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Autism spectrum disorder: Neuropathology and animal models

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Abstract

Autism spectrum disorder (ASD) has a major impact on the development and social integration of affected individuals and is the most heritable of psychiatric disorders. An increase in the incidence of ASD cases has prompted a surge in research efforts on the underlying neuropathologic processes. We present an overview of current findings in neuropathology studies of ASD using two investigational approaches, postmortem human brains and ASD animal models, and discuss the overlap, limitations and significance of each. Postmortem examination of ASD brains has revealed global changes including disorganized gray and white matter, increased number of neurons, decreased volume of neuronal soma, and increased neuropil, the last reflecting changes in densities of dendritic spines, cerebral vasculature and glia. Both cortical and non-cortical areas show region-specific abnormalities in neuronal morphology and cytoarchitectural organization, with consistent findings reported from the prefrontal cortex, fusiform gyrus, frontoinsular cortex, cingulate cortex, hippocampus, amygdala, cerebellum and brainstem. The paucity of postmortem human studies linking neuropathology to the underlying etiology has been partly addressed using animal models to explore the impact of genetic and non-genetic factors clinically relevant for the ASD phenotype. Genetically modified models include those based on well-studied monogenic ASD genes (*NLGN3*, *NLGN4*, *NRXN1*, *CNTNAP2*, *SHANK3*, *MECP2*, *FMR1*, *TSC1/2*), emerging risk genes (*CHD8*, *SCN2A*, *SYNGAP1*, *ARID1B*, *GRIN2B*, *DSCAM*, *TBR1*), and copy number variants (15q11-q13 deletion, 15q13.3 microdeletion, 15q11-13 duplication, 16p11.2 deletion and duplication, 22q11.2 deletion). Models of idiopathic ASD include inbred rodent

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strains that mimic ASD behaviors as well as models developed by environmental interventions such as prenatal exposure to sodium valproate, maternal autoantibodies and maternal immune activation. In addition to replicating some of the neuropathologic features seen in postmortem studies, a common finding in several animal models of ASD is altered density of dendritic spines, with the direction of the change depending on the specific genetic modification, age and brain region. Overall, postmortem neuropathologic studies with larger sample sizes representative of the various ASD risk genes and diverse clinical phenotypes are warranted to clarify putative etiopathogenic pathways further and to promote the emergence of clinically relevant diagnostic and therapeutic tools. In addition, as genetic alterations may render certain individuals more vulnerable to developing the pathological changes at the synapse underlying the behavioral manifestations of ASD, neuropathologic investigation using genetically modified animal models will help to improve our understanding of the disease mechanisms and enhance the development of targeted treatments.

Keywords

autism spectrum disorder; cerebral cortex; genetically modified animal models; neuronal morphology; synapse; idiopathic autism models

Introduction

Autism spectrum disorder (ASD) is typically diagnosed within the first three years of life, and is defined by persistent deficits in social communication and interaction, as well as restrictive and repetitive behaviors [8]. ASD affects 1 in 68 children in the United States and affects boys 4.5 times more often than girls [68]. Behavioral interventions produce significant results in some cases, with early diagnosis being critical for better prognosis [7, 268]. No cure is currently available and current pharmacological treatments do not address the core ASD behaviors but target comorbid conditions such as seizures, anxiety, depression and attention deficit hyperactivity disorder (reviewed in [88, 125]).

Despite increased research efforts, the pathogenesis of ASD is not fully understood. ASD without a known specific cause is referred to as idiopathic ASD and constitutes the majority of cases. Various environmental factors have been proposed that contribute to idiopathic ASD, such as maternal immune activation and prenatal exposure to toxins. Among the known genetic causes of ASD are *de novo* mutations identified in approximately 20–30% of cases [81, 286]. The genetic abnormalities associated with ASD may be grouped into three classes: (1) at least 5% are caused by single gene mutations, such as those found in *SHANK3*, *FMR1*, or *MECP2*, (2) approximately 10% are copy number variants (CNVs) including duplications, large deletions, inversions, and translocations of chromosomes, and (3) many are polygenic risk factors due to accumulation of common variants, each contributing to a portion of the risk. Recent advances in genetic techniques, especially whole-exome sequencing applied to large cohorts, have uncovered forms of genetic variation contributing to risk and enabled the identification of additional risk genes [83, 165, 166, 254, 261, 309].

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A recent prospective magnetic resonance imaging (MRI) study found that early enlargement of cortical surface area between 6 to 12 months of age could predict for ASD diagnosis in infants, and that the rate of volume change in the second year correlates with ASD severity [151]. Functional neuroimaging studies have been crucial for highlighting the presence of altered activation patterns in specific brain regions of patients with ASD, such as those involved in emotional regulation and social interaction (for reviews, see [241] and [320]). However, given the technical limitations of neuroimaging studies, such as low spatial resolution and the need for higher functioning individuals as subjects, these studies need to be complemented by other approaches. Two complementary approaches address the limited resolution of neuroimaging studies to detect changes at the cellular level. First, neuropathologic explorations in human postmortem tissue allow for investigation at the cellular and cytoarchitectural levels from individuals with ASD across the spectrum. Second, the use of genetic manipulations to create ASD model organisms enables the identification of molecular pathways central to the pathogenesis of ASD. The consistent presence of changes in the cerebellum revealed by neuropathologic investigations in ASD [35, 385, 386] and the identification of synaptic dysfunction in ASD based on studies of mouse models constitute successful examples of each of these approaches [39].

In the first part of the review, we summarize the existing knowledge regarding cortical and non-cortical brain areas most consistently implicated in the neuropathology of ASD (Figure 1) in postmortem human studies. In the second part, we provide an overview of the most significant contributions to the understanding of ASD neuronal and synaptic pathology using mouse models genetically modified or derived through manipulation of their environment. Finally, to highlight possible strategies to overcome some of the limitations of both research approaches, we discuss the current views on the interplay between neuropathology and disease mechanisms.

Neuropathology of ASD: recent findings from human studies

Investigating the neuropathology of ASD enables the identification of its impact on the subtle characteristics of the human brain that cannot be assessed through neuroimaging studies, such as differentiation and migration processes, neuronal morphology and spatial distribution or cytoarchitectural alterations. Many neuropathology studies have been carried out in ASD (see [272] for review) and we present here an update on these findings. However, with the extreme etiological and phenotypic heterogeneity of ASD and the small sample size (average number of cases/study = 7.43), all reports should be considered provisional and awaiting replication.

Cortical areas: abnormalities in differentiation and migration, and alterations in cytoarchitecture and neuronal numbers

Whole brain changes—Global brain developmental abnormalities manifest in the archicortex, cerebellum, brainstem, and other subcortical structures, with region-specific severity of neuropathology, in young children with ASD [376, 377]. The brain size as well as the head circumference of a subset of subjects with ASD is increased compared to normal age-based values (see meta-analysis in [308]). The increase in brain size in many subjects

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with ASD may be due to various underlying reasons as discussed below, including an increase in the number of neurons or no change in neuron numbers but increased neuropil.

The microarchitecture of cortical areas commonly implicated in ASD was assessed to determine qualitative patterns of developmental disparities between subjects with ASD and controls [379]. Overall, there is a disorganization of gray and white matter in the form of thickening in the subependymal layer. Disorganized cortical structure (dysplasia) and nodules of misplaced neurons (heterotopia) can be detected in individuals with ASD; in some cases dysplasia and heterotopia are observed concomitantly in the same individual [59, 339, 379]. These defects reflect alterations in neuronal maturation and migration processes in subjects with ASD. Expression of Reelin, a protein necessary for neuronal migration during cortical lamination, is decreased in brains from subjects with ASD, although the density of layer I neurons expressing Reelin in the superior temporal cortex of subjects with ASD is comparable to that in controls [48]. Subjects with ASD have increased volume of white matter hypointensities around ventricles and in deep subcortical areas as compared to controls, the changes remaining static with age suggesting a developmental origin [34]. A study from one subject with ASD described finding pencil fibers consisting of oligodendrocytes, astrocytes and glia that disrupted cortical lamination in the prefrontal cortex (PFC) [147].

Minicolumns represent a functional structure where afferent, efferent and local connections of pyramidal projection neurons come together in the neocortex [55, 246]. Minicolumnopathy may be related to macroencephaly [76], abnormal connectivity, and early age of onset of ASD [54]. Patients with ASD have smaller and more numerous minicolumns with more dispersed neurons in Brodmann's areas 9, 21, and 22 [57, 58], and patients with Asperger's syndrome have smaller, less compact minicolumns in the temporal neocortex [56]. Differences in frontal minicolumnar growth trajectory show narrower minicolumns in the dorsal and orbital frontal cortex in ASD [46], which are not present in the primary visual cortex, suggesting that these changes may be regionally specific. However, all these studies of minicolumn changes in ASD originated from one group that uses an indirect method of quantification from 2-dimensional images rather than 3-dimensional stereological techniques; hence, these findings need to be interpreted with caution until they are independently replicated. Moreover, the concept of minicolumns as a fundamental unit of neocortical organization is being challenged by recent findings of lateral expansion during neurogenesis in the primate neocortex that disrupt the radial unit hypothesis underlying columnar organization during corticogenesis (for review see [220]).

Perikaryal abnormalities originate from volume differences in both the neuronal nucleus and soma cytoplasm, both of which have altered trajectories in ASD due to defects in neuronal development and maturation. Young children with ASD have significantly reduced neuronal and cytoplasmic volumes in the majority of examined areas compared to age-matched controls [376, 377]. The distribution of neuronal sizes becomes more comparable between control and ASD in adulthood [376]. This is likely a result of opposing developmental trajectories, with nuclear volume increasing with age in ASD in half of the examined brain areas, whereas nuclear volumes are reduced in most brain areas of controls as they aged [377]. Region-specific neuronal soma alterations are characteristic of both idiopathic ASD

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and some syndromic forms [378]; however, the trajectories are unique. Thus, cell number and volume of pyramidal neurons in layers II and III and layers V and VI of the anterior superior temporal cortex are unchanged in ASD [194] and lower cell density is found in the septal, but not striatal, subventricular zone in ASD when epilepsy is not a comorbidity [205]. Although neuronal volume reduction is most apparent in young children with idiopathic ASD, persistent reduction of neuronal volume is observed in subjects with 15q11.2-13.1 duplication syndrome through young adulthood [378] and in subjects with Rett syndrome through adolescence [25].

Subjects with ASD also have a significant increase in neuropil, comprising the dendrites, non-myelinated axons, synapses, vasculature and glial cell processes present in between cell bodies, in the frontopolar region and the anterior cingulate gyrus, but not in the primary visual, motor, and somatosensory and dorsolateral prefrontal association cortices [54]. Neurons show reduced dendritic branching in the hippocampus of ASD subjects compared to controls [296] and fewer dendrites as detected by microtubule-associated protein 2 immunoreactivity in the PFC [248]. Although a qualitative study reported decreased spine density on apical dendrites of pyramidal neurons in the neocortex [387], subsequent studies that quantified spine density showed greater spine density on apical dendrites of layer II pyramidal neurons in the frontal, temporal, and parietal cortex as well as layer V neurons of the temporal cortex of subjects with ASD compared to controls [162]. Slower pruning of spines in the temporal lobe results in the difference in spine densities between ASD and controls being greater in adolescence than in early childhood [349]. Higher density of axons has been described in the serotonin pathways of subjects with ASD compared to controls [17]. Additionally, loss of cortical lamination in the distribution of axons in the fusiform cortex as well as abnormal morphology such as thick axons and varicosities are observed in the temporal cortex, as well as the amygdala, and hippocampus in ASD [16]. Stereological quantification would be required to confirm these findings. The presence of nestin-immunoreactive pericytes and CD-34-immunoreactive endothelial cells indicates that vascular remodeling persists in the superior temporal cortex, fusiform cortex, midbrain and cerebellum in ASD beyond childhood when they usually decline in controls [15]. The pathology of microglia in ASD has also been assessed in a few patient studies. One study quantified microglia density in the frontoinsular cortex and the visual cortex, and found significantly increased density in both, concluding that this increase in microglia is likely present throughout the brain [352]. Others have found region-specific neuron-glia abnormalities (see section on ‘Prefrontal cortex’ below), although whether the alterations are widespread or distinct to certain areas and what their role is in ASD, is yet to be resolved. For example, whereas no changes in the number of microglia, astrocytes or oligodendrocytes were observed in the amygdala in ASD, a small subset of the cases showed increased microglial activation and greater number of oligodendrocytes [244], and glial fibrillary acidic protein-immunoreactive ependymal cells in the striatum [205].

