Animal Models of Autism

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Over the past, the prevalence of autism spectrum disorders has progressively increased, however, no clear diagnostic markers and specifically targeted medications for autism have emerged. As a result, neurobehavioral abnormalities, neurobiological alterations in autism spectrum disorder (ASD), and the development of novel ASD pharmacological therapy necessitate multidisciplinary collaboration.

autism spectrum disorder (ASD) ASD animal model neurobiological mechanisms

1. Introduction

Autism is a collection of genetically variant neurodevelopmental disorders that manifest as early social intercourse dysfunction and impaired repetitive behaviors and interests [1]. Based on estimates from the Centers for Disease Control and Prevention's Autism and Developmental Disabilities Monitoring Network, approximately 1 in 44 children are diagnosed with an autism spectrum disorder (ASD) [2]. The prevalence of autism is over four times greater among boys than girls, and it commonly has co-occurring conditions, including epilepsy, depression, anxiety, and attention deficit hyperactivity disorder, as well as challenging behaviors such as sleep and self-harm [3]. Autistic individuals have atypical cognitive deficits, like impaired social cognition and perception, executive dysfunction, and atypical perception and information processing. These features are underpinned by atypical neurodevelopment at the systems level [4].

Both genetics and environmental factors early in development play a vital role in the etiology of autism [5]. Genetic variation in genes dramatically increases ASD risk. Features of autism may be detected in early childhood, but the diagnosis of autism is usually not made until much later. Early diagnosis requires a joint multidisciplinary assessment, and targeted behavioral interventions and pharmacological treatment can only somewhat reduce the social impairment and emotional instability-induced aggression and decrease the complications, but cannot completely cure them. Current research has not identified clear neuropathological markers of autism that can provide a basis for diagnostic criteria, and at this stage, it is speculated that abnormal behavior in autism is associated with alterations in emerging properties of brain function. Thus, studying the physiological mechanisms and potential pathogenesis of brain circuits is crucial for future diagnostic treatments.

2. Technological Advances in Animal Models of Autism

Autism is a lifelong neurodevelopmental disorder with a vital hereditary element, but environmental factors, including toxicants, insecticide, infections, and medications, have been familiar to contribute to autism

susceptibility. Current animal models of ASD include genetic animal models, CNVs -induced syndrome ASD animal models, idiopathic animal models and environmentally induced types, which have different advantages and individual limitations in generating ASD among different models [6][7]. Researchers can improve drugs and optimize treatment regimens by thoroughly analyzing the mechanics of various animal models. Meanwhile newly developed devices facilitate combination of valproic acid (VPA) with other techniques to probe the neural basis of complex behaviors associated with ASD.

2.1. Genetic Animal Models

Based on family and population studies, ASD heritability is about 50% that of mental illness and is even higher in identical twins [7]. Using present techniques, the genetic cause of the presence of ASD is clear in approximately only 20% cases [8]. In this part, researchers outline the neuropathological abnormalities reported in animal models following various gene ablation and assess the importance of transgenic models in ASD.

Neurexins (NRXNs): NRXN genes encode α and β-neurexin proteins, which assume a significant part in synaptic adhesion, differentiation and maturation as presynaptic binding partners of NLGN [7][9][10]. NRXN1, one of the susceptibility genes for ASD, has point mutations that are often associated with loss of function [11]. NRXN3 is also correlated with abnormal ASD function [12]. KO animals with all three NRXN genes knocked out or double knockout of NRXN1/2 were observed to have reduced inhibitory synapses in the brainstem and cortex. Contact protein-associated protein 2 (CNTNAP2), a member of the NRXN family, encodes contact protein-associated protein-like 2 (CASPR2). recessive mutations or chromosomal inversions in CNTNAP2 have been observed in ASD individuals. CASPR2 is required for dendritic arborization, stabilization of dendritic spines, and α-amino-3-hydroxyl-5-methyl-4isoxazole-propionic acid (AMPA) receptor trafficking. CNTNAP2 knockout mice also showed abnormalities in the corpus callosum and in somatosensory cortex neuronal migration.

Neuroligins (NLGNs): Trans-synaptic complexes formed by postsynaptic NLGNs and presynaptic neurexins are considered to facilitate synaptic stability. Different compositions of these cell adhesion molecules have been associated with formation of glutamate or γ-aminobutyric acid (GABA) synapses. MRI scans revealed that Nlgn3-knockout mice have a smaller brain volume than controls [13]. Nlgn3 knock-in (KI) mice showed increased postnatal turnover of excitatory spines in layer II and III pyramidal neurons of the prefrontal cortex and elevated expression of saccadic GABA transporters in somatosensory cortical neurons, but no changes in inhibitory synapse counts or ultrastructure [14][15]. The volume of multiple brain regions is reduced in $Nlgn3^{R451C}$ KI mice that exhibit the $Nlgn3^{R451C}$ mutation seen in persons with ASD [16]. The $Nlgn3^{R704C}$ KI animal model, mimics an ASD-related mutation, demonstrates reduced AMPA receptor-mediated hippocampal neurotransmission but no similar reduction for N-Methyl-D-aspartic acid (NMDA) or GABA, perhaps due to increased AMPA receptor internalization [17].

