenesis and synaptic plasticity *via* providing and receiving ions and cytokines. These astrocytes' functions suggest their involvement in the pathophysiology of ASDs, which is also referred to as a synaptopathy.[24,25] Indeed, astrocytes are persistently activated in the cerebellum of a mouse model of Fragile X syndrome.[26] However, a report shows that morphology and number of astrocytes do not change in postmortem brains of ASD patients.[27] Furthermore, few studies have investigated astrocyte-related genes and molecules in ASD animal models. Thus, further investigation is necessary to clarify the role of astrocytes in the pathogenesis of ASDs.

2. Therapeutic targets for ASD treatment and possible involvement of microglia

To date, two drugs, risperidone and aripiprazole, are approved by the US FDA to be used for the treatment of ASD symptoms.[28] Some reports have suggested that these drugs modulate microglial functions. For example, risperidone inhibits the production of inflammatory cytokines in microglia *in vitro,* while aripiprazole reduces microglial activation in an MIA model mouse.[29,30] In the serum and cerebrospinal fluid (CSF) of ASD patients, inflammatory cytokines such as IL-1β and IL-6 are expressed at higher levels, which implies immunological dysfunction in ASDs.[31] This chapter discusses the use of immunomodulatory agents as a potential therapeutic strategy for ASDs (listed in Table 1). We also introduce how these agents possibly modify microglial functions. In the latter part, we discuss cell therapy and its future prospects.

2.1 Drug therapy

2.1.1 Luteolin

Luteolin is a naturally occurring flavonoid. Luteolin decreases the serum level of tumor necrosis factor (TNF) and IL-6 and has been shown to improve the social interaction of ASD patients.[32,33] In an MIA model mouse, luteolin also ameliorates elevated anxiety and impaired sociability.[34] Additionally, BV-2 microglial activation and subsequent neurotoxicity induced by lipopolysaccharide (LPS) are inhibited by luteolin application *in vitro*.[59]

Table 1 Drug therapies for ASDs.

Drug	Subject species	Administration route Dosage	Finding	Reference
Luteolin	Human ASD patients	Orally 100 mg	Decreased serum levels of IL-6 and TNF Improved communication and social skills	32
	Human ASD patients	Orally 200 mg/10 kg	Improved GI and allergy symptoms Increased social interaction	33
	IL-6-induced ASD model mice	Orally 10 mg/kg/day	Decreased TNF-α and IL-1β in brains Decreased repetitive behavior Improved social interaction	34
	BV-2 microglia *in vitro*	~50 μM	Inhibited proinflammatory pathway Enhanced anti-inflammatory pathway	35
Minocycline	Human ASD patients	50 mg twice/day	Decreased hyperactivity and Irritability	36
	VPA-induced ASD model rats	Orally 25 and 50 mg/kg	Decreased BBB permeability Improved social interaction Decreased repetitive behavior	37
	Microglia prepared from Poly(I:C)-induced ASD model mice *in vitro*	Orally 3 mg/kg/day	Increased phagocytic capacity	38
Suramin	Human ASD patients	Intravenously 20 mg/kg	Improved social interaction Decreased repetitive behavior	39
	Poly(I:C)-induced ASD model mice	Intraperitoneally 20 mg/kg	Improved social interaction Improved hippocampus-dependent memory	40
Vitamin D	Human ASD patients	Orally 300 IU/kg/day	Improved social interaction Decreased irritability	41
	Mouse microglia *in vitro*	10 nM	Decreased proinflammatory cytokines Increased anti-inflammatory cytokines	42

Continued

Table 1 Drug therapies for ASDs.—cont'd

Drug	Subject species	Administration route Dosage	Finding	Reference
Gut microbiota	Poly(I:C)-induced ASD model mice	Orally ND	Corrected GI deficits Decreased repetitive and anxiety behavior Improved communicative behavior	43
Immunoglobulin	Human ASD patients	Intravenously 0.4 g/kg	Reduced hyperactivity and irritability Improved communication ability	44
	Mouse microglia *in vitro*	20 μM	Increased Aβ clearance by microglia	45
Pioglitazone	Human ASD patients	Orally 30 mg/day	Decreased hyperactivity and irritability	46
	Human ASD patients	Orally 30 or 60 mg/day	Decreased hyperactivity, irritability and stereotypy	47
Pioglitazone	APP/PS1	Orally ∼150 mg/kg/day	Increased Aβ phagocytosis by microglia	48
	HAIP microglia	∼20 μM	Inhibited proinflammatory cytokine expression induced by LPS	49
Sulforaphane	Human ASD patients	Orally 50–150 μmol	Reduced hyperactivity, irritability and repetitive behavior Improved communication ability	50
	BV2 microglia Primary mouse microglia	2.5 μM	Reduced proinflammatory cytokine expression induced by LPS	51
	N9 microglia	∼10 μM	Reduced proinflammatory cytokine expression and ROS production induced by LPS	52

Celecoxib	Human ASD patients	Orally 100–300 mg/day	Improved social interaction Decreased irritability and repetitive behavior	53
	Rats	Intraperitoneally 20 mg/kg	Decreased the number of activated microglia in substantia nigra and striatum	54
Lenalidomide	Human ASD patients	Orally 2.5 mg/day	Decreased serum levels of TNF-α Improved social interaction Decreased irritability and repetitive behavior	55
	Parkinson's disease model mice BV-2 microglia	NS 100 mg/kg 250 μg/mL	Decreased microgliosis in striatum Reduced proinflammatory cytokine expression induced by α-synuclein	56
Spironolactone	Human ASD patients	Orally 2 mg/kg/day	Decreased irritability, hyperactivity and repetitive behavior	57
	Rats	Intrathecally ND	Suppressed microglial secretion of inflammatory cytokines induced by chronic compression of the dorsal root ganglion	58

ND, not determined; *NS*, not shown.