Prefrontal cortex—The PFC is known for its role in cognitive control, as it coordinates memory, planning and executive activity of other individual brain areas towards behavioral outcomes [116, 238]. The PFC has consistently been shown to have abnormal overgrowth in young children with ASD from about the age of 2 to 5 years [52, 53, 152, 319, 334].

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Although one study reported a 67% increase in neuron number in the entire PFC of boys with ASD, with a striking 79% increase in the dorsolateral PFC and a 29% increase in the medial PFC, these results need to be interpreted with caution as the method used to estimate the volume of reference for the regions of interests in order to derive total cell counts is difficult to assess based on the reported available materials. This increase is limited to neurons, as the difference in glial number is insignificant [77]. The number of parvalbumin-immunoreactive interneurons, but not those expressing calbindin or calretinin, is decreased in the medial PFC [148], which was recently confirmed to be due to fewer chandelier interneurons [12].

Patches of abnormalities, identified by a decrease in neurons expressing layer- or cell-specific markers typically present in fully differentiated cortical neurons, have been identified in the dorsolateral PFC of subjects with ASD [339]. Such patches in laminar architecture are distinct in each individual but are most often characterized by reduced excitatory cortical neuron markers, although there is no overall reduction of neuron density. Glia-specific markers remain unchanged, suggesting there is no global downregulation of expression, but rather a cell- and region-specific abnormality in subjects with ASD. However, previous studies have found altered spatial organization in microglia, observing their distinct gathering around neurons in the dorsolateral PFC in ASD [245].

The inferior frontal cortex is involved in connecting words to convey and infer relationships and concepts, imitation, language production, empathy and social processing networks, and changes in any of these functions may contribute to the ASD core symptom of social communication deficits [43, 218]. In the inferior frontal cortex, subjects with ASD have significantly smaller pyramidal neurons, although the number of pyramidal neurons and the volume of layers is comparable between groups [173]. The significantly reduced size of pyramidal neurons suggests that long-range communication may be impaired, which is supported by similar neuropathological findings in regions that communicate with the inferior frontal cortex [318, 362].

Fusiform gyrus—The fusiform gyrus (FG) plays a major role in our ability to process faces and therefore is a crucial part of our capacity to interact appropriately in social situations. Even though not conclusive (see for example, [137]), most functional magnetic resonance imaging studies report a hypoactivation of the FG in ASD [38, 185, 283–285]. The hypoactivation may be a reflection of reduced mitochondrial energy metabolism, seen in the temporal cortex of ASD compared to controls [350]. The FG was stereologically assessed from a neuropathologic standpoint [362], with the hypothesis that FG hypoactivation may reflect alterations in neuron density, total neuron number, and mean perikaryal volume. Subjects with ASD have significantly lower neuron densities in layer III, significantly lower total neuron numbers in layers III, V, and VI, and significantly smaller mean perikaryal volumes in layers V and VI of the FG (Figure 2), compared to controls. The primary visual area and the whole cortical gray matter show no differences between subjects with ASD and controls, confirming that the observed changes are FG-specific. The posteroinferior occipitotemporal gyrus (PIOTG) is involved in visual object recognition, language and word processing, and face processing [60, 61], but does not show significant differences in pyramidal neuron number and size or in layer volume, between ASD and

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control groups [358]. Although the PIOTG is within the facial processing network that includes the FG and amygdala, both of which present with neuropathology, the typical cellular architecture in the PIOTG suggests that the neuropathology seen in subjects with ASD is specific to the FG.

These results support neuroimaging data showing abnormal activation of the FG in ASD putatively associated with the social deficits observed in this disorder [317]. In fact, layer III is primarily a corticocortical projection layer, layer V axons typically extend to subcortical areas, and layer VI sends efferents principally to the thalamus [182]. The neuropathology in the FG suggests that there may be a similarly reduced connectivity between the FG and its cortical inputs, primarily from areas that visually analyze faces [149, 290], and outputs, to areas that evaluate facial expression and reward value [170, 171]. Subcortically, the FG projects to the amygdala, which is thought to underlie understanding of the emotional significance of stimuli [101, 355]. Consistent with a disconnect between these regions, the amygdala has an overall decrease in neurons in ASD [318]. A more recent study reexamining the FG did not find numerical or cytoarchitectural differences between subjects with ASD and controls [262], possibly owing to the study design that grouped layers instead of analyzing them separately. Also, a difference in the age-range of the examined cases should be taken into account given that the earlier study assessed young children and young adult ASD subjects, whereas the later study focused on adolescence to adulthood. To understand the implication of the FG in ASD further, a developmental approach is warranted and future studies would benefit from exploring narrower age-ranges and comparing findings among different age groups.

Frontoinsular and cingulate cortex—The presence of consistently impaired social skills in ASD has prompted an intense exploration of cortical areas implicated in emotional regulation and awareness of oneself and others [95, 131, 133, 241]. Interestingly, a particular population of distinct neurons, the von Economo neurons (VENs), present almost exclusively in the frontoinsular (FI) and anterior cingulate cortices (ACC) have been found to be consistently affected in ASD [4, 104, 256, 258]. Given their distribution in these cortical areas, these neurons have been suggested to subserve circuits involved in social cognition, autonomic regulation, and sense of self [5, 6, 323]. VENs are large, vertical bipolar neurons found in layer V of the FI and ACC (Figure 3) [256, 258, 366] and, in fewer numbers, in area 9 of the PFC [104]. Their cortical distribution as well as the bipolar shape of their dendritic tree is linked to a possible role in the integration of homeostatic information (processed in the FI) and goal-directed cognitive assessments (processed in the ACC and PFC) in the fast intuitive evaluation of complex social situations [5, 6, 375]. The selective disruption of VENs in disorders marked by social deficits [188, 195, 321–323] supports their hypothesized role in social cognition and suggested that VENs may be affected in ASD. An early study quantifying VENs in ASD [192] reported a trend-level significant increase in VEN number in subjects with ASD, consistent with a recent observation [6] of increased VEN density in layer V of the FI and their abnormal presence in layer VI and in the white matter in subjects with ASD.

More recently, Santos and colleagues [311] used a stereologic approach to quantify VENs and pyramidal neurons in layer V of the FI. They showed that children with ASD

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consistently have a significantly higher ratio of VENs to pyramidal neurons than control subjects. This result may reflect the presence of neuronal overgrowth in young subjects with ASD and may also be related to alterations in migration, cortical lamination, and apoptosis. Qualitatively, the same study reported abnormalities in VEN morphology (Figure 3d) and cortical lamination (Figure 4), which may underlie disrupted information processing.

The ACC, which functions in concert with the FI in the processing of emotions and their integration in decision-making [132], can be grossly subdivided into anterior and posterior parts. Consistent alterations in activation patterns in the ACC have been demonstrated through imaging studies of subjects with ASD [21, 95, 105–107, 153, 263, 384], and this region is unusually coarse with poor lamination in ASD [191]. The ACC was qualitatively assessed, with the hypothesis that areas in which neuronal size and density were altered may represent abnormal neuronal development or circuitry [327]. Neuronal densities are higher in layers I-III of area 24a in the left hemisphere, neuronal size is reduced in all layers of area 24b, and neuronal packing density is reduced in layers V-VI in area 24c, consistent with previously reported reductions in neuronal density in the FG and amygdala [318, 362]. In line with the heterogeneity of the disorder itself, these authors found distinct subgroups in VEN density in comparison to controls, with some reporting significantly higher VEN density in areas 24a and 24b, or significantly lower density of VENs in 24a, b and c. Similar to the effect of increased numbers and an atypical cortical lamination in the FI, a disruption in information processing of the ACC may result in a reduction in the ability to modulate social interaction. Although difficult to perform, the unveiling of neuropathologic distinctions between subgroups, subsequently explored by neuroimaging studies, could provide insights into the correlations between activation patterns and behavioral or symptomatic manifestations potentially useful in clinical settings.

The anterior midcingulate cortex (aMCC) is involved in decision-making during uncertain situations, and is also home to VENs in layer V [107, 365]. One of the defining characteristics of a child with ASD is his/her resistance to change and routine, suggesting that the aMCC may be impaired in ASD. Young children with ASD do present with neuropathology in the aMCC, showing a positive correlation between ASD severity and number of pyramidal neurons and VENs [360]. Additionally, young children with ASD have significantly smaller pyramidal neurons than controls. Although these differences are not seen in adolescents, the developmental trajectory of pyramidal neuron size in subjects with ASD is opposite to that of controls: pyramidal neuron size reduces with age in controls, whereas in subjects with ASD it slightly increases with age [376]. The decreased pyramidal neuron size may reflect a potential reduction in the functional connectivity of the aMCC, which does not recover through development.

Although not yet widely studied from a neuropathologic standpoint, the posterior cingulate cortex (PCC) is involved in the processing of the salience of events and faces and is activated by emotionally significant stimuli in neuroimaging studies [223–225]. No overall significant differences have been found in densities of pyramidal neurons and interneurons in the PCC between subjects with ASD and controls [262]; however, individual cases display changes in neuron density, size, and distribution, as well as poor lamination, warranting further studies in larger cohorts.

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Hippocampus—Several studies have reported decreases in neuronal size with an increased cell packing density as well as the presence of less complex dendritic arborization in the hippocampus of subjects with ASD, putatively indicative of disrupted neuronal maturation [19, 191, 296]. Subjects with Fragile X syndrome, 30% of whom meet criteria for ASD (reviewed in [138, 299]), have focal thickening in the CA1 field and abnormalities in the dentate gyrus [128]. The expansions in CA1 contain an increased number of pyramidal neurons, whereas adjacent areas have a decrease in pyramidal neurons. In addition to pyramidal cell increase, a stereological study in the anterior body of the hippocampus showed increased packing density of specific subtypes of γ-aminobutyric acid (GABA)-ergic interneurons occurs in subjects with ASD compared to age- and gender-matched controls [211]. Density of calbindin-immunoreactive interneurons is significantly increased in the dentate gyrus, whereas parvalbumin-immunoreactive interneurons occur at a greater density in CA1 and CA3, and CA1 also presents with a higher density of calretinin-immunoreactive interneurons. In light of a possible role of these calcium-binding proteins in the modulation of calcium signaling in the hippocampus, these alterations may underlie some of the behavioral abnormalities observed in ASD. A caveat while interpreting these studies is that comparison of the volume estimates between ASD and controls are necessary to confirm whether the observed differences in cell density translates to a difference in total cell count in the hippocampus and its subfields. A subset of individuals with ASD also present with dysplasia in the entorhinal cortex and CA1 field, whereas abnormal migration and distortion of layers were observed in the dentate gyrus [379]. At the cellular level, subjects with ASD present with swollen axon terminals (termed spheroids) in the entorhinal cortex and all CA subfields in the hippocampus [382].

Non-cortical regions consistently altered in ASD

Amygdala—Another area closely tied into the neural circuitry thought to subserve social behavior is the amygdala, most well-known for its role in emotional learning [47, 212, 282]. Early assessments of the amygdala in ASD report decreased size and increased neuron density in the medial, central, and cortical nuclei [23, 191], although more recent quantifications showed a significant reduction in neuronal numbers in the amygdala as a whole [318] or in the lateral nucleus of the amygdala [318, 376] in subjects with ASD. When cases were grouped by age, the decrease in amygdala neuron numbers was not apparent in adolescents but was detected in adults with ASD [244]. The reduction in the number of neurons may originate from fewer neurons being generated during development, or from cell loss due to abnormal degeneration that may occur after a typical early development. The stark differences between the results of these studies may be accounted for by the presence of epileptic comorbidity in many of the cases in early studies, as well as by the differences in quantification methodologies. Nonetheless, neuropathology in this region as well as connected areas (i.e., the FG [362]) suggests abnormalities in specific pathways in ASD. Future studies focusing on the trajectory of neuronal changes in specific pathways (such as the amygdalo-sensory input pathway) [295] and throughout development will better assess the role of amygdala neuropathology in ASD.