SH3 and multiple ankyrin repeat domains protein 3 (SHANK3): SHANK3 is a postsynaptic density (PSD) protein that interacts with and binds with several ionotropic and metabotropic glutamate receptors. ASD and Phelan-McDermid syndrome are linked to SHANK3 mutations [18][19]. SHANK3 mutations and chromosomal rearrangements at 22q13.3 can cause Phelan-McDermid syndrome [20]. It has been suggested that SHANK3-

deficient heterozygotes are associated with the defects observed in ASD and Phelan–McDermid syndrome [21]. Consistently, the current work shows that mouse models having distinct SHANK3 isoforms exhibit ASD behavior [22]. Models that ablate the full-length SHANK3 isoform by deleting exons 4–9 show decreased glutamate receptor 1 immunoreactive spots in hippocampal CA1 [23]. In SHANK3/- mice lacking Shank 3α and β dendritic length and complexity were increased, while PSD length and spiny neuron thickness were lessened. The striatum of KO mouse models disrupting all SHANK3 isoforms show abnormal spine density and PSD. Similarly, reinstating SHANK3 expression in mature SHANK3 deficient mice has been demonstrated to salvage dendritic spine loss and excitatory synaptic function in the striatum [20]. Finally, SHANK3 is critical in coordinating integration of the numerous glutamate receptors of the PSD and linking synaptic signaling to spinal movements.

Tuberous sclerosis complex 1/2 (TSC1/2): Tuberous sclerosis complex (TSC) is an autosomal dominant disorder with a feature of benign tumor nodules in a variety of organs and an elevated risk of malignancy [24]. The prevalence of ASD among TSC mutation carriers is between approximately 36% and 50% [25]. In TSC patients, abnormalities in neuronal migration and differentiation in and around cortical nodules have been observed [26]. Research in TSC2 mutant subjects suggest that cortical nodules have abnormal neurons and aberrant stratification, along with cell loss leading to hippocampal sclerosis and cerebellar atrophy [27][28]. Tsc1 knockout in forebrain pyramidal neurons has little effect on somatic cell size or dendritic shape but increases the spine density of temporal lobe cortical neurons. The spine density and pruning of Tsc2+/− mice layer V neurons in the temporal cortex increased with age. In Tsc2+/− Eker rats, hippocampal neurons show an increase in spine length but a decrease in spine width and number of excitatory synapses. Researches have shown that TSC1/2 gene mutations lead to abatement of its inhibitory effect on mammalian rapamycin (mTOR) protein, causing misregulation of mTOR signaling [29].

Fragile X Mental Retardation 1 gene (FMR1): Fragile X is generated by amplification or uncommon point mutation in the promoter of cytosine-guanine-guanine trinucleotide in the fragile X mental retardation gene [30]. Fragile X syndrome mental retardation protein (FMRP) is an RNA-binding protein that modulates synaptic plasticity by interacting with specific mRNAs in the brain [31][32]. Furthermore, roughly 22% of FMR1 mutation carriers and 30% of men in this category match the ASD diagnostic criteria [23]. It was found that subjects with fragile X syndrome had increased spine density and length in the temporal lobe and visual cortex, predominantly in the immature spine. In the visual cortex of adult FMR1−/− mice, there were more immature spines and less mature spines [33]. Nevertheless, the opposite is true in CA1. FMR1−/− mice have increased spine density in the somatosensory cortex 4–7 days after birth. However, it is not present in the hippocampus. The above experimental results consider that FMR1 has brain region-specific effects in synaptic maturation. It has also been observed that FMR1 shows varying effects on synaptic development with age. Hippocampal neurons in FMR1 KO pups are dominated by the occurrence of short spines, while adult mice have more occurrence of long spines. The variation in spine morphology observed may be caused by changes in spinal flip. Adult FMR1 mice exhibit a higher rate of spine renewal in the visual cortex. The neurological and behavioral damage in FMR1 knockout mice can be rescued to some extent by removing p70 s6 kinase 1 or by treatment with polyunsaturated fatty acids [34]. Pietropaolo's study revealed that daily supplementation with omega-3 fatty acids (n-3 PUFA) also improved social interaction,

emotional and non-spatial memory, and normalized some of the symptoms in FMR1-Ko mice. It caused a reduction in some neuroiflammatory changes in the brain.

Methyl-CpG-binding protein 2 (MECP2): MECP2 gene mutations lead to Rett syndrome, an X-linked neurodevelopmental disorder primarily affecting females [35]. More than 61% of Rett syndrome patients match the criteria for ASD, including repetitive hand movements, social withdrawal, and loss of verbal communication [36][37]. Many human symptoms are generalized by mouse models lacking in MECP2, making them a suitable experimental paradigm for exploring the underlying processes of ASD behavior. Patients with Rett syndrome have reduced brain size, smaller neurons, increased neuronal accumulation, and reduced neuronal dendritic complexity in frontal and motor cortex layers III and V. Some mouse models have shown neocortical thinning. An increase in cell density and smaller neuronal cell bodies were noticed in several brain regions [38][39]. In MECP2-deficient mice, the spinal heads in the dentate gyrus and hippocampal CA1 regions are smaller, and axonal direction in the motor cortex is altered. These changes appear to result from the delayed neuronal growth and synapse formation induced by MECP2 haploinsufficiency, a developmental defect that does not improve with age. MECP2 deficiency leads to fewer glutamatergic synapses and higher baseline levels of AMPA, indicating an activity-dependent failure of synaptic receptor transport [40]. Mutant mice encoding the transcriptional regulator MECP2 gene present autism-like behavioral traits typical of Rett syndrome. Guy et al. found that re-expression of the MECP2 gene manipulated by gene-editing techniques in a mouse model of autism reversed behavioral changes similar to autism, as well as the typical neurological abnormal symptoms of Rett syndrome [34].