2.1.2 Minocycline

Minocycline is a tetracycline analog and is widely used to suppress microglial activation, although its mechanism of action remains to be revealed. Minocycline has been suggested as an effective adjuvant of risperidone because patients cotreated with minocycline and risperidone show greater improvements in hyperactivity and irritability than patients treated with risperidone alone.[60] In a valproic acid (VPA)-induced rat model of ASDs, which mimics a drug-induced ASD, minocycline ameliorates ASD-like behaviors and disruption of the blood-brain-barrier (BBB).[37] Loss of BBB integrity is one of the pathological features of ASDs, and it is shown that BBB disruption causes microglial activation *in vivo*.[61,62] Minocycline has also been shown to rescue the decreased phagocytic ability of microglia prepared from MIA model mice *in vitro*.[38] Thus, minocycline possibly normalizes synaptic density in the ASD brain *via* inducing engulfment of surplus synapses by microglia.

2.1.3 Suramin

Suramin is a polyanionic compound with an unknown mechanism of action. Intravenous administration of suramin improves social interaction and repetitive behavior in ASD patients.[39] MIA model mice also show improved social interaction and memory after suramin treatment.[40] Suramin inhibits microglial activation in the spinal cord *in vivo*.[63] Additionally, suramin inhibits P2X7-dependent secretion of IL-6, CCL2 and TNF-α from mouse primary microglia.[64] A recent report has shown that P2X7 drives ASD behaviors in MIA model mice.[35] Thus, P2X7 can be a possible therapeutic target for ASDs.

2.1.4 Vitamin D

In ASD patients, the serum levels of vitamin D and its receptor are reduced.[36] Vitamin D supplementation ameliorates irritability and impaired social interaction in ASD patients.[41] Vitamin D decreases the expression of inflammatory cytokines such as IL-6 and TNF-α, while it increases the expression of antiinflammatory cytokine IL-10 from mouse primary microglia stimulated by LPS or IFN-γ.[42]

2.1.5 Gut microbiota

Because many ASD patients suffer from gastrointestinal disturbances, the gut microbiota has been considered.[65] Microbiota transfer therapy improves irritability, communication skills and sociability in ASD patients.[43] The gut microbiota regulates microglial development and homeostasis.[66] For example, microglia in mice without gut microbiota were hyper-ramified

and expressed higher levels of maturation and activation marker, while their immune response to LPS infection was diminished.[66] In a mouse model of MIA-induced ASDs, the composition of microbiota is disrupted, and tight junctions of intestinal mucosa become fragile.[67] Transplantation of human commensal *Bacteroides fragilis* into a mouse model of MIA-induced ASDs improves repetitive and anxiety behaviors.[67]

2.1.6 Immunoglobulin

Intravenous administration of immunoglobulin reduces hyperactivity and irritability and elevates communication ability in ASD patients.[44] Furthermore, IgG has been shown to promote Aβ phagocytosis by murine microglia *in vitro*.[45] Thus, it is possible that IgG induces engulfment of increased synapses by microglia in the ASD brain.

2.1.7 Pioglitazone

Pioglitazone is a thiazolidinedione with antidiabetic properties and potential antineoplastic activity. Oral administration of pioglitazone reduces hyperactivity, irritability and repetitive behaviors in ASD patients.[46,47] Pioglitazone enhances Aβ phagocytosis by microglia in Alzheimer's disease model mice, while it suppresses secretion of IL-6, TNF-α, and iNOS from microglia stimulated by LPS *in vitro*.[48,49] Therefore, pioglitazone could promote engulfment of excess synapses in the ASD brain by microglia without inflammation and neurodegeneration.

2.1.8 Sulforaphane

Sulforaphane is the most characterized isothiocyanate. Oral administration of sulforaphane reduces hyperactivity, irritability and repetitive behaviors in ASD patients in addition to improving their communication ability.[50] Sulforaphane inhibits LPS-induced microglial activation processes, such as proinflammatory cytokines (IL-1β, IL-6 and TNF-α) expression and reactive oxygen species (iNOS) release, in murine microglial cultures.[51,52]

2.1.9 Celecoxib

Celecoxib is a pyrazole derivative and a selective cyclooxygenase 2 inhibitor. Celecoxib, in combination with risperidone, ameliorates irritability, repetitive behavior and impaired social interaction in ASD patients.[53] The LPS-induced increase in the number of activated microglia is inhibited by celecoxib in neonatal rats.[54]

2.1.10 Lenalidomide

Lenalidomide is a phthalimide and piperidone derivative that has immunomodulatory and antiangiogenic properties. Lenalidomide reduces the serum levels of TNF-α in ASD patients.[55] In addition, ASD patients have shown improved irritability, repetitive behavior and social interaction after lenalidomide administration.[55] Furthermore, lenalidomide inhibits the expression of inflammatory cytokines such as IL-1β, IL-6 and TNF-α, while it increases the expression of antiinflammatory cytokine IL-10 in Parkinson's disease model mice and α-synuclein-stimulated activation of microglia *in vitro*.[56]

2.1.11 Spironolactone

Spironolactone is a potassium-sparing diuretic that acts by antagonism of aldosterone in the distal renal tubules. Oral administration of spironolactone reduced irritability, hyperactivity and repetitive behavior in ASD patients.[57] Spironolactone also suppresses microglial secretion of inflammatory cytokines such as IL-1β and TNF-α induced by chronic compression of the dorsal root ganglion in rats.[58]

2.2 Cell-transplantation-based therapy

Cell-transplantation-based therapies may provide us with some clues regarding the future application of microglial transplantation as a therapeutic strategy for ASDs. Injection of bone marrow-derived mononuclear cell (BMMNC) intrathecally or subcuntaneously[68,69] and embryonic stem (ES) cells intravenously[70,71] to ASD patients rescues ASD behaviors, including hyperactivity, repetitive behavior and impaired social communication. Additionally, intravenous injection of umbilical cord blood cells improved social communication in ASD patients.[72] Transplantation of cord blood mononuclear cells to veins in combination with umbilical cord-derived mesenchymal stem cells reduces repetitive behavior and improved sociability.[73]