Cerebellum—Initially thought to be mostly involved in the coordination of motor actions, the role of the cerebellum in the regulation of emotion has progressively come to light. The

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study of clinical cases revealed that lesions in the cerebellum result in neuropsychiatric impairments, including attention deficit hyperactivity disorder, obsessive compulsive disorder, bipolar disorder, depression, anxiety, panic disorder and ASD [315]. In this context, the presence of cerebellar alterations in ASD became an area of interest and the cerebellum is certainly one of the most extensively explored brain regions in the field of ASD neuropathology, even though the results are still controversial. Most studies have assessed the number, size, or density of Purkinje cells. The mean size of Purkinje cells in subjects with ASD is significantly smaller compared to controls [108, 380]. Although Purkinje cell density is not significantly different in subjects with ASD and controls as a whole [385], a subgroup of subjects with ASD have a reduction in Purkinje cell number [329, 376]. A subsequent study quantified the inhibitory GABAergic basket and stellate interneurons that innervate Purkinje cells [386]. No significant difference was found between ASD subjects and controls in packing density of either type of interneuron or in their ratio to Purkinje neurons. The loss of Purkinje cells in subjects with ASD, despite a normal amount of innervating interneurons, was interpreted as a neurodevelopmental process with Purkinje cells present initially in normal numbers and distribution but degenerating later, probably between 32 weeks of gestation and early postnatal life [35]. A study in Fragile X syndrome confirmed this trend by demonstrating a significant decrease in the number of Purkinje cells, and abnormalities such as incorrect orientation and abnormal clustering of Purkinje cells, undulations of the internal granular layer, and the presence of astrocytes in white matter. Atrophy in the anterior and posterior parts of the vermis was also quantitatively determined [128]. Other groups confirmed these results, consistent with the hypothesis of compromised cerebellar development in subjects with ASD, including flocculonodular dysplasia [380], cerebellar hypoplasia [379], and spheroids [382]. Although there is not a clear mechanistic relationship between the neuropathologic alterations observed in the cerebellum in ASD and the symptomatic manifestations of the disorder, the previously discussed studies implicate this region in ASD pathology.

Brainstem—The medial superior olive, an auditory brainstem structure, shows significant disruption of cell morphology, particularly in cell body shape and orientation, in subjects with ASD [207]. In young children with ASD, deep cerebellar nuclei and inferior olivary neurons appear enlarged, but with age these neurons become reduced in size [191]. A case study also reported the presence of marked alterations in the brainstem of a 21-year-old subject with ASD [303], including an almost complete lack of facial nucleus and superior olive as well as a global shortening of the brainstem between the trapezoid body and inferior olive. Similarly, a subset of subjects with ASD have olivary dysplasia and abnormally placed neurons, relative to the olivary complex [19]. Spheroids are observed in the periaqueductal gray matter and reticular formation of the midbrain, the dorsal raphe, locus coeruleus and interpeduncular nucleus, and the sensory nuclei of the medulla [382]. Although not conclusive given the low number of cases and the lack of precise quantitative data, these findings provide a strong case for further exploration of the possible implication of the brainstem in ASD.

The need for neuropathologic studies with a higher number of subjects and the use of precise quantitative methodologies are essential, considering the inconsistent results of different

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studies focusing on each brain region. Also, except for the studies on cortical thickness in the PFC, and size and density of cell in the cerebellum, many of the findings on ASD neuropathology have not been replicated or are reported mostly from one group. However, beyond methodological concerns, several studies emphasize the importance of looking at ASD as a heterogeneous condition.

Lessons from animal models

Animal models provide an advantage over human studies by allowing for controlled testing of the effects of specific disease-causing factors on neuropathology, synaptic function and behavioral outcomes. For the purposes of this review, we focus on *in vivo* mammalian models of ASD, based on known Mendelian genes and novel risk genes for ASD, natural selection of animals with autism-like phenotypes, or environmental manipulations, in which neuropathology or synaptic morphology is available.

Animal models based on single ASD genes

The genetic basis of ASD was appreciated as early as 30 years ago. Twin, family and population-based studies have shown ASD to be one of the most heritable of all psychiatric disorders, with heritability estimated at ~50% (see reviews in [119, 310]) and monozygotic twin concordance rates between 70 to 90% [1, 113, 139, 304, 344]. Using current methods, only 20% of ASD cases have an identified genetic cause, often involving chromosomal rearrangements, CNVs or point mutations. Albeit rare, *de novo* mutations have emerged as substantial contributors to ASD risk [83, 165, 166, 254, 261, 309].

Most of the known genetic alterations contributing to ASD risk affect the expression or function of proteins with established roles in the formation, function and maintenance of synapses or in chromatin remodeling [82]. Ablation of such genes in animals can result in a behavioral phenotype reminiscent of ASD, with specific impairments in social interaction, communication and repetitive behaviors. The characterization of genetically modified animal models created based on these discoveries has led to the identification of biochemical pathways likely to be relevant to a wider population and has yielded promising results with regard to the development of novel ASD drugs. This section provides an overview of recent findings with respect to neuropathology and neural systems involved in animal models with modification of a single gene or chromosomal region, as summarized in Supplementary Table 1, and will evaluate the contribution of genetically modified animal models in deciphering common underlying biochemical pathways. We compare the clinical features shown by carriers of each gene to the observed behaviors in the respective animal models in Supplementary Table 2.

Neuroligins (NLGNs)—Postsynaptic NLGNs and presynaptic neurexins together form a trans-synaptic complex, thought to mediate synaptic stabilization. Various combinations of these cell adhesion molecules are implicated in the differentiation of glutamatergic or GABAergic synapses. Of the five *NLGN* genes (*NLGN1-3, 4X and 4Y*) expressed in humans, mutations in *NLGN3* and *NLGN4* are associated with non-syndromic X-linked ASD and intellectual disability and rodent models replicate these behavioral phenotypes (see Supplementary Table 2).

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MRI scans showed that whole brain volume (excluding olfactory bulb, cerebellum and brainstem) is reduced in *Nlgn3*-knockout (KO) mice compared to controls [293]. *Nlgn3*^{R451C} knock-in (KI) mice, that express the *Nlgn3*^{R451C} mutation found in individuals with ASD [174], also show reduction in volume of the hippocampus, striatum, thalamus, cerebral peduncle, corpus callosum, fimbria/fornix and internal capsule [97]. Synaptic changes in the *Nlgn3* KI mice include greater postnatal turnover of excitatory spines in layer II and III pyramidal neurons in the anterior frontal cortex [172], and increased vesicular GABA transporter expression without changes in inhibitory synapse number or ultrastructure in neurons in the somatosensory cortex [345]. In the hippocampus, increased dendritic complexity in the stratum radiatum, unaltered spine density or postsynaptic density (PSD) length, as well as decreased presynaptic bouton size, vesicle numbers and spine area are observed in *Nlgn3* KI mice [99]. Another mouse model mimicking an ASD-related mutation [393], the *Nlgn3*^{R704C} KI shows decreased α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid (AMPA), but not *N*-Methyl-D-aspartic acid (NMDA) or GABA, receptor-mediated hippocampal neurotransmission [100], possibly due to increased internalization of AMPA receptors [62]. Although inhibitory markers, such as vesicular GABA transporter and gephyrin, are increased in these animals, there is no change in the number of synapses. Taken together, these data support the notion that neuroligins play a pivotal role in regulating the balance between inhibitory and excitatory transmission and that the enhanced inhibitory transmission in *Nlgn3* KIs is due to increased strength of inhibitory synapses rather than a change in the number of synapses [345].

Nlgn4 KO mice have decreased volume of the whole brain, as well as the cerebellum and brainstem, as detected by MRI [175]. These mice have decreased excitatory PSD-95-immunolabeled puncta in the stratum lucidum as well as lower density of $GABA_A$ receptor and gephyrin-immunoreactive synapses in the stratum pyramidale of the CA3 field [140]. However, these findings need to be interpreted with caution when extrapolating to the human disease as the mouse *Nlgn4* gene is evolutionarily divergent from human *NLGN4* [37].

Neurexins (NRXNs)—The *NRXN* genes encode α- and β-neurexin and, as the presynaptic binding partners of NLGNs, are important in adhesion, differentiation and maturation of synapses. Subjects with CNVs and point mutations in *NRXN1* present with intellectual disability, developmental delay, language problems, ASD, seizures, hypotonia and facial dysmorphism and have a higher risk for schizophrenia. *NRXN3* mutations are rarer, but also associated with ASD.

Nrxn1 contains two promoters, which produce two major classes of isoforms, *Nrxn1α* and *Nrxn1β*. Targeting the first coding exon produces mice lacking *Nrxn1α* isoforms. Behavioral studies have been performed in *Nrxn1α* and *Nrxn2α* KO mice (see Supplementary Table 2), but most of the available neuropathological evidence comes from triple KO mice, where all three *Nrxnα* genes have been ablated, or from *Nrxn1α/2α* double KO mice. Triple and double KO mice show fewer inhibitory synapses in the brainstem and neocortex, respectively, compared to the wild-type (WT) controls [242]. The *Nrxn1α/2α* double KO mice show no gross anatomical changes but increased cell density and decreased dendritic length in the visual cortex, with no changes in synaptic ultrastructure, compared to *Nrxn2α* KO [89]. Further studies using *Nrxn1* or *Nrxn3* single KO mice and the

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development of KI mice carrying the ASD mutations would enable more direct comparison to the observations in subjects with ASD who carry these mutations.

CNTNAP2 is a member of the NRXN family and encodes Contactin Associated Protein-Like 2 (CASPR2). Recessive mutations or chromosomal inversions of the *CNTNAP2* gene have been observed in individuals with intellectual disability and/or ASD. CASPR2 is necessary for development of dendritic arborization [9], stabilization of dendritic spines [122] and AMPA receptor trafficking [363]. When compared to WT controls, $Cntnap2^{-/-}$ mice show abnormal migration of neurons in the corpus callosum and somatosensory cortex, have fewer parvalbumin-immunoreactive interneurons in the hippocampus and striatum, fewer GABAergic interneurons in the somatosensory cortex [278] and fewer oxytocin-expressing neurons in the hypothalamic paraventricular nucleus [279].

SH3 and multiple ankyrin repeat domains protein 3 (SHANK3)—SHANK3 (or ProSAP2) is a postsynaptic density (PSD) protein that interacts with a variety of ionotropic and metabotropic glutamate receptors and links them to the actin cytoskeleton [252]. Mutations in *SHANK3* have been associated with ASD and Phelan-McDermid syndrome. Phelan-McDermid syndrome can result from chromosomal rearrangements (in most cases, terminal deletions) at 22q13.3 spanning *SHANK3* or point mutations (reviewed in [145]). The genetic heterogeneity of Phelan-McDermid syndrome underlies the variable clinical manifestations, which include global developmental delay, intellectual disability, delay or absence of speech, and ASD, as well as low motor tone and minor dysmorphic features. Several rodent models targeting different isoforms of Shank3 have been generated. Although they display some discrepancies, they consistently show behaviors indicative of ASD, with little impact of genetic background (see Supplementary Table 2).

The existence of different Shank3 isoforms, whose expressions vary by brain region and age [373], complicates the analyses in animal models. Studies across various $Shank3^{+/-}$ mouse models have consistently documented deficits in glutamatergic transmission. For example, a model with ablation of the full-length isoform of *Shank3* (*Shank3α*) through deletion of exons 4–9 shows reduced glutamate receptor 1-immunoreactive puncta in the hippocampal CA1 [39]. The CA1 of these mice also show increased perforated synapses at 5 weeks of age (Figure 5), which do not persist with age and may be a compensatory mechanism for non-functional synapses [359]. A mouse model generated by an independent group, but with a similar design, also shows reduced spine density in the CA1 at 4 weeks of age, which becomes comparable to that in WT at 10 weeks of age. This mouse model has longer dendritic spines in the CA1 at 4 weeks and shorter spines at 10 weeks of age when compared to spine lengths in WT mice [372]. Deletion of the two longer *Shank3* isoforms (*Shank3α* and *β*) in the $Shank3B^{-/-}$ mice is associated with increased dendritic length and complexity but reduced spine density, PSD length and thickness in striatal medium spiny neurons [277]. Purkinje cells in $Shank3^{+/\Delta C}$ mice, which have a C-terminus deletion of *Shank3*, are unchanged in terms of density but have greater dendritic complexity distal to the soma and reduced spine density [202]. These mice have reduced expression of NMDA receptors in the PFC, without changes in spine density [90]. Dendrites on the CA1 neurons of $Shank3^{\Delta C/\Delta C}$ mice do not show any changes in the complexity or spine density, although LTP and NMDA/AMPA ratio are decreased in the hippocampus [206]. Decreased spine density, PSD length

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and PSD thickness are also observed in the striatum, but not the hippocampus, of a KO mouse model ablating all Shank3 isoforms (Shank3$^{\Delta e4\text{-}22-/-}$ mouse) [371]. More recently, restoration of Shank3 expression in adult *Shank3*-deficient mice was shown to reverse dendritic spine loss and excitatory synaptic function in the striatum [235]. The above findings all confirm a role for Shank3 in orchestrating the assembly of multiple glutamate receptors at the PSD and coupling synaptic signaling to spine dynamics. One caveat of several of these studies is their focus on the homozygous *Shank3* KO, despite *Shank3*-deficient heterozygotes being more representative of the deficit observed in Phelan-McDermid syndrome.