Emerging potential genetic models of ASD: In the following discussion, several models are ASD-related genetic models emerging in recent years, and although some of following have been validated for the ASD phenotype, relevant neuropathological data are less available. Thus, there is growing interest in potential emerging transgenic animal models.

The chromodomain helicase DNA-binding protein 8 (CHD8) gene encodes a chromatin-modified gene on chromosome 14q11.2, which has been identified as a high-risk gene for ASD. In a cohort of approximately 6000 autistic patients, 0.2% had specific ab initio CHD8 mutations [41]. Patients with CHD8 mutations have head-size differences, as well as presenting developmental delays, cognitive impairment, motor deficiencies, and anxiety [42] [43]. CHD8 has been found to mediate the transcription of ASD risk factors in human neural progenitor cells, as well as brain development pathways including neuronal differentiation, synaptic development, cell adhesion, and axonal guidance [41]. In P23-25, a 7-nucleotide deletion in exon 1 resulted in CHD8 single-fold resistant (CHD8+/−) animals with somewhat reduced social interaction and diminished preference for social novelty. Relevant MRI analysis indicated that CHD8+/− mice have larger brain volumes than wild-type controls [44]. CHD8 exon 5 was deleted in order to create a strain of CHD8+/del5 mice that showed transcriptional changes in neurodevelopmental disease pathways such as neurogenesis, synaptic processes, and neuroimmune signaling. The anterior cortical and neocortical areas were increased after birth [45].

Sodium channel power-gated type II subunit (SCN2A) mutations are correlated with epilepsy, intellectual disability, and ASD without epilepsy [46]. Generally, mutations altering neuronal sodium channel structure, function, or

expression lead to epilepsy and neurological disease, and SCN2A stop codon mutations lead to termination of protein translation in autism [47]. It has been estimated that 7.5 out of every 100,000 births are diagnosed with a pathogenic mutation in SCN2A. In 50% of patients diagnosed with SCN2A syndrome, symptoms are similar to the more familiar forms of ASD, including reduced social interaction and repeated behaviors [48]. Adult subtypes of Scn2ain mice showed neuronal hyperexcitability [49]. Mice with the Scn2aGAL879-881QQQ mutation exhibited neuronal loss and glial hyperplasia in the hippocampus.

Synaptic GTPase-activating protein 1 (Syngap1) is a Ras-GTPase-activating protein that exists in the postsynaptic density of glutamatergic neurons and participates in dendritic spine formation, glutamate receptor transport, and synaptic function [50]. Syngap1 mutations are linked to various neurodevelopmental diseases, including non-syndromic intellectual disability and ASD [51]. Compared to WT mice, Syngap1 deficiency resulted in early dendritic branching, premature pruning, and larger dendritic spines in somatosensory cortical pyramidal neurons at key times in development. The density of mushroom spines in the hippocampus CA1 of Syngap1+/− mice was enhanced compared with WT mice, but there was no change in the density of fine or thick spines. Selective induction of Syngap1 deficiency in GABAergic neurons causes reduced presynaptic neuron density in somatic cells and decreased axon terminal branching of interneurons on cortical interneurons [52][53]. Syngap1 expression is not expressed in the development of glutamatergic neurons in the forebrain, whereas it is expressed in GABAergic neurons, causing cognitive impairment in mice [23].

Glutamate receptor ionotropic NMDA2B (Grin2b) is linked to epileptic encephalopathy, ASD, and other neurological disorders in which Grin2b haploinsufficiency is often an important factor [54][55]. GluN2B deletion disrupts protein-dependent homeostatic plasticity, according to in vitro electrophysiological investigations. In vitro inhibition of Grin2b causes delayed migration of cortical neurons, as well as increases in dendritic length and branching. The involvement of GluN2B in modulating synaptic maturation has been established in rat cortical and spinal cord co-cultures, with synaptic elimination reduced when postsynaptic GluN2B is lacking. Dendritic spine density is decreased in mice with conditional GluN2B ablation in CA3 pyramidal neurons and CA1 pyramidal neurons [56].