It has also been suggested that BMMNC injection increases glucose metabolism at the prefrontal cortex and the hippocampus in ASD patients.[68,69] Glucose metabolism regulates activity-dependent synaptic vesicle transmission, suggesting the possible contribution of BMMNCs to the normalization of activity-dependent signal transmission.[74] This idea is supported by the transplantation of bone marrow mesenchymal stem cells into the cerebellum of a mouse model of Niemann-Pick disease: Purkinje cells increase spontaneous activity, promoting the formation of functional neural circuits after transplantation.[75] Recently, it has been reported that microglia-like cells can be generated from iPSCs, ES, and blood-derived monocytes.[76–79]

These microglia-like cells can be engrafted into the brain parenchyma, and they exhibit phagocytic properties.[76] Thus, it might be possible that microglia-like cells eliminate surplus synapses in the ASD brain. Moreover, microglia-like cells are suggested to be useful for drug screening.[78,79]

3. Synaptic deficits in ASDs

As mentioned above, immunomodulatory agents that suppress immune responses, such as luteolin and sulforaphane, are expected to be options for the treatment of ASDs; however, these strategies are symptomatic therapies. Furthermore, patients are usually treated with these agents chronically, which could cause multiple side effects. Another issue in ASD treatment is that it is difficult to determine the criteria for ASD severity because ASDs are spectrum disorders. In general, medication is prescribed if patients exhibit symptoms interfering with daily life, such as hyperactivity, aggression and self-injurious behavior. However, patients without those problems but with ASD-related problems, including deficits in social communication and their family, would also wish treatment for higher quality of life. These limits of medication led us to discover another therapeutic strategy.

In developing brains where synaptic pruning is robustly carried out, neural-activity-dependent signal transmissions are promoted, while they are suppressed after the critical period of developmental plasticity.[80,81] Deficits in developmental synapse elimination have been suggested to cause ASDs, but the mechanisms remain elusive. ASD patients usually suffer impaired processing of higher-order cognitive functions, but many reports imply abnormalities in primary sensory processing.[82] In sensory cortexes, including the visual and auditory cortices, neural activity induced by sensory stimuli causes neural circuits to form.[83] This circuit formation is regulated by modulation of synaptic maturation and function. Thus, it is possible that activity-dependent synaptic maturation and further circuit formation are not normally conducted in ASD brains.[84] Previous reports have demonstrated that microglia likely phagocytose less active synapses, indicating that microglia are able to decipher the strength of neuronal activity.[85] These findings have motivated us to examine whether microglia-dependent reorganization of synaptic connections could be a therapeutic strategy for ASDs; we hypothesize that microglia are unable to select synapses to phagocytose due to weak contrast between activities of each synapse in ASD brains.

Most animal models of ASDs are based on mutations of synapse-related genes, especially those that impair activity-dependent signal transmission (listed in Table 2).[95] For example, knockout of a member of the neurexin

Table 2 Animal models for ASDs.

Gene	Associated disease Function	Mutation Animal species	Behavioral abnormality	Neuroanatomical abnormality	Reference
NRX1	ASDs, schizophrenia, epilepsy, Pitt-Hopkins syndrome Presynaptic adhesion molecule	Nrxn1α knockout C57BL6/SV129 mice	Decreased social interaction	NA	86
SHANK	ASDs Postsynaptic scaffolding protein	Shank3 knockout C57 mice	Decreased social interaction, increased repetitive behavior	Decreased cortico-striatal synaptic transmission, striatal hypertrophy	87
		Shank2 knockout C57BL/6J mice	Decreased social interaction, impaired communication, enhanced anxiety	Impaired NMDAR function	88
FMR1	Fragile X syndrome mRNA trafficking, dendrite maturation, synaptic plasticity	Fmr1 knockout C57BL/6J mice	NA	Increased spines in hippocampal CA1, decreased microglia-dependent synaptic engulfment	15
		Fmr1 knockout C57BL/6 mice	NA	Dysregulation of glutamatergic signaling maturation	89
		Fmr1 knockout C57BL/6 mice	Impaired social interaction, increased repetitive behavior	Enhanced LTD and increased spines in hippocampal CA1	90

MECP2	Rett syndrome Transcriptional regulation	MeCP2 S421A knock-in C57BL/6 mice	Impaired social interaction	Increased dendritic complexity and increased mIPSC amplitude in visual cortex	91
PTEN	ASDs Inhibition of mTOR activation	Nse-cre; $Pten^{loxP/loxP}$ C57/BL6 mice	Decreased social interaction, decreased pre-pulse inhibition, decreased anxiety	Hippocampus hypertrophy, increased spines and ectopic dendrites in dentate gyrus	92
TSC1/2	Tuberous sclerosis Inhibition of mTOR activation	TSC1/2 hetero knockout C57BL/6J mice	Impaired context discrimination	Decreased LTD in hippocampal CA1	93
UBE3A	ASDs, Angelman syndrome	Ube3A knockout	NA	Increased Arc expression and decreased number of AMPAR at synapses in hippocampus	94

NA, not analyzed.

family, NRX1α, leads to decreased social interaction,[86] which suggests the possible involvement of deficits in neural activity and synapse formation in ASDs because the structural binding of neurexin and neuroligin, which are located at the presynaptic and postsynaptic membranes, respectively, promotes synaptic maturation associated with neural activity.[96,97] SHANK2, a scaffold protein found in the postsynaptic densities of excitatory synapses, is crucial for normal long-term potentiation (LTP) and long-term depression (LTD).[87] SHANK2 knockout mice exhibit impaired social interaction, but the abnormal behavior is improved by restoring synaptic plasticity *via* NMDA receptor stimulation. Mutations in another SHANK-family protein, SHANK3, weaken excitatory neuronal transmission in corticostriatal synapses, dysfunctions of which are implicated in ASDs.[88] Risk genes for symptomatic ASDs, including fragile X syndrome and Rett syndrome, are also related to activity-dependent synaptic modulation. In fmr1 knockout mice, a model of fragile X syndrome, activity-dependent synaptic maturation and pruning are impaired.[89,90] Recently, fmr1 knockout has been revealed to reduce microglia-dependent synaptic pruning,[15] indicating the possible involvement of microglia in ASD-related symptoms. The Rett syndrome risk gene MECP2 is necessary for synaptic maturation.[91] Knockout of PTEN, an ASD risk factor gene, reduced synaptic pruning in the hippocampus.[92] Mutations of the tuberous sclerosis risk gene TSC1/2, which encodes a downstream protein of PTEN, hinder activity-dependent gene translation and synaptic plasticity.[93] In an Angelman model, mice with mutations in UBE3A, which is also an ASD risk gene, exhibit reduced activity-dependent gene expression and excitatory neural transmission.[94]

4. Physical exercise, possibly a potent therapeutic strategy for ASDs

Mild therapeutic strategies for ASDs, as opposed to drug or cell-transplantation therapies, are ideal. Recently, exercise has attracted attention as a potential therapeutic strategy for abnormal behaviors in neurodevelopmental disorders, including ASDs, but the cellular and molecular mechanisms by which exercise ameliorates the symptoms remain unrevealed.[98–100] In the following paragraphs, we discuss the possibility that physical exercise is a viable treatment to induce synaptic pruning by microglia, resulting in improved ASD-related behaviors.