Methyl-CpG-binding protein 2 (MECP2)—Mutations in the *MECP2* gene cause Rett syndrome, an X-linked neurodevelopmental disorder primarily affecting females. Subjects with Rett syndrome show normal development until 6 to 18 months of age and then developmental regression sets in, resulting in loss of sensory, motor and cognitive functions. Over 60% of patients with Rett syndrome meet the criteria for ASD, with features such as repetitive hand movements, social withdrawal and loss of verbal communication. As constitutive *Mecp2* deletion in mice causes embryonic lethality, several different conditional-deficient or null mouse models expressing the deletion in selected tissues and/or selected time points have been generated. These models recapitulate many of the human symptoms (see Supplementary Table 2), thus presenting valuable experimental systems for understanding the mechanisms underlying ASD behaviors.

Many of the neuropathologic changes observed in subjects with Rett syndrome, such as reduced brain size [177], smaller neurons, increased packing of neurons [25], decreased dendritic complexity of neurons in layers III and V of the frontal and motor cortex [13], and reduced spine density in layer II and III of the neocortex [29] and CA1 of the hippocampus [65], are recapitulated in these models. Loss of MeCp2 results in reduced brain weight [67, 126], with thinning of the neocortex described in some mouse models [115, 200, 302]. Increased cell density was observed in the neocortex [115, 200, 341], olfactory bulb, hippocampus and cerebellum [178]. Reduced size of the cell body has been detected in neurons from layers II and III of the neocortex [200], layer V of the motor cortex [300], layer V of the somatosensory cortex [370], CA1 [126] and CA2 of the hippocampus [67], locus coeruleus [348], and cerebellum [67] of various *Mecp2*-deficient mouse models. Dendrites are shorter in layer V pyramidal neurons of the neocortex [341] as well as primary motor cortex neurons [302] but not in the medial PFC [313], and dendritic complexity is reduced in neocortical layers II and III [200] in the somatosensory cortex [178, 370] and primary motor cortex [302, 341] when MeCp2 is deficient. Reduced spine density was observed in neurons of the somatosensory cortex [115], primary motor cortex [302, 341], medial PFC [313], hippocampus CA1 [30], dentate gyrus [330] and cerebellum [202] in *Mecp2*-deficient mice. Spine heads are smaller in the dentate gyrus and hippocampus CA1 field and axon orientation in the motor cortex is disrupted in *Mecp2*-deficient mice [30]. These changes are apparently due to a delay in development and synapse formation in neurons resulting from *Mecp2* haploinsufficiency [115, 330], which are abnormalities in development that do not recover with age. MeCp2 deficiency results in reduction of glutamatergic synapses [63] and increased basal levels of AMPA, indicating a failure in

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activity-dependent synaptic trafficking of the receptor [217]. *Mecp2*-deficient spines also fail to increase in volume upon induction of long-term potentiation, indicating the loss of activity-dependent synaptic plasticity [217].

Male individuals with *MECP2* duplications present with a syndromic manifestation that can include intellectual disability, language deficits, anxiety and ASD behaviors [294]. The ASD behaviors are replicated in mouse models though the cognitive deficit is not (see Supplementary Table 2). Mice overexpressing *Mecp2* (*Mecp2*^{Tg1}) do not have changes in size of neuronal soma [370], although both dendritic length and complexity [180] as well as glutamate receptor density are increased [63]. Overall, studies in the *Mecp2* animal models indicate that neuronal and dendritic morphology changes may appear transiently during development or be variously affected, depending on developmental stage and MeCp2 gain- or loss-of-function [64, 180].

Fragile X Mental Retardation 1 gene (*FMR1*)—Fragile X syndrome is caused by expansion of a cytosine-guanine-guanine (CGG) trinucleotide in the promoter of the fragile X mental retardation (*FMR1*) gene or, more rarely, due to point mutations [18]. The expansion of the CGG repeat over 200 copies leads to the transcriptional silencing of the gene and loss of the fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein known to interact with specific mRNAs in the brain and regulate synaptic plasticity-dependent local protein synthesis [18, 80, 275]. Clinical symptoms affect boys more severely and include intellectual disability, delayed speech, anxiety, attention deficit disorder and hyperactivity, seizure and body dysmorphism. Besides, approximately 22% of all carriers and 30% of males with *FMR1* mutations meet the diagnostic criteria for ASD.

The first neuropathologic studies in the neocortex of subjects with fragile X syndrome noted the presence of long spines and reduced PSD length, almost no stubby spines and the absence of differences in synaptic vesicle density [305, 389]. Cortical lamination and neuronal density were normal [158]. Subsequently, increased spine density and spine length, with more immature spines and fewer mature spines was quantitatively confirmed in the temporal and visual cortices of subjects with fragile X syndrome compared to controls [169].

The increased spine length and density observed in human postmortem studies were replicated in *Fmr1* KO mice [74, 167–169], although differences were noted dependent on age and brain area. For instance, in the somatosensory cortex of postnatal day 4–7 *Fmr1*$^{-/-}$ mice, the spine length is comparable to WT mice, but spine density is higher in the mutant [79]. For the same brain area in *Fmr1*$^{-/-}$ mice between the ages of 7–25 days, contradictory findings indicate either no change in spine length and density [78, 118, 144, 353] or increased spine length and transiently increased spine density at 1 week of age [257]. However, these changes were not fully replicated in the hippocampus. In the dentate gyrus, although the mutant has longer spines at the earlier ages, the age-related increase in spine density is comparable between the *Fmr1*$^{-/-}$ mice and WT from 10 to 60 days of age [129]. Higher incidence of shorter spines was reported in hippocampal neurons of 2-week old *Fmr1* KO pups, and more long spines in 10-week old and adult mice [288]. Also, there is no

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change in spine density in the CA1 [130]. In the nucleus accumbens of adult *Fmr1*$^{-/y}$ mice, the incidence of long spines is greater [255].

Similar to the observations from postmortem studies on fragile X syndrome brains, the distribution of spine types tended towards decreased maturity in *Fmr1* KO mice. Thus, in the somatosensory cortex of 10–12 day old as well as in older *Fmr1*$^{-/-}$ mice, more filopodia and fewer mature spines are observed [78]. Increased immature spines and fewer mature spines are also observed in the visual cortex of adult *Fmr1*$^{-/-}$ mice [233]. The mutant has more thin spines and fewer mushroom and stubby spines in the dentate gyrus [129] as well as the CA1, but not the CA3 [214]. However, one study reported more mature spines and fewer immature spines in the CA1 [130].

The observed differences in spine morphology may be a result of changes in spine turnover. Postnatal day 4–7 *Fmr1*$^{-/-}$ mice show motility and turnover of spines comparable to WT mice in the somatosensory cortex [79], but the spine turnover rate increases in 10–12 day old mice [78] as well as in older *Fmr1*$^{-/-}$ mice [118, 273, 353]. Spine turnover rate is also higher in the visual cortex of adult *Fmr1*$^{-/-}$ mice [251].

In addition to studies on spine dynamics, other morphological parameters assessed in *Fmr1*$^{-/-}$ mice include dendritic arborization and synaptic ultrastructure. Analysis of dendritic complexity in the somatosensory cortex has revealed no overall change [353], except for a directional increase in dendrite branching towards the septa, but not the barrel hollow, in *Fmr1*$^{-/-}$ mice compared to WT [117]. Presynaptic deficits including a smaller active zone and fewer docked vesicles are seen in the CA3 of adult *Fmr1*$^{-/-}$ mice [201]. In the nucleus accumbens of adult *Fmr1*$^{-/y}$ mice, PSD density is increased, indicating more excitatory synapses, but PSD length is unaltered and the incidence of long spines is greater [255], whereas in the amydgala, fewer inhibitory synapses are detected [266]. Overall, these findings indicate a role for *FMR1* in the maturation of synapses, with age- and brain-area specific effects.

Tuberous sclerosis complex 1/2 (*TSC1/2*)—Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterized by benign tumors called tubers in multiple organs, including the brain and kidneys, as well as increased risk for developing malignant tumors. TSC is caused by mutations in the *TSC1* gene encoding the hamartin protein or the *TSC2* gene encoding tuberin, resulting in the loss of their inhibitory action on the mammalian target of rapamycin (mTOR) protein and dysregulation of mTOR signaling. The neurological phenotype includes seizures, intellectual disability, and ASD. The estimated prevalence of ASD in carriers of *TSC* mutations is 36%.

Neuropathologic observations in human subjects with TSC indicate abnormal migration and differentiation of neurons within and proximal to the tubers in the cerebral cortex [163] and the presence of abnormally large cells, which show features of either neurons or astrocytes or both, indicating a defect in differentiation [243]. Studies in subjects with *TSC2* mutations revealed cortical tubers with dysmorphic neurons and abnormal lamination [36, 70], and loss of cells resulting in hippocampal sclerosis and cerebellar atrophy [36].

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Conditional KO of Tsc1 in forebrain pyramidal neurons (*Tsc1*^{CKO} mice) does not result in change in soma size or dendritic arborization but does increase spine density in basal dendrites of temporal cortex neurons. This defect is due to reduced synaptic pruning during development [349]. In contrast to these findings, *Tsc1*$^{c-SynICre+}$ mice, in which synapsin-driven knockout of *Tsc1* occurs developmentally, show disrupted cortical layers and enlarged cells but no cell loss in neocortex, hippocampus, thalamus, hypothalamus, or brainstem [236]. *In vitro* Cre recombinase-induced KO of *Tsc1* results in increased neuronal size, spine length, and spine head width, and decreased spine density in hippocampal neurons prepared from transgenic mice carrying a *lox*P-flanked *Tsc1* allele [351]. In the *Tsc1*$^{fl/mut}$ mouse model, where cortical Tsc1 expression is knocked out by *in utero* electroporation in a heterozygote carrying a *Tsc1* mutation, soma size is increased and tuber-like structures are seen in the brain [110]. Similar increase in cell size, together with altered migration patterns and increased dendritic branching, is also observed in the olfactory bulb [109]. A Purkinje cell-specific KO of *Tsc1* in *L7*^{Cre}*;Tsc1*$^{flox/flox}$ mice results in postnatal loss of Purkinje cells as well as increase in size of the neuronal soma and density of dendritic spines [356].

Layer V neurons in the temporal cortex of *Tsc2*$^{+/-}$ mice show increased spine density and decreased pruning with age but comparable cell size and dendritic branching to the WT [349]. Hippocampal neurons in the *Tsc2*$^{+/-}$ Eker rat show increased spine length, and decreased spine diameter and number of excitatory synapses [395]. When *Tsc2* is knocked out in cerebellar cells (*Tsc2f/−;Cre* mice), a reduction in Purkinje cell density is observed [298].

Emerging monogenic models of ASD—Although some of the models discussed in the following section have either not been validated for ASD phenotypes or have very little neuropathologic data available, there is growing interest in them as potential models of subsets of subjects carrying specific ASD risk genes.

Chromodomain helicase DNA-binding protein 8 (*CHD8*), encoding a chromatin modifying gene on chromosome 14q11.2, is one of the high-penetrance ASD risk genes recently identified by sequencing studies [83, 165, 261, 347]. Knock-down of the *CHD8* ortholog results in macrocephaly in zebrafish [32, 343] and increased brain weight and volume [187, 287] in mice, phenotypes that reflect clinical observations in subjects with loss-of-function mutations in the gene [32]. *CHD8* knockdown in human neural progenitor cells results in downregulation of neuronal development and cell adhesion genes, several of which have been associated with ASD [343]. *In utero* knockdown of CHD8 in layers II and III neurons of mice at embryonic day 13 results in reduced neural proliferation, dendritic arborization and spine density [91]. Germline Chd8 knockdown mice have normal cortical lamination in the somatosensory cortex [287].