2.2. Syndromic ASD Animal Models Caused by CNVs

15q11-q13 deletions and 15q13.3 microdeletions: Genomic deletions within the chromosome 15q11-13 locus cause different neurodevelopmental syndromes. Prader-Willi and Angelman syndromes are the most common, with Prader-Willi syndrome caused partly by a deletion on the paternal copy of chromosome 15q11.2-q13, uniparental dimorphism, or imprinting center defects. Patients with Prader-Willi syndrome have abnormal dentate and olivary nuclei distribution, dentate nucleus neurodegeneration, cerebellar ectopia, expanded ventricles, volume reduction in the parieto-occipital lobe, numerous cerebellar gyri in the lateral fissure, and smaller cerebellum and brainstem. Angelman syndrome is caused by maternal copy deletion, chromosome 15q11.2-q13 abnormalities or the E6-AP ubiquitin protein ligase (UBE3A/E6AP) gene mutation [57]. Approximately 34% of those affected exhibit autistic-like characteristics. Angelman syndrome patients have smaller brains, cerebellar atrophy, and reduced white matter integrity. In maternal illness models, many and thick neural spines are found, as well as decreased presynaptic GABA vesicle density at inhibitory and excitatory synapses [25][58]. 15q13.3 deletion is associated with an increased

risk of ASD, intellectual disability, schizophrenia, and epilepsy [59]. A newly produced heterozygous D/+ mouse model with a homozygous microdeletion had larger brains and lateral ventricles in adulthood, and this microdeletion has also been linked to head enlargement in humans.

16p11.2 deletion and duplication syndromes: Duplications and deletions of the 16p11.2 gene can result in ASD and other neurological problems [60][61]. According to recent research, 20% of patients with duplications and 16% with deletions had ASD-like behavior [62][63]. Subjects with 16p11.2 deletions displayed large head malformations, whereas duplicated carriers displayed small head deformities [64]. Compared to WT controls, the 16p11.2 deletion model in mice results in decreased brain weight, cortical dimensions and disrupted cortical compartmentalization [65]. Another 16p11+/− mouse model demonstrates an increase in the relative volume of the nucleus ambiguus and pallidum, as well as a decrease in dopaminergic cells in cortical layers V and VI. Although the relative brain volume alterations in certain animal models may not match those reported in people with CNV in this chromosomal region, their usage can reveal cellular pathways. The relative volume changes in animals modeled with 16p11.2 repeats (DP/+) are the inverse of those seen in DF/+ mice. Although certain animal models cannot replicate the abnormalities reported in CNV patients in this chromosomal region, employing these models can show the molecular pathways that cause brain volume changes.

22q11.2 deletion syndrome: Subjects carrying the 22q11.2 deletion are more likely to develop DiGeorge syndrome and are predisposed to various neuropsychiatric diseases [66]. A meta-analysis estimated the prevalence of ASD in deletion bearers to be 11%. When individuals with ASD having the 22q11 deletion were comparing with controls, the right amygdala volume was increased. More medium-sized multispiny neurons and interneurons clustered in the caudate nucleus in the subcortical white matter. In hippocampal CA1, dendritic complexity, spine density and PSD length are not affected in the Df1/+ mouse model with 22q11.2 deletion. In vitro cultures of hippocampal neurons from DfA++ animals revealed lower numbers of mushroom spines, diminished spine length, fewer glutamatergic synapses, and reduced presynaptic vesicle density. The numbers of cells in layers II and V were decreased in DfA++/− animals, as were inhibitory neurons in layer V. The length and intricacy of the basal dendrites were reduced.

2.3. Idiopathic Animal Model

ASD is a neurological illness produced by a combination of circumstances, and mutations in single genes do not adequately duplicate all the clinical symptoms of ASD. Inbred strains of mice and rats displayed substantial and well-replicated ASD-related social impairments and repetitive behaviors in recent years. It is thought to be a model of idiopathic autism.

BTBR-T+ tfl/J (BTBR): In terms of core ASD behavioral traits, BTBR mice are the most fully studied and are the most frequently reproduced inbred breed [67]. The lack of the corpus callosum and significant shrinking of the hippocampal confluence characterize BTBR animals [68]. When BTBR mice were compared to controls, their brain volume was lowered [69]. These findings are consistent with an increase in gray matter volume with time in ASD and are strongly correlated with the seriousness of symptoms. Other notable differences between BTBR mice and

control mice included more neuronal expression of 5-hydroxytryptamine in the median and dorsal caudal spine and fewer axonal terminals in hippocampal CA1 [70], but increased postnatal turnover of excitatory synapses in the prefrontal cortex.

BTBR mice performance is connected with genetic alterations in the brain, including brain-derived neurotrophic factor (BDNF) and synaptophysin. Steinmetz et al. showed that injecting insulin-like growth factor 2 prior in BTBR mice before behavioral testing can reverse abnormal behavior and memory deficits [71]. Social interaction and communication in BTBR mice were increased by administering beta carotene according to Avraham et al. [72]. Silverman et al. reported that by injecting BTBR mice with GRN-529, a modulator selectively metabolizing glutamate receptor subtype 5, excitatory neurotransmission was manipulated, and ASD-like symptoms were improved. As mentioned above, immune system dysfunction was thought to be a factor contributing to ASD-like behavior in BTBR mice. Indeed, Schwarzer discovered that irradiating BTBR mice, achieving bone marrow ablation, then injecting bone marrow cells from normal C57BL mice improved mice's social competence. Studies that proved transplanting bone marrow cells from BTBR mice into C57BL mice and observed that C57BL mice exhibited an increased number of repetitive grooming activities, thus demonstrating the importance of the immune system in social behavior. The advantage of these animal models above is that they allow for molecular and pathological studies of specific brain changes, along with gene editing in an attempt to reverse these ASD-like behavioral changes [73].