Previous studies have reported that exercise may help improve cognitive performance in humans and rodents, probably through enhancing the

production of neurotrophic factors and increasing hippocampal neurogenesis.[101–104] The hippocampus and prefrontal cortex regulate cognition and social behavior, deficits of which are the main symptoms of ASD, highlighting these regions as possible target brain regions for ASD treatment.[105] Consistent with this possibility, exercise increases neural activity in multiple regions including the hippocampus and prefrontal cortex in humans and rodents.[106,107] These regions show increased expression of brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1) in response to exercise.[108,109] BDNF is secreted depending on neural activity, promoting synaptic maturation.[110] IGF-1 also stabilizes synapses by increasing the expression of synapse-related molecules such as Synapsin I and PSD95.[111,112] These molecules may accelerate activity-dependent synaptic maturation after exercise.

Another major effect of exercise is the enhancement of adult neurogenesis in the hippocampus.[104] Adult-born hippocampal granule cells exhibit higher excitability than preexisting mature granule cells, which may generate synaptic competition between newborn and existing cells.[113] Thus, it is possible that microglia detect differences in neural activity therein. It was recently revealed that in MIA-induced ASD model mice, hippocampal neurogenesis is inhibited during both the immediate postnatal period and adulthood, leading to the impaired maturation of newborn granule cells.[114] Thus, inadequate neurogenesis may underlie ASD development. The activity of neural precursor cells is also enhanced by exercise, and this process is modulated by the CX3CL1-CX3CR1 axis, one of the important signaling pathways regulating microglia-neuron interaction, suggesting an active role of microglia in the exercise-induced activity of neural precursors.[115]

In order to generate contrast in synaptic activity, which is necessary for microglia-dependent synaptic pruning, it will be also useful to focus on inhibitory neurotransmission, as GABAergic signaling has been shown to regulate synaptic pruning in the cerebellum.[116] In addition, impaired inhibitory neurotransmission has been reported in some animal models of ASDs.[117–119] Furthermore, ASD patients express reduced levels of glutamic acid decarboxylase (GAD) and GABA receptor subunits, which implies a causal relationship between impaired inhibitory neural transmission and ASDs.[120–122] Exercise increases the numbers of interneurons and inhibitory synapses, enhancing the secretion of GABA.[123,124] Therefore, exercise is expected to induce synaptic competition by modulating both excitatory and inhibitory neural transmission.

Neural activity promotes the dynamics of microglial processes *in vivo*.[125] Therefore, elevation of neural activity by exercise might induce microglial contact with synapses, resulting in the elimination of less active synapses by microglia. It has been reported that exercise decreases the expression of MHC-II and CD68 in microglia, both of which are regarded as markers of activated microglia.[126,127] These results indicate that exercise induces synaptic pruning without overactivating microglia. We are now testing the hypothesis that exercise induces microglia-dependent synaptic pruning through synaptic competition. Our study will link exercise to microglia-dependent changes in the status of the brain.

5. Concluding remarks

We discussed the involvement of microglia in ASDs and the possibility of microglia as the therapeutic targets for ASDs. As introduced in the first section, several researches have suggested that microglia are likely involved in the pathogenesis and development of ASDs. In the second section, we introduced immunomodulatory agents that could attenuate ASD-related behaviors probably *via* regulation of microglial functions. Some of them are under clinical trial, but the side effects should be carefully addressed before the actual clinical use. We also mentioned the cell-transplantation-based therapy, but the method itself is invasive and could result in undesired activation of innate microglia. In the third and fourth sections, we further focused on synaptic remodeling by microglia in the ASD brain. Specifically, we mentioned the possibility that exercise cures ASD behaviors *via* inducing synaptic pruning by microglia. Though it should be noted that some ASD patients have difficulties in exercise or may feel stress by compulsive exercise, exercise, if it is effective, is useful in that it can be basically a noninvasive treatment and the exercise intensity can be changed according to the severity of ASD symptoms. A few clinical studies have shown that various types of exercise actually ameliorate ASD-related behaviors. We are currently examining the cellular and molecular mechanisms underlying exercise-induced modulation of ASD behaviors.

Acknowledgment

This work was supported in part by a Grant-in-Aid for Scientific Research (B) (17H03988 to R.K.) from JSPS, by JST PRESTO (JPMJPR18H4 to R.K.), and by JST ERATO (JPMJER1801 to Y.I.).