Sodium channel, voltage-gated type II α subunit (*SCN2A*) mutations have previously been associated with infantile seizures, epileptic encephalopathy, epileptic syndromes, as well as intellectual disability and ASD without epilepsy. *In vitro* studies have demonstrated that the $Na_v1.2$ subunit encoded by *Scn2a* localizes to the axon initial segment in cerebellar granule cells [267] and that pharmacological modulation of voltage-gated sodium channel activity in

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cortical neurons influences neurite length and complexity as well as density of spines and excitatory synapses [123, 124]. Alternative splicing of *Scn2a* results in differential expression of the various isoforms during development and adulthood [121], possibly as a means of controlling neuronal excitation. Expression of the adult isoform of *Scn2a* in mice results in neuronal hyperexcitability [120]. Mice with *Scn2a* GAL879-881QQQ mutation show neuron loss and gliosis in the hippocampus [190]. How the ASD-linked mutations affect *Scn2a* function during development and whether this results in altered neuronal structure and connectivity remains to be investigated.

Synaptic GTPase activating protein 1 (SynGAP1) is a Ras-GTPase activating protein localized in the PSD in glutamatergic neurons [66, 197] and involved in dendritic spine maturation, glutamate receptor trafficking and synaptic function [11]. Mutations resulting in *SYNGAP1* haploinsufficiency are associated with several neurodevelopmental disorders, including non-syndromic intellectual disability in 100% and ASD in 50% of carriers, with symptoms including encephalopathy, epilepsy, hypotonia, stereotypical behaviors and aggression. Studies using genetically modified mice that mimic the *Syngap1* deficit have revealed precocious dendritic arborization, early formation and premature pruning of dendritic spines as well as enlarged dendritic spines in layer V pyramidal neurons of the somatosensory cortex during a critical period of development, compared to WT mice [2]. Maturation and size of spines in the hippocampal dentate gyrus of *Syngap1* heterozygous mice follow a similar trend to that observed in the somatosensory cortex, although dendritic complexity is not affected in this cortical area [72]. In the hippocampal CA1 of adult mice, the density of mushroom spines, but not those of thin or stubby spines, is increased in $Syngap1^{+/-}$ mice compared to WT mice [51], although another study using young adult mice reported no change in spine densities or size [22]. Studies using *in vitro* systems and KO mice have shown that SynGAP expression is inversely correlated to the levels of AMPA receptors, but not NMDA receptors, at the synapse [306]. *Syngap1* deficiency selectively induced in GABAergic neurons results in decreased density of presynaptic boutons terminating on the soma as well as reduced terminal branching of axons on interneurons of the cortex [33]. Specific reduction of Syngap1 expression in developing forebrain glutamatergic, but not GABAergic, neurons induces cognitive deficits in mice [269], although whether similar effects are seen on the core ASD-related behaviors or on neuron or synapse morphology is yet to be determined.

AT-rich interactive domain containing protein 1B (*ARID1B* or *BAF250B*) is a member of the SWItch/Sucrose NonFermentable (SWI/SNF) chromatin remodeling complex. *ARID1B* haploinsufficiency is associated with Coffin-Siris syndrome, presenting with developmental delay, hypotonia, feeding problems, and physical features such as anomalous nails or shorter fingers, hirsutism, sparse scalp hair and facial dysmorphism and ASD. *In utero* knockdown of *Arid1B* in mice results in reduced dendritic length and complexity in pyramidal neurons of the neocortex and hippocampus in mice as well as decreased spine density and spine head size in cortical neurons [183]. No behavioral testing has been done in this model; hence further studies are needed to assess whether the behavioral phenotype induced by *Arid1B* deficiency in mice recapitulates the cognitive impairments and the social and communicative deficits observed in individuals carrying *ARID1B* mutations.

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Glutamate receptor ionotropic, NMDA 2B (*GRIN2B*) encodes the GluN2B subunit of the NMDA receptor and haploinsufficiency of *GRIN2B* has been associated with an array of neurodevelopmental disorders, including epileptic encephalopathy, intellectual disability, and ASD. *Grin2b* full KOs have no suckling response and therefore die shortly after birth [208]. As there is an experience-driven switch between GluN2B- and GluN2A-containing NMDA receptors during postnatal development [31, 324], functional implications of GluN2B loss during development have been studied using mice where *Grin2b* is replaced with *Grin2a* or conditionally knocking out the gene in specific brain regions. *In vitro* electrophysiological studies confirm that GluN2B loss results in disruption of protein-dependent homeostatic plasticity [369]. *In utero* knockdown of *Grin2b* results in delayed migration of cortical neurons, together with increased dendritic length and branching [179]. The role of GluN2B on control of synapse maturation has been demonstrated experimentally in rat cortical and spinal cord co-cultures, where synapse elimination is decreased when postsynaptic GluN2B is absent [264]. Dendritic spine density is decreased in CA3 pyramidal neurons [3] as well as in CA1 pyramidal neurons in mice with conditional ablation of GluN2B [41].

Down syndrome cell adhesion molecule (DSCAM) is a neuronal surface receptor involved in the targeting of axon collaterals, dendrite branching and synapse formation during development [154, 216], encoded by an ASD risk gene. Absence of Dscam in $Dscam^{del17}$ mutant mice results in a transient decrease in the thickness of upper cortical layers. These mice also showed altered dendritic morphology of layer V pyramidal neurons compared to WT controls, starting with an early increase in apical dendritic branching, reduced dendritic length and higher spine density, which were normalized by adulthood. Although young $Dscam^{del17}$ mutant mice have fewer long spines and more spines with small head size, the number of long and large spines increases with age [230]. $Dscam^{del17}$ mutant mice exhibit severe hydrocephalus, [392] but whether ASD phenotypes are replicated in mice with a Dscam deficiency remains an open question.

T-brain-1 (TBR1) is a T-box transcription factor expressed specifically in the brain, and its expression is increased in response to neuronal activity [71]. Mutations in *TBR1* are associated with intellectual disability and ASD, and functional implications will likely impact its activity as a transcriptional regulator, its cellular localization or its interaction with co-regulators. Complex interactions of several genes with *Tbr1* determine the corticothalamic projection of layer VI neurons, callosal projection of neurons in the cortical superficial layers [335] and subcortical projection of layer V neurons [50, 142, 232]. *Tbr1* in turn regulates the expression of rostrocaudal and layer-specific markers in the cerebral cortex, the former being partly through activation of the autism gene *AUTS2* [28]. The laminar positioning of cortical GABAergic interneurons during embryonic development is disrupted in *Tbr1* KO mice [93]. $Tbr1^{-/-}$ mice have defective cortical morphology, neuronal migration, and axonal projections [156, 157], which are not detected in $Tbr1^{+/-}$ mice that model the haploinsufficiency observed in patients more faithfully. $Tbr1^{+/-}$ mice exhibit a small or absent posterior region of the anterior commissure, possibly a result of defective neuronal differentiation in amygdala neurons [161].

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Animal models of syndromic ASD caused by CNVs

15q11-q13 deletions and 15q13.3 microdeletions—Prader-Willi and Angelman syndromes are two different neurodevelopmental syndromes caused by the deletion of the same imprinted genomic material within the 15q11-13 chromosomal locus [160]. The manifestation of one syndrome versus the other depends on the parental origin of the deleted allele. Angelman syndrome is caused by *de novo* deletions on the maternal copy, uniparental disomy of the paternal copy, or defective imprinting on chromosome 15q11.2-q13 or by mutations in the *UBE3A* gene, which result in expression deficits of UBE3A. UBE3A is an E3 ubiquitin ligase that flags proteins, including itself, for degradation [260] and the *UBE3A* gene is located in the 15q11–q13 chromosomal region. The development delay in Angelman syndrome is manifested at 6 to 12 months of age. Symptoms include ataxia or tremor, speech and motor deficits, inappropriate happiness with hand flapping, seizures, microcephaly, hypotonia, misaligned eyes, drooling, protruding tongue, disturbed sleep and anxiety, as well as ASD in 34% of patients. Prader-Willi syndrome is caused by deletion, uniparental disomy, or an imprinting center defect on the paternal copy of chromosome 15q11.2-q13. Subjects with Prader-Willi syndrome have extremely low motor tone. The difficulty in feeding during infancy switches to uncontrolled eating and the development of obesity in early childhood. Affected individuals are cognitively impaired and approximately 25% of patients show several ASD behaviors, including social deficits and increased repetitive and ritualistic behaviors. Animal models with both maternally or paternally derived alleles have been generated.

A neuropathological study of a subject with Angelman's syndrome revealed a smaller brain, with no gross morphological changes, except for cerebellar atrophy, due to loss of Purkinje and granule cells [176]. Another study reported the absence of cerebellar changes and smaller frontal and temporal lobes [209]. Dendrites in layer III and V neurons of the visual cortex are shorter, less complex, and have fewer spines [176]. MRI and diffusion tensor imaging studies have revealed impaired white matter integrity in subjects with Angelman's syndrome [281, 354, 388], likely due to a delay in myelination [146]. Similar to the observation of small brain size in human samples from Angelman's syndrome, the total brain weight, as well as the weight of the cortex and cerebellum, is lower in a mouse model with maternal *Ube3a* deleted (*Ube3a*$^{m-/p+}$) [181]. Also, the density of presynaptic vesicles, as well as spine density and length, in hippocampal CA1 neurons is lower compared to WT controls [342]. In the visual cortex of *Ube3a*$^{m-/p+}$ mice, cortical thickness and cell density in layers II, III and V are unaffected [312], cell density of inhibitory interneurons is unaffected in layers II and III [368], spine density is decreased in layer V after 21 days of age [86, 196, 312], maintenance of spine density after sensory stimulation is deficient [196, 394], with more thin spines and fewer stubby spines in the disease model than in the WT [196] and presynaptic GABA vesicle density is decreased at both inhibitory and excitatory synapses [368]. In the cerebellum, cell density and dendritic complexity are unaffected in *Ube3a*$^{m-/p+}$ mice [86]. Expression of the *UBE3A* T485A variant, identified in a patient with ASD abolishes phosphorylation of UBE3A at the mutated site, results in increased activity of the protein and causes increased spine density on basal dendrites in the somatosensory cortex [396]. These studies indicate that depending on type of mutation, the gain- or loss-of-function of *UBE3A* in ASD can have varying effects at the level of the synapse.

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Neuropathological findings in subjects with Prader-Willi syndrome include abnormal distribution of cells in the dentate and olivary nuclei, nerve degeneration in the dentate nucleus, cerebellar heterotopia [150], enlarged ventricles, reduced volume of parieto-occipital lobe, multiple small gyri in the sylvian fissure, incompletely operculated insula [239], and smaller cerebellum and brainstem [222]. *In vitro* studies using overexpression of *Snrpn* show that the gene reduces dendrite length, increases spine density and alters spine distribution on cortical neurons [215]. Deletions in patients with Prader-Willi syndrome can differ considerably in size and location, with the common deletions occurring in the *SNURF-SNRPN* gene and the adjacent Imprinting Center, *MAGEL2* and *NECDIN*, and the *SNORD116* gene cluster. Specific KO mouse models have been generated for all or some of those genes. *Magel2*-null mice show decreased volume of parietal and temporal cortex, hippocampus, amygdala, nucleus accumbens, corpus callosum, and olfactory bulbs [237]. Mice with a deletion of *Snord116* have reduced gray matter volume in the ventral hippocampus [210]. *Necdin*-deficient mice have small brainstem nuclei and disorganized axonal tracts in the medulla [271].

A 15q13.3 microdeletion has been associated with increased risk for ASD, intellectual disability and other disorders including schizophrenia and epilepsy. A recently developed heterozygous deletion D/+ mouse model with a homologous microdeletion has enlarged brains and lateral ventricles in adulthood [204]; enlarged head size has been observed in subjects with the microdeletion [272]. Although neuron number and dendritic morphology were not quantified in the D/+ mice, no gross differences were observed [204].

15q11-13 duplication—Duplications of the 15q11-13 chromosomal region, spanning *UBE3A* and the genes encoding $GABA_A$ receptor α5 and β3 subunits, often of maternal origin, are associated with an array of neurodevelopmental and neurological phenotypes with features of both Prader-Willi syndrome and Angelman syndrome. Clinical features include hypotonia, speech disorder, developmental delay, seizures and ASD.