2.4. Environmental Models

Models of environmentally induced ASD-like behaviors attempt to treat offspring directly by affecting the mother or early after birth. Generally divided into infectious/inflammatory means and specific chemicals (valproic acid) promoting autism-like behavioral traits.

Specific chemicals (valproic acid) exposure: VPA is a short-chain fatty acid used extensively as an antiepileptic and mood stabilizer [74]. Clinical studies have shown an increased risk of numerous neural tube defects, extracerebral malformations, developmental delays, cognitive impairment and autism when VPA is taken during pregnancy [75]. Fetal valproic acid syndrome (FVS) develops from prenatal exposure to VPA, and children with FVS display a significantly increased incidence of developmental problems, decreased verbal intelligence, and often comorbid communication problems associated with ASD. Intriguingly, rodents exposed to VPA before birth exhibited deficient behavior comparable to that of autistic individuals. Thus, the VPA rodent model has been widely used as a common model for studying the neurobiology of autism and screening new drugs [76].

There is now substantial evidence that maternal challenge with VPA in rodents is a favorable animal model for autism. A possible link between maternal exposure to VPA and offspring ASD was first described by Christianson et al. Later, larger studies confirmed the association between intrauterine exposure to VPA and autism. Based on the Diagnostic and Statistical Manual of Mental Disorders criteria, statistics found that 8.9% of 56 children with prenatal exposure to VPA in the monotherapy study developed autism or Asperger's syndrome. A large study based on the Danish population revealed a 2-fold increase in the prevalence of ASD among 508 kids prenatally exposed to VPA.

Prenatal exposure to VPA in rodents is associated with behavioral and neuroanatomical deficits, including reduced social interactions, greater repetitive behaviors, and more anxiety, accompanied by a reduction in the number of cerebellar Purkinje cells, nucleus damage, and cortical synaptic changes like those observed in ASD humans.

Acute higher doses of VPA exposure resulted in decreased brain weight, increased cortical layer thickness, cell density in the prefrontal and somatosensory cortices, and hippocampus. On the other hand, lower doses had no effect on brain weight, but affected cortical thickness and neocortical cell density. VPA exposure leads to thinning of the early prefrontal cortex (PFC), basolateral amygdala, and hippocampal CA1 [77][78]. VPA exposure leads to a decrease in microalbumin-expressing interneurons in the parietal and occipital cortices and a decrease in the number of motor neurons in some brainstem motor nuclei [79]. VPA-treated rats showed an increase in microglia in the medial PFC and astrocytes in the hippocampus [80].

Current studies have reported that using antioxidants such as astaxanthin, piperine, and green tea extract are effective in preventing VPA-induced ASD-like behavior in rats and mice [81]. The polyunsaturated fatty acids α linoleic acid (ALA) or γ linoleic acid (GLA) attenuate neurobehavioral changes in VPA rat offspring by reducing oxidative stress marker concentrations [82]. A recent study found that VPA rat offspring, injected with guanfacine (an endogenous NMDA receptor antagonist) at one-half hour prior to behavioral testing, alleviated ASD-like symptoms. Interestingly, repetitive behaviors in VPA rat offspring were prevented by low-dose injections of donepezil [83]. A current study found that repetitive behaviors were significantly reduced and social skills improved using human adipose-derived stem cells injected into VPA rat offspring ventricles [84]. However, these drugs must be administered continuously to be effective. There are no data to support successful prevention of VPA-induced neurobehavioral impairment in humans.

Maternal immune activation: Maternal immune activation during pregnancy is highly associated with ASD incidence in offspring [85]. Studies have showed that maternal immune activation leads to alterations in levels of multiple interleukin-like factors in the fetal brain, accompanied by morphological abnormalities in different brain regions [34]. Maternal mid-pregnancy injection with endotoxin and polysaccharide polycyclic acid induced autism-like behaviors in offspring. Viral influenza infection in pregnant mice at mid-gestation resulted in decreased social competence in the offspring. Infection with Borna virus in rats leads to increased stereotypic behaviors associated with autism in offspring. Pioglitazone, an agonist of peroxisome proliferator-activated receptor gamma with anti-inflammatory effects, has been shown to attenuate autistic-like behavioral changes in the offspring of endotoxin-treated rats [86]. These models can be utilized to explore neurological and behavioral changes along with the molecular and biochemical mechanisms involved in neurological pathology, particularly in the hippocampus and cerebral cortex. The majority of these animal models assess inflammatory processes rather than specific viruses.Animal models of genetic mutations in ASD reveal differential effects of genetic variation in individual genes on ASD-related phenotypes. But ASD is heterogeneous, and it is difficult for a single transgenic model to recapitulate all its symptoms. At the same time, transgenic models are susceptible to the influence of genetic background. CNV animal models have high genetic penetrance and a strong association with ASD manifestations. But CNVs are less common in the general patient population. Idiopathic animal models mimic many of the aberrations found in the ASD population. But the behavior of this strain of mice must be compared with that of other unrelated mice [87]. The

rodent with VPA is an important model of autism and may be more representative of many cases of idiopathic autism due to environmental/exogenous causes than autism models carrying mutations in a single associated gene. However, most people with autism are not exposed to the drug before birth. Therefore, the validity of the VPA rodent model may be limited to cases of autism resulting from exposure to drugs that act as histone deacetylase (HDAC) inhibitors.