References

1. Huttenlocher PR. Morphometric study of human cerebral cortex development. *Neuropsychologia*. 1990;28(6):517–527.
2. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 2011;333(6048):1456–1458.
3. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. *Nat Neurosci*. 2011;14(3):285–293.
4. Stevens B, Allen NJ, Vazquez LE, et al. The classical complement cascade mediates CNS synapse elimination. *Cell*. 2007;131(6):1164–1178.
5. Koyama R, Ikegaya Y. Microglia in the pathogenesis of autism spectrum disorders. *Neurosci Res*. 2015;100:1–5.
6. Morgan JT, Chana G, Pardo CA, et al. Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol Psychiatry*. 2010;68(4):368–376.
7. Tetreault NA, Hakeem AY, Jiang S, et al. Microglia in the cerebral cortex in autism. *J Autism Dev Disord*. 2012;42(12):2569–2584.
8. Suzuki K, Sugihara G, Ouchi Y, et al. Microglial activation in young adults with autism spectrum disorder. *JAMA Psychiatry*. 2013;70(1):49–58.
9. Morgan JT, Chana G, Abramson I, Semendeferi K, Courchesne E, Everall IP. Abnormal microglial-neuronal spatial organization in the dorsolateral prefrontal cortex in autism. *Brain Res*. 2012;1456:72–81.
10. Corbett BA, Kantor AB, Schulman H, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. *Mol Psychiatry*. 2007;12(3):292–306.
11. Singh V. Elevation of serum C-reactive protein and S100 proteins for systemic inflammation in autistic children. *J Spec Educ Rehab*. 2005;6:117–125.
12. Zhu F, Zheng Y, Liu Y, Zhang X, Zhao J. Minocycline alleviates behavioral deficits and inhibits microglial activation in the offspring of pregnant mice after administration of polyriboinosinic-polyribocytidilic acid. *Psychiatry Res*. 2014;219(3):680–686.
13. Hui CW, St-Pierre A, El Hajj H, et al. Prenatal immune challenge in mice leads to partly sex-dependent behavioral, microglial, and molecular abnormalities associated with schizophrenia. *Front Mol Neurosci*. 2018;11:13.
14. Fernández de Cossío L, Guzmán A, van der Veldt S, Luheshi GN. Prenatal infection leads to ASD-like behavior and altered synaptic pruning in the mouse offspring. *Brain Behav Immun*. 2017;63:88–98.
15. Jawaid S, Kidd GJ, Wang J, Swetlik C, Dutta R, Trapp BD. Alterations in CA1 hippocampal synapses in a mouse model of fragile X syndrome. *Glia*. 2018;66(4):789–800.
16. Heo Y, Zhang Y, Gao D, Miller VM, Lawrence DA. Aberrant immune responses in a mouse with behavioral disorders. *PLoS One*. 2011;6(7): e20912.
17. Han S, Tai C, Jones CJ, Scheuer T, Catterall WA. Enhancement of inhibitory neurotransmission by GABAA receptors having α2,3-subunits ameliorates behavioral deficits in amouse model of autism. *Neuron*. 2014;81(6):1282–1289.
18. Tang G, Gudsnuk K, Kuo SH, et al. Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. *Neuron*. 2014;83(5):1131–1143.
19. Kim HJ, Cho MH, Shim WH, et al. Deficient autophagy in microglia impairs synaptic pruning and causes social behavioral defects. *Mol Psychiatry*. 2017;22(11):1576–1584.
20. Zhan Y, Paolicelli RC, Sforazzini F, et al. Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat Neurosci*. 2014;17(3):400–406.
21. Filipello F, Morini R, Corradini I, et al. The microglial innate immune receptor TREM2 is required for synapse elimination and normal brain connectivity. *Immunity*. 2018;48(5):979–991.

22. Werling DM, Parikshak NN, Geschwind DH. Gene expression in human brain implicates sexually dimorphic pathways in autism spectrum disorders. *Nat Commun*. 2016;7:10717.
23. Weinhard L, Neniskyte U, Vadisiute A, et al. Sexual dimorphism of microglia and synapses during mouse postnatal development. *Dev Neurobiol*. 2018;78(6):618–626.
24. Petrelli F, Pucci L, Bezzi P. Astrocytes and microglia and their potential link with autism spectrum disorders. *Front Cell Neurosci*. 2016;10:21.
25. Petrelli F, Bezzi P. mGlu5-mediated signalling in developing astrocyte and the pathogenesis of autism spectrum disorders. *Curr Opin Neurobiol*. 2018;48:139–145.
26. Pacey LK, Guan S, Tharmalingam S, Thomsen C, Hampson DR. Persistent astrocyte activation in the fragile X mouse cerebellum. *Brain Behav*. 2015;5(10): e00400.
27. Lee TT, Skafidas E, Dottori M, et al. No preliminary evidence of differences in astrocyte density within the white matter of the dorsolateral prefrontal cortex in autism. *Mol Autism*. 2017;8:64.
28. Marchezan J, Winkler Dos Santos EGA, Deckmann I, Riesgo RDS. Immunological dysfunction in autism spectrum disorder: a potential target for therapy. *Neuroimmunomodulation*. 2018;25(5–6):300–319.
29. Kato T, Monji A, Hashioka S, Kanba S. Risperidone significantly inhibits interferon-gamma-induced microglial activation in vitro. *Schizophr Res*. 2007;92(1–3):108–115.
30. Sato-Kasai M, Kato TA, Ohgidani M, et al. Aripiprazole inhibits polyI:C-induced microglial activation possibly via TRPM7. *Schizophr Res*. 2016;178(1–3):35–43.
31. Masi A, Quintana DS, Glozier N, Lloyd AR, Hickie IB, Guastella AJ. Cytokine aberrations in autism spectrum disorder: a systematic review and meta-analysis. *Mol Psychiatry*. 2015;20(4):440–446.
32. Tsilioni I, Taliou A, Francis K, Theoharides TC. Children with autism spectrum disorders, who improved with a luteolin-containing dietary formulation, show reduced serum levels of TNF and IL-6. *Transl Psychiatry*. 2015;5: e647.
33. Theoharides TC, Asadi S, Panagiotidou S. A case series of a luteolin formulation (NeuroProtek®) in children with autism spectrum disorders. *Int J Immunopathol Pharmacol*. 2012;25(2):317–323.
34. Parker-Athill E, Luo D, Bailey A, et al. Flavonoids, a prenatal prophylaxis via targeting JAK2/STAT3 signaling to oppose IL-6/MIA associated autism. *J Neuroimmunol*. 2009;217(1–2):20–27.
35. Horváth G, Otrokocsi L, Beko K, et al. P2X7 receptors drive poly(I:C) induced autism-like behavior in mice. *J Neurosci*. 2019;39(13):2542–2561.
36. Altun H, Kurutaş EB, Şahin N, Güngör O, Fındıklı E. The levels of vitamin D, vitamin D receptor, homocysteine and complex B vitamin in children with autism Spectrum disorders. *Clin Psychopharmacol Neurosci*. 2018;16(4):383–390.
37. Kumar H, Sharma B. Minocycline ameliorates prenatal valproic acid induced autistic behaviour, biochemistry and blood brain barrier impairments in rats. *Brain Res*. 1630;2016:83–97.
38. Mattei D, Ivanov A, Ferrai C, et al. Maternal immune activation results in complex microglial transcriptome signature in the adult offspring that is reversed by minocycline treatment. *Transl Psychiatry*. 2017;7(5): e1120.
39. Naviaux RK, Curtis B, Li K, et al. Low-dose suramin in autism spectrum disorder: a small, phase I/II, randomized clinical trial. *Ann Clin Transl Neurol*. 2017;4(7):491–505.
40. Naviaux JC, Schuchbauer MA, Li K, et al. Reversal of autism-like behaviors and metabolism in adult mice with single-dose antipurinergic therapy. *Transl Psychiatry*. 2014;4: e400.
41. Saad K, Abdel-Rahman AA, Elserogy YM, et al. Randomized controlled trial of vitamin D supplementation in children with autism spectrum disorder. *J Child Psychol Psychiatry*. 2018;59(1):20–29.