MRI analysis in a case of confirmed 15q13.3 duplication presenting with ASD detected heterotopia in the parietal and occipital cortices, likely due to abnormal neuronal migration [26]. Mice with maternally derived duplication (*matDp*/+), but not those with the paternal duplication (*patDp*/+), show increased expression of *Ube3a* and *Gabra5* in the hippocampus. Neither of these mice have any gross morphological or histological abnormalities in the brain [253]. However, reduced volume was detected by MRI in the stratum granulosum of the hippocampus, the inferior and superior colliculi, the hypothalamus, the thalamus, the pons and the midbrain of *patDp*/+ mice [98]. *PatDp*/+ mice, but not *matDp*/+ mice, show increased postnatal turnover of excitatory spines in the somatosensory and anterior frontal cortex [172]. Further investigation of neuronal development and migration in these mouse models will reveal the role played by this chromosomal region in ASD.

16p11.2 deletion and duplication syndromes—16p11.2 deletions and duplications are recurrent copy number variants in ASD, schizophrenia and other neurodevelopmental manifestations. Speech and language delays, cognitive impairment and facial dysmorphy are some of the features shown by these patients. ASD occurs in 16% of subjects with deletion

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and 20% of those with duplication of this chromosomal region. Macrocephaly is observed in subjects with the 16p11.2 microdeletion, whereas carriers of the duplication show microcephaly [227, 292, 326, 337].

When compared to WT controls, mice that model the deletion (*df*/+) have increased relative volume as detected by MRI in the basal forebrain, superior colliculus, fornix, hypothalamus, mammillothalamic tract, midbrain and periaqueductal grey [159]. However, another model of the deletion, the 16p11.2del mouse, shows reduced brain weight and decreased cortical size with perturbed cortical lamination [291]. In a third model (the *16p11*$^{+/-}$ mice) brain weight is comparable to controls, although relative volumes of the nucleus accumbens and globus pallidus are increased. The number of cells expressing dopamine receptor D2 is increased in the striatum, with no change in dendritic complexity or spine density, and fewer dopaminergic cells in cortical layers V and VI are found in these mice [289]. Relative volumetric changes in mice modeling the 16p11.2 duplication (*dp*/+) are in the opposite direction to those observed in the *df*/+ mice [159]. Although the relative brain volume changes in some of the mouse models do not reflect those seen in people with CNVs of this chromosomal region, the cellular mechanisms leading to altered brain volumes can be uncovered using these models.

22q11.2 deletion syndrome—Velocardial facial or DiGeorge syndrome is manifested in individuals carrying 22q11.2 deletions, with congenital or late-onset features including mild dysmorphism, inability to seal the nasopharynx, heart defects, and impaired cognition. These patients are at high risk for a number of neuropsychiatric disorders, including attention deficit hyperactivity disorder, schizophrenia, intellectual disability and ASD. A meta-analysis has estimated the prevalence of ASD in carriers of the deletion to be at 11%.

Imaging studies in 22q11.2 syndrome have revealed smaller brain volume [361], decreased cortical thickness in the superior parietal, right parietooccipital and inferior frontal gyrus, heterotopias and fewer gyri [27, 193, 314], and reduced volume in the hippocampus [84, 85], cerebellum [49] and amygdala [85], some of which varies based on the presence of schizophrenia or cognitive deficits [20, 316]. When cases with 22q11 deletion with ASD were compared to those without, volumes of the whole brain as well as prefrontal cortex, cerebellum and left amygdala were unchanged, but that of the right amygdala was enlarged [10]. Neuropathological observations include more numerous medium spiny neurons in the caudate and interstitial neurons in subcortical white matter [390].

In the *Df(16)*$^{1/+}$ mouse model of the 22q11.2 deletion, dendritic complexity, spine length, spine width, spine density and PSD length are unaffected in the hippocampal CA1 [92]. Other studies show fewer mushroom spines, decreased spine head diameter and length, fewer glutamatergic synapses and decreased presynaptic vesicle density using *in vitro* cultures of hippocampal neurons from *Df(16)A*$^{+/-}$ mice [249]. *In vivo* analyses show reduced total and mushroom spine density and decreased spine head diameter but not length [336]. In the medial prefrontal cortex of *Df(16)A*$^{+/-}$ mice, there is a decrease in cell numbers in layers II and V, with fewer inhibitory neurons in layer V. Cell size is normal, basal dendritic length and complexity is decreased [391] and length of the primary apical dendrite is reduced in these animals. Spine turnover in these animals is higher than in WT, with

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smaller spine width but comparable spine density and length on apical dendrites [112] and decreased spine density, increased spine width and fewer mushroom spines on the basal dendrites [391]. Another mouse model of 22q11.2 deletion syndrome shows a reduction in basal progenitor cells, leading to fewer cells in the medial supragranular cortical layers [234]. Mouse models with knock out of single genes within the deleted interval show morphological alterations similar to the changes observed with the 22q11 deletion. Some examples include fewer layer II and III cortical neurons and reduced spine width, but not spine length, on basal dendrites in prefrontal cortical neurons of *Dgcr8*$^{+/-}$ mice [111] and reduced proliferation of basal progenitor cells, leading to fewer layer II and III projection neurons in *Ranbp1*$^{-/-}$ embryos [274]. However, which of these genes is relevant to ASD behaviors is an open question.

Animal models of idiopathic ASD

As multiple factors may contribute to ASD, genetic models expressing a single mutation cannot perfectly model all the pathological features of ASD. Hence, several inbred mouse and rat strains have been selected because they were found to have robust and well-replicated behavioral features reminiscent of ASD such as social deficits and repetitive behaviors (summarized in Supplementary Table 3). These inbred strains are considered to be models of idiopathic autism, as their ASD-relevant behaviors are not caused by known genetic mutations.

Inbred mice that replicate ASD phenotypes

BTBR-T^{+} tf/J: BTBR mice are the most extensively characterized and well-replicated inbred strain for the core behavioral characteristics of ASD (see Supplementary Table 3). The most striking neuroanatomical features of BTBR mice are the absence of corpus callosum and the extremely reduced hippocampal commissure, observed by diffusion tensor tractography and on histologic preparations [87, 96, 367]. Patients with agenesis of corpus callosum show difficulties in language and social communication [45, 276, 357], and decreased volume of the corpus callosum has been found in ASD (see meta-analysis in [114]). Other white matter changes in BTBR mice include abnormal posterior interhemispheric connectivity [240] and skewed orientation of glial cells in some of the affected white matter tracts [338]. BTBR mice have decreased brain volume compared to controls [96]. MRI studies have revealed decreased gray matter volume in the dorsofrontal, cingulate, retrosplenial, occipital and parietal cortex, as well as in subcortical areas including lateral and posterior thalamus, posterior hypothalamus and ventral hippocampus, but increased volume in the olfactory bulbs, medial prefrontal and insular cortex, amygdala and dorsal hippocampus [87, 270]. These findings agree with the reduction of gray matter volume over time in ASD, which is associated with increased severity of symptoms [143]. The presence of more serotonin-expressing neurons in the caudal portions of the median and dorsal raphe but decreased axon terminals in the hippocampal CA1 [134], decreased adult neurogenesis in the dentate gyrus [338], upregulation of postnatal turnover of excitatory synapses in the anterior frontal cortex [172], and unaltered synaptic density and PSD thickness in the somatosensory cortex [381] are other noteworthy features when comparing BTBR to control mice.

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BALB/cByJ (BALB): BALB is another strain of inbred mice that present with significant social deficits compared to inbred mouse strains with high sociability, such as C57BL/6J and FVB/NJ mice (see Supplementary Table 3). The size of the corpus callosum in decreased in BALB mice [102, 103].

Environmental models

Prenatal sodium valproate (VPA) exposure: VPA, or 2-propylpentanoic acid, is frequently prescribed as an anti-epileptic drug or mood stabilizer, and is known as a human teratogen. Exposure of pregnant women to VPA, but not other anti-epileptic treatments, nearly triples their risk for having a child with ASD [44, 69] and ASD-like behavioral phenotypes have been replicated in rodent models of prenatal VPA exposure (see Supplementary Table 3).

Neuropathology in prenatal VPA models depends on the dose of VPA or the age at which neuropathology was assessed [42]. Exposure to a higher dose of VPA in rodents results in reduced brain weight [250, 265], whereas a lower dose does not affect brain weight [331]. Acute exposure at a high dose reduces cortical thickness [250], whereas chronic exposure to a low dose increases cortical thickness [307]. VPA exposure leads to decreased thickness of the PFC and the basolateral amygdala at early ages, and of the hippocampal CA1 at all ages [332]. Other brain areas that are affected include the cerebellum which decreases in size [247], and the amygdala which increases in size [265].

Cell density is increased in the neocortex with a low dose [307] and decreased in the prefrontal and somatosensory cortex with a high dose of VPA [186]. Cell density is increased in the hippocampus [94], but it is decreased in the cerebellum [164] and the superior olivary nuclei of the brainstem [221] following prenatal VPA exposure. VPA exposure *in utero* leads to fewer parvalbumin-expressing interneurons in the parietal and occipital cortex [127], fewer glutamate decarboxylase 67-immunoreactive interneurons in the dentate gyrus, in the cerebellum and in the cortex [374], and loss of motor neurons in some of the motor nerve nuclei in the brainstem [303].

Ectopic cells and reduced size and complexity of dendritic branching of Purkinje cells are seen in VPA-exposed rodents [228]. Basal dendrites in rodent models of prenatal VPA exposure show no change in length on layer II pyramidal neurons in the motor cortex [331], decreased length and complexity in the medial PFC [297] and the hippocampal CA1, and increased length and complexity in the nucleus accumbens and basolateral amygdala [42]. Reduced dendritic branching but not length in the orbital PFC was reported in one study [297], whereas another showed decreased dendritic length in the same brain region [250]. In the case of apical dendrites, complexity, but not length, is increased on layer II pyramidal neurons in the motor cortex [331], decreased in the medial PFC [297], or is unchanged in layer V pyramidal neurons of the somatosensory cortex following VPA exposure in rodents [301].

Spine density is reduced in the PFC [42, 250, 297], orbital PFC [250, 297], cerebellum [250], and in the dorsal hippocampus [42, 346], but increased in the ventral hippocampus, basolateral amygdala and nucleus accumbens [42]. Fewer postsynaptic densities are observed in the hippocampus of VPA exposed rodents [199], whereas more and thicker

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postsynaptic densities are present in the medial PFC [198]. VPA rats have increased microglia in the medial PFC and more astrocytes in the hippocampus [73]. Increased glial proliferation [213] and altered astrocyte and microglial densities are observed in the hippocampus and cerebellum following VPA exposure [189].

Maternal autoantibodies: Prenatal exposure to maternal antibodies against fetal brain proteins has been implicated in autism [40, 328] (see behavioral phenotype of animal models in Supplementary Table 3). Experimental mice exposed to maternal autoantibodies *in utero* show increased cell proliferation during cortical neurogenesis [184, 229] and increased neuronal and brain size in adulthood [229]. In a similar model generated in rhesus monkeys, brain volume is increased [24], as has been described in children with autism born to mothers with the autoantibodies [259].

Maternal immune activation: Early prenatal exposure to maternal infection or fever has been associated with autism [14, 397]. Several autism models of prenatal exposure to maternal infection (maternal immune activation, MIA) have been developed in rodents and non-human primates (see Supplementary Table 3). MIA mice are induced by influenza-infection or synthetic double-stranded RNA (poly I:C), as a mimic of viral infection in dams. MIA mice have delayed cell development in the neocortex [333] and reduced cell density and abnormal developmental migration in the cerebellum [325]. The offspring of pregnant rhesus monkeys treated with poly I:C have thinner apical dendrites with more proximal dendritic branching in the dorsolateral prefrontal cortex [383].

Discussion

The field of ASD research has expanded remarkably in recent years. This growth has been mainly driven by the development and application of new genomic approaches that have allowed genetic analyses on large cohorts and brought enormous progress in our understanding of ASD genetics. However, analyses addressing the neuropathology of ASD using postmortem human brains face several challenges related to the heterogeneous nature of the disorder, the frequent presence of confounding comorbid diagnoses and the small sample sizes. The dearth of samples is currently being addressed by the development of brain banks, made possible by the invaluable contribution of samples from families of ASD subjects and the support of associations and funding agencies. The greater the availability of resources, the more accurately researchers will be able to parse out neuropathology of ASD subgroups, based on genetic and behavioral classification.