3. Importance of Multidisciplinary Assessment of Animal Models of Autism

At present, clinical ASD is mainly diagnosed through the basis of behavioral disorders and no specific neurodiagnostic markers are available. Therefore, tools to assess the development of ASD-like behaviors must be created. Currently established ASD rodents assess ASD-like behaviors in humans primarily through the following behavioral tests. In order to explore potential tools that may engage in the neurobiological mechanisms of human ASD, a multi-dimensional approach is proposed to study animal models of ASD, combining core behaviors, neuroimaging, neuropathology, and neurochemistry in animal models.

3.1. ASD Core Behavioral Testing

Core behaviors in ASD mainly show behavioral cognitive deficits of social behavior impairment, repetitive behaviors, and cognitive rigidity. It is mainly assessed by ultrasonic vocalization test, open-ended test, social novelty preference test, maze experiment, and novelty recognition test.

Ultrasonic vocalization in mice may be a valuable marker to differentiate between the control and ASD models [88]. Autism-like disorders can be detected in interactions between offspring and their mothers. The number of ultrasonic vocalizations decreased after separation of prenatal VPA-exposed pups from their mothers in isolation testing, while the duration of vocalization events consistently increased [89]. Where calls had a lower amplitude, there were more flat calls, a change in the number of complex and downward calls, less variety in call types, and fewer 2-syllable calls [90][91]. Instead, offspring injected with VPA between gestation days 11 and 13 exhibited increased call frequency [92]. Other flaws included lower call amplitude, complicated and downward call number alterations [90]. Although contradictory to earlier findings in the literature, it may be attributable to the divergent dose ranges of VPA employed in various experiments.

Open-field tests are used to evaluate changes in gross locomotor activity in rodent performance levels. The test is commonly applied in classical experiments to detect core symptoms of an ASD diagnosis including stereotyped motor behavior, repetitive self-modification, and restriction of exploration activities. The open-ended test is strongly associated with social behavior and anxiety in mice and rats with autism [93].

Three-chamber social interaction tests are used to measure social competence and preference for social novelty by calculating the ratio of time spent on novel social stimuli to time spent on familiar social stimuli. In rats and mice exposed to VPA, the three-compartment social approach revealed deficits in social interaction [94]. Markram

discovered that VPA- exposed rats and mice exhibited reduced play activity and exploration of heterotypic adventures, which lends more credence to the effect of VPA on sociality.

The T-maze test is used to assess memory and cognitive abilities in rodents. The animal's capacity to spontaneously alternate is examined using a closed apparatus, and is primarily affected by hippocampal dependent function [95]. Water T-maze serves to measure reversal learning, cognitive rigidity, and repeated behavior. The animal must suppress the first learning reaction and learn a new place on the platform [96]. Three-arm maze is used to evaluate short-term memory, measuring mainly hippocampal function in mice and rats [97].

3.2. Neuropathology

Neuropathological studies can evaluate subtle features affecting ASD patients' brains such as neuronal differentiation migration, morphology and spatial distribution. Numerous neuropathological examinations have been performed in ASD, and here an update of these results is provided.

Neuron size, number, and density: Early autopsy studies compared the number of neurons and neuroglia in various regions of the cerebral cortex of autistic patients to age- and gender-matched controls. Autistic brains had lower neuroglial/neuronal ratios across the board, but no significant differences in cell density were detected [98]. Another study discovered 79% more neurons in the DL-PFC and 29% more neurons in the M-PFC in 7 male autistic children than in 6 controls [99]. The autistic group's brain weight was slightly higher than average, indicating a pathological increase in neuron number. A stereological study [100] estimated neuronal volumes in the cortical structures, hippocampus, arches, cerebellum, and brainstem of 14 autistic individuals. Neuronal volumes were observed to be reduced in the locations studied. Significant deficits in 14 subregions were detected in four autistic patients aged 4–8 years, but volume deficits were found in only three or four of the 16 examined regions in six subjects aged 11–23 years and four subjects aged 36 years. Purkinje cells and neurons in the claustrum have consistently decreased neuronal volumes throughout their lifespan. However, the developmental trajectory of neuronal volume changes revealed an increase in neuronal volume in both adolescents and adults with autism, as well as a decrease in neuronal size in most regions in older controls, indicating an abnormal neuronal growth trajectory.

Neuronal migration impairment: It has been proposed that cerebral cortical abnormalities found on magnetic resonance imaging examination of autistic individuals are caused by a malfunction in neuron migration to the cerebral cortex during the first 6 months of gestation. Other signs of cortical dysgenesis identified in autism patients include thicker cortex, high neuronal density, minicolumnar changes, the presence of neurons at the molecular layer, abnormal laminar patterns, weak grey-white matter borders, and ectopic grey matter [101]. The discovery of lower levels of Reelin in post-mortem cerebellum tissue from autistic patients lends credence to the idea of disrupted neuronal migration in autism. Reelin expression was demonstrated to be decreased in the cerebral cortex of pregnant mice offspring exposed to human influenza virus in pregnancy [102].