42. Boontanrart M, Hall SD, Spanier JA, Hayes CE, Olson JK. Vitamin D3 alters microglia immune activation by an IL-10 dependent SOCS3 mechanism. *J Neuroimmunol*. 2016;292:126–136.
43. Kang DW, Adams JB, Gregory AC, et al. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome*. 2017;5(1):10.
44. Niederhofer H, Staffen W, Mair A. Immunoglobulins as an alternative strategy of psychopharmacological treatment of children with autistic disorder. *Neuropsychopharmacology*. 2003;28(5):1014–1015.
45. Magga J, Puli L, Pihlaja R, et al. Human intravenous immunoglobulin provides protection against Aβ toxicity by multiple mechanisms in a mouse model of Alzheimer's disease. *J Neuroinflammation*. 2010;7:90.
46. Ghaleiha A, Rasa SM, Nikoo M, Farokhnia M, Mohammadi MR, Akhondzadeh S. A pilot double-blind placebo-controlled trial of pioglitazone as adjunctive treatment to risperidone: effects on aberrant behavior in children with autism. *Psychiatry Res*. 2015;229(1–2):181–187.
47. Boris M, Kaiser CC, Goldblatt A, et al. Effect of pioglitazone treatment on behavioral symptoms in autistic children. *J Neuroinflammation*. 2007;4:3.
48. Yamanaka M, Ishikawa T, Griep A, Axt D, Kummer MP, Heneka MT. PPARγ/RXRα-induced and CD36-mediated microglial amyloid-β phagocytosis results in cognitive improvement in amyloid precursor protein/presenilin 1 mice. *J Neurosci*. 2012;32(48):17321–17331.
49. Ji H, Wang H, Zhang F, Li X, Xiang L, Aiguo S. PPARγ agonist pioglitazone inhibits microglia inflammation by blocking p38 mitogen-activated protein kinase signaling pathways. *Inflamm Res*. 2010;59(11):921–929.
50. Singh K, Connors SL, Macklin EA, et al. Sulforaphane treatment of autism spectrum disorder (ASD). *Proc Natl Acad Sci USA*. 2014;111(43):15550–15555.
51. Townsend BE, Johnson RW. Sulforaphane induces Nrf2 target genes and attenuates inflammatory gene expression in microglia from brain of young adult and aged mice. *Exp Gerontol*. 2016;73:42–48.
52. Eren E, Tufekci KU, Isci KB, Tastan B, Genc K, Genc S. Sulforaphane inhibits lipopolysaccharide-induced inflammation, cytotoxicity, oxidative stress, and miR-155 expression and switches to Mox phenotype through activating extracellular signal-regulated kinase 1/2-nuclear factor erythroid 2-related factor 2/antioxidant response element pathway in murine microglial cells. *Front Immunol*. 2018;9:36.
53. Asadabadi M, Mohammadi MR, Ghanizadeh A, et al. Celecoxib as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind, placebo-controlled trial. *Psychopharmacology (Berl)*. 2013;225(1):51–59.
54. Kaizaki A, Tien LT, Pang Y, et al. Celecoxib reduces brain dopaminergic neuronaldysfunction, and improves sensorimotor behavioral performance in neonatal rats exposed to systemic lipopolysaccharide. *J Neuroinflammation*. 2013;10:45.
55. Chez M, Low R, Parise C, Donnel T. Safety and observations in a pilot study of lenalidomide for treatment in autism. *Autism Res Treat*. 2012;2012:291601.
56. Valera E, Mante M, Anderson S, Rockenstein E, Masliah E. Lenalidomide reduces microglial activation and behavioral deficits in a transgenic model of Parkinson's disease. *J Neuroinflammation*. 2015;12:93.
57. Bradstreet JJ, Smith S, Granpeesheh D, El-Dahr JM, Rossignol D. Spironolactone might be a desirable immunologic and hormonal intervention in autism spectrum disorders. *Med Hypotheses*. 2007;68(5):979–987.
58. Sun YE, Peng L, Sun X, et al. Intrathecal injection of spironolactone attenuates radicular pain by inhibition of spinal microglia activation in a rat model. *PLoS One*. 2012;7(6): e39897.