Volume-based analyses undertaken using neuroimaging techniques may lack sufficient resolution to detect subtle morphological changes (see recent aggregated study in [136]). Hence, evaluation of neuropathologic changes in ASD is crucial in order to identify cellular changes that occur in ASD-related brain areas. To date, several neuropathologic abnormalities have been reported in postmortem brain of ASD subjects, including altered size of neuronal cell bodies, changes in neuronal density, and abnormal distribution of neurons during development. Notably, several groups have highlighted the presence of possibly distinct neuropathologic alterations in young subjects versus older subjects with ASD [311, 318–320, 377]. If these observations are consistently replicated as larger sample

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sizes become available, it would be important to evaluate the impact of early interventions on the development of the abnormal findings in older subjects.

Most available neuropathologic studies in ASD postmortem samples lack genetic information, with the exception of the fragile X cases discussed earlier in this review. In the syndromic cases where genetic information was assessed, cases were rarely separated based on presence or absence of ASD or only qualitative neuropathology was provided [128, 340]. Given the paucity of samples, very few studies have accounted for accurate representation of comorbidities reflecting their incidence in patient populations [329]. Alternatively, when this information was provided, studies did not have sufficient numbers within the subtypes of ASD to compare the effects of different clinical phenotypes on neuropathology within the ASD cohorts. Hence, the inclusion of genetic information and detailed clinical reports, including comorbidities, for all cases in future studies is key to enable better correlation of specific clinical phenotypes and patient genotypes to the neuropathologic observations in the laboratory.

Although studies of the human brain are the most appropriate for understanding the cellular underpinnings of complex neuropsychiatric disorders, supplementing studies using human samples with those in animal models of ASD provides a valuable tool to fill in some of the gaps in postmortem ASD studies and to eliminate heterogeneity, thus enabling a better understanding of the effect of a mutations in ASD-associated genes or genetic loci on neurological and synaptic features. Animal models also allow the study of developmental changes or expression of genetic variants in different brain areas, something that is hard to do in human postmortem brain samples. Given the heterogeneity of ASD, no single model can be expected to recapitulate the entire spectrum of symptoms. These drawbacks notwithstanding, genetically engineered mice based on monogenic forms of the disorder have greatly advanced our understanding of the biochemical pathways involved (Figure 6). Thus, mouse models, for example the neuroligin models discussed earlier in this review, have revealed how the genetic variants of a single gene can cause different effects on the ASD-related phenotype, based on differential effects in brain areas or even specific cell types. The *Shank3* mouse models have demonstrated how the presence or absence of various isoforms derived by splicing of the same gene can produce myriad outcomes in terms of neuropathology and behavior. The *Mecp2* mouse models make evident how deficiency or overexpression of the same gene can result in ASD phenotypes through very different mechanisms. However, it is important to note that changes in behavior and neuroanatomy in these models may be influenced by the genetic background of the mouse strains, as we have discussed in the previous sections. Moreover, powerful new techniques that enable selective expression or deletion of genes in specific cell types or brain areas are enabling the tracing of ASD behaviors at the cellular level, some of which we have summarized here [269, 298, 356]. Genetically modified animal models also can be used to distinguish the disease-specific pathways that are affected in ASD when the same gene is known to cause other neuropsychiatric disorders such as schizophrenia and bipolar disease, as is the case for *SHANK3* [141, 398]. Another emerging area is the contribution of non-neuronal cells to the neuropathologic changes observed in ASD, as has been observed using ASD mutations expressed specifically in glial cells [226].

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Many of the genetically modified rodent models show decreased brain weight or volume compared to controls, which appears to be contradictory to the macrocephaly seen in some subjects with ASD. Greater cell density is a shared feature in the neocortex of *Nrxn1*- and *Mecp2*-deficient mice and in the hippocampus of *Mecp2* mice, which also agrees with some of the findings from human neuropathology in both these brain regions. However, even in the absence of altered cell numbers in many of the models, a common theme emerges from studying the various genetically modified animal models of ASD: altered density of dendritic spines. Thus, a decrease in dendritic spine density is found in the neocortex of mice deficient in *Mecp2*, *Ube3a* or *Arid1b*, in the hippocampus of models of *Shank3*, *Mecp2*, *Ube3a*, *Tsc1* or *Grin2b* deficiency, in the cerebellum of *Shank3* or *Mecp2* models, and in the striatum of *Shank3*-deficient mice, whereas increased spine density occurs in the neocortex of *Fmr1*, *Tsc1*, *Tsc2*, *Chd8* and *Dscam* mice, as well as in the amygdala of *Tsc1* mice. To illuminate fully the functional implications of altered spine densities, further investigations are warranted in most of these models to determine the distribution of various spine types, which changes in response to neuronal activity, aging or disease pathology (see reviews in [155, 280]), as well as to assess whether spine dynamics and turnover are affected by specific ASD genes. These experimental systems are proving to be invaluable in uncovering mechanisms whereby ASD genes contribute or regulate the pruning and turnover of dendritic spines during development.

In conclusion, studies using human brains from subjects with ASD have provided valuable insights into alterations at the cellular level in specific areas of the brain that are associated with ASD behaviors. Complementary investigations using animal models that replicate genetic or idiopathic ASD are revealing possible therapies that can target core ASD behaviors as well as basic mechanisms underlying the disease that may enable more personalized diagnoses. As the number of available cases increases, different subgroups of subjects can be included in neuroimaging and neuropathology studies to test potential diagnostic strategies in the clinical arena, where the need for an individualized “patient-centered” treatment has been consistently emphasized [75, 135, 203, 219, 364]. This is particularly relevant for the initial diagnosis and treatment plan that, given the neurodevelopmental character of the disorder, can have a major and long-lasting impact in the progression of the symptoms for each affected child. Finally, understanding the interactions between putative genetic susceptibility in the context of neuropathologic abnormalities at the cellular and molecular levels will allow for better diagnostic and therapeutic tools.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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List of abbreviations

ASD	Autism spectrum disorders
PFC	prefrontal cortex
FG	fusiform gyrus
CA1	cornu ammonis 1
GABA	γ-aminobutyric acid
VEN	von Economo neuron
FI	frontoinsular
ACC	anterior cingulate cortex
aMCC	anterior midcingulate cortex
PCC	posterior cingulate cortex
PIOTG	postero-inferior occipitotemporal gyrus
CNVs	copy number variations
NLGN	Neuroligins
MRI	magnetic resonance imaging
KO	knockout
KI	knock-in
PSD	postsynaptic density
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionic acid
NMDA	*N*-Methyl-D-aspartic acid
NRXN	Neurexins
WT	wild-type
CNTNAP2	Contactin Associated Protein-Like 1
SHANK	SH3 and multiple ankyrin repeat domains protein
LTP	long-term potentiation
MECP2	Methyl-CpG-binding protein 2

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FMR1	Fragile X Mental Retardation 1
FMRP	Fragile X mental retardation protein
UBE3A	E6AP-E3 Ubiquitin Protein Ligase
TSC	Tuberous sclerosis
CHD8	Chromodomain helicase DNA-binding protein 8
SCN2A	Sodium channel, voltage-gated, type II alpha subunit
SYNGAP1	Synaptic GTPase activating protein 1
ARID1B	AT-rich interactive domain containing protein 1B
GRIN2B	Glutamate receptor ionotropic, NMDA 2B
DSCAM	Down syndrome cell adhesion molecule
TBR1	T-brain-1

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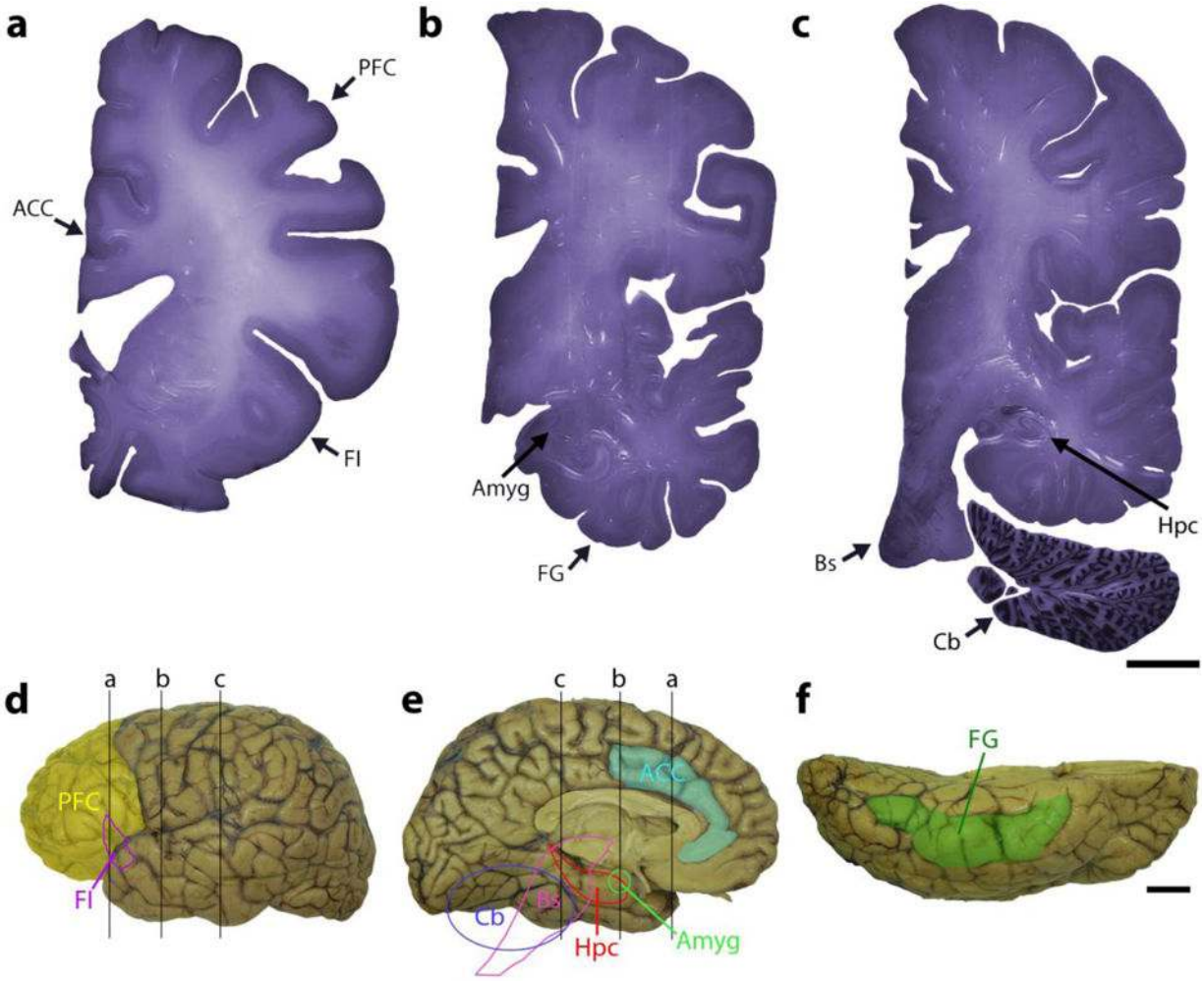

Fig. 1. Brain areas that show neuropathological changes implicated in ASD

Nissl-stained right hemispheres showing areas implicated in ASD, with panels **(a)** to **(c)** arranged in rostro-caudal order. **(a)** The anterior cingulate cortex (ACC), prefrontal cortex (PFC) and frontoinsular cortex (FI); **(b)** amygdala (Amyg) and the fusiform gyrus (FG); **(c)** brainstem (Bs; at the level of the pons), hippocampus (Hpc), and cerebellum (Cb). Human brain from the **(d)** left lateral, **(e)** mid-sagittal, and **(f)** ventral view, showing the areas represented in **(a–c)**. Brain regions visible on the displayed surface (ACC, PFC, FG) are indicated using filled areas, whereas those that are hidden beneath outer structures (FI, Amyg, Hpc) or not included in the image (Bs, Cb) are indicated by outlines approximating the location. The vertical lines indicate the approximate location of each of the sections shown in **(a–c)**. Scale bar = 1 cm for (**a–c**) and 2 cm for (**d–f**)