Other neuropathologies: Research found disturbances in cortical cell patterning in the superior temporal gyrus, dorsolateral frontal lobes, and dorsal parietal lobes, including dysplasias, related lamination disturbances, and a vaguely less defined gray/white matter border in 8 ASD participants. The most reliable indicator of an affected area was a lack of expression of excitatory cortical neuron markers [103]. These patches of aberrant laminar cytoarchitecture and cortical disarray were observed in neurons but not in glia. However, there were significant differences in which cell types and layers were most affected by the pathological features. In ASD patients, a dramatic reduction was observed in pyramidal neuron size in the inferior frontal cortex suggesting that long distance communication is hindered. This was confirmed by neuropathology [104]. Meanwhile, hypoactivation of the syrinx gyrus was observed in the temporal cortex of ASD, which may be related to reduced mitochondrial energy metabolism. The subventricular zone of the lateral ventricles is one of two neurogenic niches in the brain that are required for neural proliferation, migration, and differentiation throughout both prenatal and postnatal development. Early analysis in ASD patients with the amygdala showed reduced volume and higher neuronal density in the medial, central and cortical nuclei, whereas the most recent quantification in ASD patients showed a significant reduction in the number of neurons in the amygdala as a whole or in the lateral nucleus of the amygdala [105]. The reduction in the number of neurons might be due to less neurons formed during the developmental process or could be caused by abnormal degeneration of cells that occurs after normal early development loss.

3.3. Neuroimaging

In the past decade, in vivo MRI research has contributed many useful insights into the neural basis of ASD. Human neuroimaging research may contribute to biomarker development for ASD and other neurodevelopmental disorders, as well as novel approaches to diagnosis and treatment.

A growing number of neuroimaging studies support early atypical brain development and widespread alterations in ASD neurological connections. Normal brain development is reliant on both cellular and synaptic growth, as well as the properly timed trimming of neurons and synapses [106]. This balance appears to be compromised among some children with autism. A recent neuroimaging study addressing 6 months of age at-risk infants with ASD showed that the aforementioned children exhibit abnormal connectivity in brain trajectories. According to multiple studies, the extent of atypical connectivity at 6 months of age relates to future symptom severity [107][108]. Children with ASD exhibit a sustained expansion of cortical surface area from auditory and visual processing sensory areas beginning at 6 months to 12 months of age, with overdevelopment at 12 months to 24 months of age [109]. Similarly, the overgrowth observed in individuals with ASD may not represent new neurodevelopment because neuronal cells may not be apoptotic and pruned early in development due to neuronal overgrowth. Children with autism continue to have larger brain sizes than their counterparts from the age of 2 to 4 years. By school age, brain development has slowed and the brain volume of normally growing children is catching up to the brain volume of children with autism. Studies of connectivity have revealed persistent impairments in the way brain regions are connected throughout childhood, adolescence, and maturation. A major pooled study of functional MRI resting-state data from ASD patients compared to age-matched normal controls showed widespread low connectivity in distant cortico-cortical and hemispheric projections. In contrast, subcortical regions exhibited local hyperconnectivity [110]. Overall,

these findings indicate that higher brain activity requiring communication between brain regions are suppressed to the benefit of local circuits that may be hyperactive and difficult to interrupt.

Neuroimaging studies are advancing people in understanding the biology of ASD, however, there is no evidence to support that routine neuroimaging can confirm a diagnosis of ASD. Before neuroimaging may be considered for clinical application, further research is needed to better understand and standardize the developmental trajectory of the brain in ASD, which could eventually be used to detect children at risk of developing ASD before overt symptoms appear.

3.4. Neurochemistry

From a neurochemical perspective, brain structures and neural circuit activity are regulated by a combination of neurotransmitters. Changes in neurotransmitter concentrations and dynamics can affect neuron-related functions [111]. Growing evidence suggests disturbances in the neurotransmitter system may be associated with ASD, including mainly GABA, glutamate, serotonin, dopamine, and N-acetyl aspartate, among other agents.

Gamma aminobutyric acid (GABA), derived from glutamate by the action of glutamic acid decarboxylase, is the most frequent excitatory neurotransmitter in the developing brain and has a complex link to neuronal excitability [112][113][114][115]. Alterations in the gabaminergic and glutaminergic systems can disrupt the excitatory/inhibitory balance, which is a possible causative factor in autistic development. Elevated excitatory/inhibitory balance impairs information processing and causes social-behavioral dysfunction. In mouse models with mutations in SHANK3 and the glial-neuropilin complex, glutamate concentrations in the striatum were shown to be reduced [115]. Furthermore, in magnetic resonance spectroscopy tests, reduced GABA was observed in participants in motor, visual, auditory, and somatosensory regions, as well as in the left hemisphere lateral fissure region, resulting in aberrant information processing [116]. When compared to controls, kids with autism had markedly elevated plasma GABA and glutamate/glutamine ratios, but significantly lower plasma glutamine levels and glutamate/GABA ratios [117]. MECP2 mutant mice change synaptic physiology by decreasing glutamic acid decarboxylase-1 and -2 levels and GABA immunoreactivity, resulting in GABA dysfunction and various autism-like Rett syndrome characteristics. Several studies have highlighted links with GABA receptor single nucleotide polymorphisms [118][119].