59. Dirscherl K, Karlstetter M, Ebert S, et al. Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J Neuroinflammation*. 2010;7:3.
60. Ghaleiha A, Alikhani R, Kazemi MR, et al. Minocycline as adjunctive treatment to risperidone in children with autistic disorder: a randomized, double-blind placebo-controlled trial. *J Child Adolesc Psychopharmacol*. 2016;26(9):784–791.
61. Kealy J, Greene C, Campbell M. Blood-brain barrier regulation in psychiatric disorders. *Neurosci Lett*. 2018. https://doi.org/10.1016/j.neulet.2018.06.033.
62. Ju F, Ran Y, Zhu L, et al. Increased BBB permeability enhances activation of microglia and exacerbates loss of dendritic spines after transient global cerebral ischemia. *Front Cell Neurosci*. 2018;12:236.
63. Wu Y, Willcockson HH, Maixner W, Light AR. Suramin inhibits spinal cord microglia activation and long-term hyperalgesia induced by formalin injection. *J Pain*. 2004;5(1):48–55.
64. Shieh CH, Heinrich A, Serchov T, van Calker D, Biber K. P2X7-dependent, but differentially regulated release of IL-6, CCL2, and TNF-α in cultured mouse microglia. *Glia*. 2014;62(4):592–607.
65. Li Q, Han Y, Dy ABC, Hagerman RJ. The gut microbiota and autism Spectrum disorders. *Front Cell Neurosci*. 2017;11:120.
66. Erny D, Hrabě de Angelis AL, Jaitin D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci*. 2015;18(7):965–977.
67. Hsiao EY, McBride SW, Hsien S, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell*. 2013;155(7):1451–1463.
68. Sharma A, Badhe P, Gokulchandran N, et al. An improved case of autism as revealed by PET CT scan in patient transplanted with autologous bone marrow derived mononuclear cells. *J Stem Cell Res Ther*. 2013;3(2):1000139.
69. Sharma A, Gokulchandran N, Sane H, et al. Autologous bone marrow mononuclear cell therapy for autism: an open label proof of concept study. *Stem Cells Int*. 2013;2013:623875.
70. Bradstreet JJ, Sych N, Antonucci N, et al. Efficacy of fetal stem cell transplantation in autism spectrum disorders: an open-labeled pilot study. *Cell Transplant*. 2014;23(suppl 1):S105–S112.
71. Shroff G. Human embryonic stem cells in the treatment of autism: a case series. *Innov Clin Neurosci*. 2017;14(3–4):12–16.
72. Dawson G, Sun JM, Davlantis KS, et al. Autologous cord blood infusions are safe and feasible in young children with autism spectrum disorder: results of a single-center phase I open-label trial. *Stem Cells Transl Med*. 2017;6(5):1332–1339.
73. Lv YT, Zhang Y, Liu M, et al. Transplantation of human cord blood mononuclear cells and umbilical cord-derived mesenchymal stem cells in autism. *J Transl Med*. 2013;11:196.
74. Ashrafi G, Ryan TA. Glucose metabolism in nerve terminals. *Curr Opin Neurobiol*. 2017;45:156–161.
75. Bae JS, Han HS, Youn DH, et al. Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration. *Stem Cells*. 2007;25(5):1307–1316.
76. Abud EM, Ramirez RN, Martinez ES, et al. iPSC-derived human microglia-like cells to study neurological diseases. *Neuron*. 2017;94(2)278–93.e9.
77. Tsuchiya T, Park KC, Toyonaga S, et al. Characterization of microglia induced from mouse embryonic stem cells and their migration into the brain parenchyma. *J Neuroimmunol*. 2005;160(1–2):210–218.
78. Ohgidani M, Kato TA, Kanba S. Introducing directly induced microglia-like (iMG) cells from fresh human monocytes: a novel translational research tool for psychiatric disorders. *Front Cell Neurosci*. 2015;9:184.

79. Ryan KJ, White CC, Patel K, et al. A human microglia-like cellular model for assessing the effects of neurodegenerative disease gene variants. *Sci Transl Med*. 2017;9(421). https://doi.org/10.1126/scitranslmed.aai7635.
80. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108 (suppl 3):511–533.
81. Hensch TK. Critical period regulation. *Annu Rev Neurosci*. 2004;27:549–579.
82. Marco EJ, Hinkley LB, Hill SS, Nagarajan SS. Sensory processing in autism: a review of neurophysiologic findings. *Pediatr Res*. 2011;69(5 pt 2):48R–54R.
83. Doll CA, Broadie K. Impaired activity-dependent neural circuit assembly and refinement in autism spectrum disorder genetic models. *Front Cell Neurosci*. 2014;8:30.
84. Morrow EM, Yoo SY, Flavell SW, et al. Identifying autism loci and genes by tracing recent shared ancestry. *Science*. 2008;321(5886):218–223.
85. Schafer DP, Lehrman EK, Kautzman AG, et al. Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron*. 2012;74(4):691–705.
86. Grayton HM, Missler M, Collier DA, Fernandes C. Altered social behaviours in neurexin 1α knockout mice resemble core symptoms in neurodevelopmental disorders. *PLoS One*. 2013;8(6): e67114.
87. Won H, Lee HR, Gee HY, et al. Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. *Nature*. 2012;486(7402):261–265.
88. Peça J, Feliciano C, Ting JT, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*. 2011;472(7344):437–442.
89. Harlow EG, Till SM, Russell TA, Wijetunge LS, Kind P, Contractor A. Critical period plasticity is disrupted in the barrel cortex of FMR1 knockout mice. *Neuron*. 2010;65(3):385–398.
90. Bhattacharya A, Kaphzan H, Alvarez-Dieppa AC, Murphy JP, Pierre P, Klann E. Genetic removal of p70 S6 kinase 1 corrects molecular, synaptic, and behavioral phenotypes in fragile X syndrome mice. *Neuron*. 2012;76(2):325–337.
91. Cohen S, Gabel HW, Hemberg M, et al. Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron*. 2011;72(1):72–85.
92. Kwon CH, Luikart BW, Powell CM, et al. Pten regulates neuronal arborization and social interaction in mice. *Neuron*. 2006;50(3):377–388.
93. Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. *Nature*. 2011;480(7375):63–68.
94. Greer PL, Hanayama R, Bloodgood BL, et al. The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. *Cell*. 2010;140(5):704–716.
95. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature*. 2013;493(7432):327–337.
96. Chubykin AA, Atasoy D, Etherton MR, et al. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. *Neuron*. 2007;54(6):919–931.
97. Choi YB, Li HL, Kassabov SR, et al. Neurexin-neuroligin transsynaptic interaction mediates learning-related synaptic remodeling and long-term facilitation in aplysia. *Neuron*. 2011;70(3):468–481.
98. Arida RM, Scorza CA, Scorza FA, Gomes da Silva S, da Graça Naffah-Mazzacoratti M, Cavalheiro EA. Effects of different types of physical exercise on the staining of parvalbumin-positive neurons in the hippocampal formation of rats with epilepsy. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31(4):814–822.
99. Anderson-Hanley C, Tureck K, Schneiderman RL. Autism and exergaming: effects on repetitive behaviors and cognition. *Psychol Res Behav Manag*. 2011;4:129–137.
100. Dillon SR, Adams D, Goudy L, Bittner M, McNamara S. Evaluating exercise as evidence-based practice for individuals with autism spectrum disorder. *Front Public Health*. 2016;4:290.

101. Aberg MA, Pedersen NL, Torén K, et al. Cardiovascular fitness is associated with cognition in young adulthood. *Proc Natl Acad Sci USA*. 2009;106(49):20906–20911.
102. Gomes da Silva S, Unsain N, Mascó DH, et al. Early exercise promotes positive hippocampal plasticity and improves spatial memory in the adult life of rats. *Hippocampus*. 2012;22(2):347–358.
103. de Almeida AA, Gomes da Silva S, Fernandes J, et al. Differential effects of exercise intensities in hippocampal BDNF, inflammatory cytokines and cell proliferation in rats during the postnatal brain development. *Neurosci Lett*. 2013;553:1–6.
104. Pereira AC, Huddleston DE, Brickman AM, et al. An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci USA*. 2007;104(13):5638–5643.
105. Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends Neurosci*. 2008;31(3):137–145.
106. Kelly NA, Wood KH, Allendorfer JB, et al. High-intensity exercise acutely increases substantia nigra and prefrontal brain activity in Parkinson's disease. *Med Sci Monit*. 2017;23:6064–6071.
107. Clark PJ, Bhattacharya TK, Miller DS, Rhodes JS. Induction of c-Fos, Zif268, and Arc from acute bouts of voluntary wheel running in new and pre-existing adult mouse hippocampal granule neurons. *Neuroscience*. 2011;184:16–27.
108. Inoue T, Ninuma S, Hayashi M, Okuda A, Asaka T, Maejima H. Effects of long-term exercise and low-level inhibition of GABAergic synapses on motor control and the expression of BDNF in the motor related cortex. *Neurol Res*. 2018;40(1):18–25.
109. Uysal N, Agilkaya S, Sisman AR, et al. Exercise increases leptin levels correlated with IGF-1 in hippocampus and prefrontal cortex of adolescent male and female rats. *J Chem Neuroanat*. 2017;81:27–33.
110. Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol*. 2010;70(5):304–322.
111. Shcheglovitov A, Shcheglovitova O, Yazawa M, et al. SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients. *Nature*. 2013;503(7475):267–271.
112. Griesi-Oliveira K, Acab A, Gupta AR, et al. Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons. *Mol Psychiatry*. 2015;20(11):1350–1365.
113. Danielson NB, Kaifosh P, Zaremba JD, et al. Distinct contribution of adult-born hippocampal granule cells to context encoding. *Neuron*. 2016;90(1):101–112.
114. Zhang Z, van Praag H. Maternal immune activation differentially impacts mature and adult-born hippocampal neurons in male mice. *Brain Behav Immun*. 2015;45:60–70.
115. Vukovic J, Colditz MJ, Blackmore DG, Ruitenberg MJ, Bartlett PF. Microglia modulate hippocampal neural precursor activity in response to exercise and aging. *J Neurosci*. 2012;32(19):6435–6443.
116. Nakayama H, Miyazaki T, Kitamura K, et al. GABAergic inhibition regulates developmental synapse elimination in the cerebellum. *Neuron*. 2012;74(2):384–396.
117. Peñagarikano O, Abrahams BS, Herman EI, et al. Absence of CNTNAP2 leads to epilepsy, neuronal migration abnormalities, and core autism-related deficits. *Cell*. 2011;147(1):235–246.
118. Han S, Tai C, Jones CJ, Scheuer T, Catterall WA. Enhancement of inhibitory neurotransmission by GABAA receptors having α2,3-subunits ameliorates behavioral deficits in a mouse model of autism. *Neuron*. 2014;81(6):1282–1289.
119. Mao W, Watanabe T, Cho S, et al. Shank 1 regulates excitatory synaptic transmission in mouse hippocampal parvalbumin-expressing inhibitory interneurons. *Eur J Neurosci*. 2015;41(8):1025–1035.
120. Fatemi SH, Halt AR, Stary JM, Kanodia R, Schulz SC, Realmuto GR. Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biol Psychiatry*. 2002;52(8):805–810.

121. Fatemi SH, Reutiman TJ, Folsom TD, Thuras PD. GABA (A) receptor down-regulation in brains of subjects with autism. *J Autism Dev Disord*. 2009;39(2):223–230.
122. Fatemi SH, Folsom TD, Reutiman TJ, Thuras PD. Expression of GABA (B) receptors is altered in brains of subjects with autism. *Cerebellum*. 2009;8(1):64–69.
123. Gomes da Silva S, Doná F, da Silva Fernandes MJ, Scorza FA, Cavalheiro EA, Arida RM. Physical exercise during the adolescent period of life increases hippocampal parvalbumin expression. *Brain Dev*. 2010;32(2):137–142.
124. Schoenfeld TJ, Rada P, Pieruzzini PR, Hsueh B, Gould E. Physical exercise prevents stress-induced activation of granule neurons and enhances local inhibitory mechanisms in the dentate gyrus. *J Neurosci*. 2013;33(18):7770–7777.
125. Wu Y, Dissing-Olesen L, Mac Vicar BA, Stevens B. Microglia: dynamic mediators of synapse development and plasticity. *Trends Immunol*. 2015;36(10):605–613.
126. Jiang T, Zhang L, Pan X, et al. Physical exercise improves cognitive function together with microglia phenotype modulation and remyelination in chronic cerebral hypoperfusion. *Front Cell Neurosci*. 2017;11:404.
127. Kohman RA, Bhattacharya TK, Wojcik E, Rhodes JS. Exercise reduces activation of microglia isolated from hippocampus and brain of aged mice. *J Neuroinflammation*. 2013;10:114.