Glutamate is the major excitatory neurotransmitter in the mammalian cerebral cortex. N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), and metabotropic glutamate receptors (mGluRs) are the three main categories of glutamate receptors [120]. NMDARs and AMPARs are thought to be associated with ASD [121]. It has been shown that overexpressing NMDA receptor subunits in rodent models of autism enhances synaptic currents mediated by NMDA receptors, thereby enhancing postsynaptic plasticity [122]. Additionally, modifications to the AMPA Receptor 2 (GluA2) subunit can profoundly affect neuronal excitability, which is associated with neuropsychiatric disorders including mental retardation and Rett syndrome. Mouse models of autism containing Cyclin-dependent kinase like-5 deficiency showed significant reductions in GluA2 in the hippocampus [123]. Recent studies have reported that the cerebellum is associated with autism spectrum disorders [124]. Interestingly, it was demonstrated for the first time that the cerebellar granule layer

was altered in the islet brain-2 (IB2) KO mouse model and triggered autistic symptoms and severe delayed motor deficits. The IB2 KO mouse model has high activity and plasticity of NMDA receptors, which determines an increased excitatory/inhibitory balance and enhanced long-term potentiation of mossy fibers and granule cells [125]. Also, early correction of NMDAR dysfunction in a mouse model showed dramatic improvements in autistic-like behavior [126]. Mutations in synapse-formation and -maintenance genes as well as protein-targeting genes, have been linked to development of autistic traits and glutamatergic dysfunction [127].

Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter that influences a variety of brain activities including memory and learning capacity [128]. moreover, acting as a sleep and mood regulator [129]. Serotonin transporter protein or serotonin levels are higher in children with autism and animal models than in controls. Studies have indicated that whole-brain 5-HT synthesis is reduced during childhood in children with autism, but gradually increases between the ages of 2 and 15 years, reaching 1.5 times the normal adult value [130][131]. Polymorphisms in the serotonin transporter protein gene (SLC6A4), which encodes platelet and neuronal transport of 5-HT, have been associated with autism. Higher 5-HT levels found in ASD are abundant in children with the SLC6A4 polymorphism [132]. Obviously, valuable animal evidence suggests that embryos developing in SLC6A4+/− mothers are less resistant to prenatal stress, which increases the risk of offspring developing ASD-like traits [133]. Multiple studies have examined platelet hyperosmolarity in ASD subjects, with a mean increase of 20% to 50%. Intriguingly, this increase appears to be unique to autism, as it has not been observed in intellectual disability or other neuropsychiatric disorders.

Dopamine, in addition to controlling locomotion, influences social cognitive and behavioral characteristics via the central cortical circuit [134]. Several investigations have discovered that ASD is connected to dopamine dysfunction [135][136]. According to research, disfunction of mesocorticolimbic circuit causes social impairment in autism, whereas dysfunction of nigrostriatal circuit results in stereotyped behavior [137]. Drug-induced nigrostriatal pathway dysfunction produced stereotypic behavior in mice [138]. Indeed, D1 dopaminergic receptor antagonists were given and these behaviors were reduced. A recent study supports the idea that mesocortical brain circuit can influence social behavior through bidirectional control of dopaminergic projections from the ventral tegmental area to the nucleus accumbens. Optogenetic stimulation of neurons in the dopaminergic ventral tegmental region activates D1 receptors, increasing the amount of time the animal spends on social interactions [139]. Genetic research has demonstrated that autism is linked to polymorphisms in several genes related to the dopaminergic pathway, such as the dopamine receptors DR3 and DR4, or the dopamine transporter protein (DAT) [140]. One recent study concerning a mouse model emphasized the mutation in DAT that triggers abnormal dopamine efflux, leading to an autism-like behavioral phenotype [141].

Acetylcholine is a neurotransmitter and neuromodulator in the central nervous system that is the major neurotransmitter of motor neurons and the parasympathetic nervous system at the neuromuscular junction [142]. Abnormalities in the cholinergic system induce ASD [143]. Current ASD autopsy has revealed substantial decreases in nicotinic subtype acetylcholine receptors (nAChRs) in brain tissues from the parietal and frontal cortex [144]. Another research found a decrease in α4 nAChRs in the cerebellum, considering that it may be related to the loss of Purkinje cells and a compensatory increase in α7 nAChRs. Several investigations on ASD animal models have

revealed that nAChRs have a role in controlling social and repetitive behaviors [145]. α4 nAChR subunit knockout and β2 nAChR subunit knockout mice exhibit increased anxiety and abnormal sleep patterns. α7 nAChR receptors are abundantly expressed in the hippocampus and frontal cortex [144], and activation of this receptor has a cognitive-promoting impact in animal models [146]. Furthermore, choline supplementation during pregnancy enhances the fetal brain response to maternal immunological stimulation and avoids certain caused behavioral abnormalities in the offspring.

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