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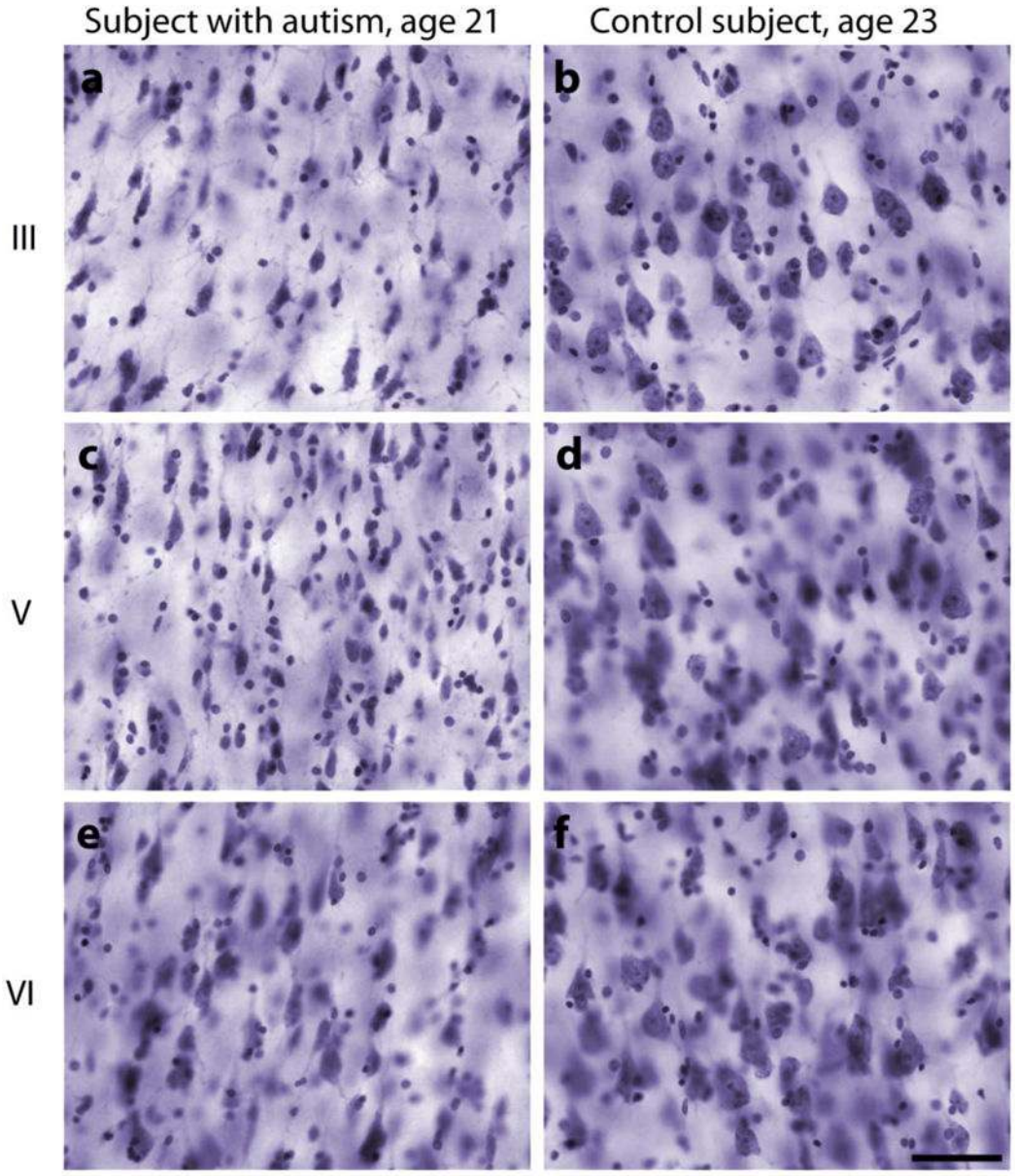

Fig. 2. Decreased perikaryal size in ASD

Neuropathologic changes in layers III, V, and VI of the fusiform gyrus in a subject with ASD compared to an age-matched control subject. Note the marked decrease perikaryal size in layers III **(a)** and V **(c)** and the less prominent decrease in perikaryal size in layer VI **(e)** in the subject with ASD, compared to the respective layers in the control subject **(b)**, **(d)**, and **(f)**. Scale bar = 50 μm

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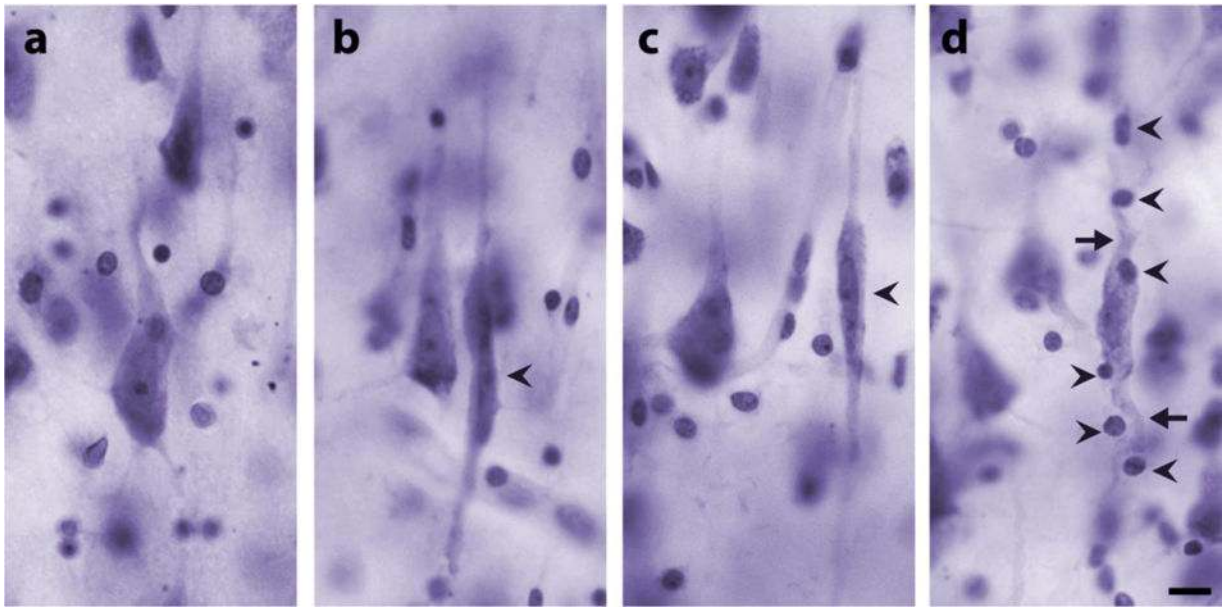

Fig. 3. Abnormal neuronal morphology in ASD

Typical and abnormal morphology of von Economo neurons (VENs). **(a)** Typical pyramidal cell in a control subject; **(b, c)** typical VEN (arrowhead) alongside a pyramidal cell; **(d)** abnormal morphology of VENs found in subjects with ASD: note the corkscrew dendrites (arrows), swollen soma and surrounding oligodendrocytes (arrowheads). Scale bar = 10 μm

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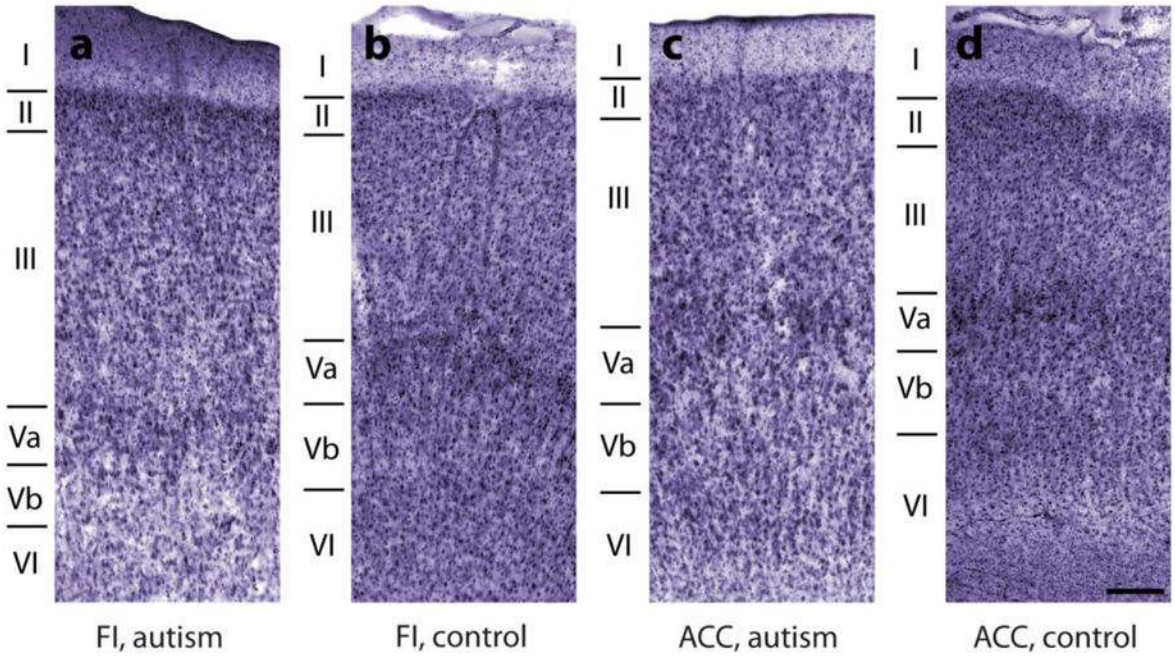

Fig. 4. Altered cell distribution in ASD

Cortical layers I–VI of subjects with ASD and control subjects in two areas implicated in ASD. Lamination is slightly less distinct in both the frontoinsular cortex **(a)** and anterior cingulate cortex **(c)** in subjects with ASD compared to controls **(b)** and **(d)**, respectively. However, no immediately obvious differences are visible at this magnification between ASD and control materials, stressing the importance of rigorous quantitative studies to reveal regional and laminar alterations in the cellular integrity and architecture of the cerebral cortex in ASD. Scale bar = 200 μm

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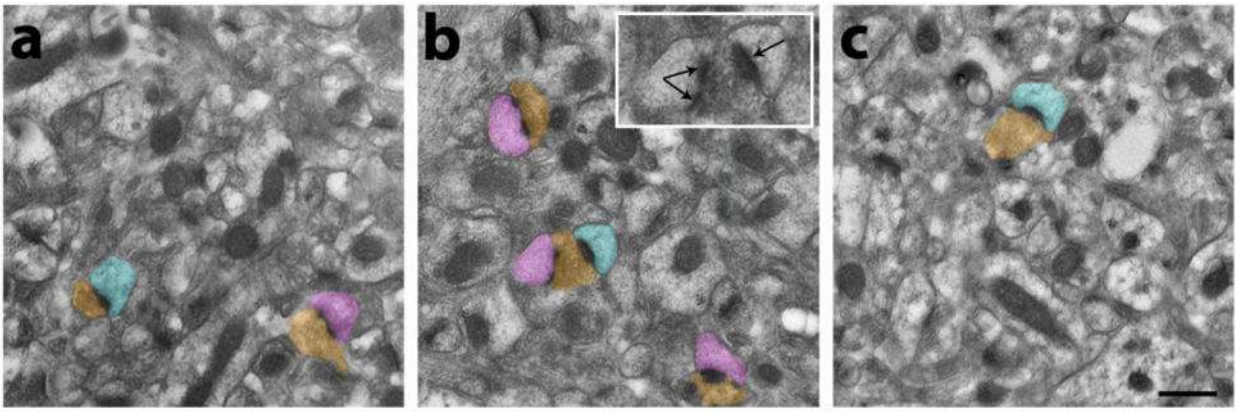

Fig. 5. Altered ultrastructure of synapses in a mouse model of monogenic ASD

Perforated synapse density was higher in the hippocampus CA1 field of young *Shank3*-deficient heterozygotes **(b)** compared to *Shank3*-homozygotes **(c)** and WT controls **(a)**. PSDs are visible as electron-dense areas on the postsynaptic side and are discontinuous in perforated synapses (postsynaptic side shown in pink), but continuous in non-perforated synapses (blue). The enlarged inset in **(b)** has arrows pointing from the postsynaptic side towards a discontinuous (double arrows) PSD and a continuous PSD (single arrow). Presynaptic terminals are indicated in orange. Scale bar = 500 nm. The difference was only apparent at 3 weeks and perforated synapse densities in both groups were comparable by the age of 5 months. (Modified from [359])

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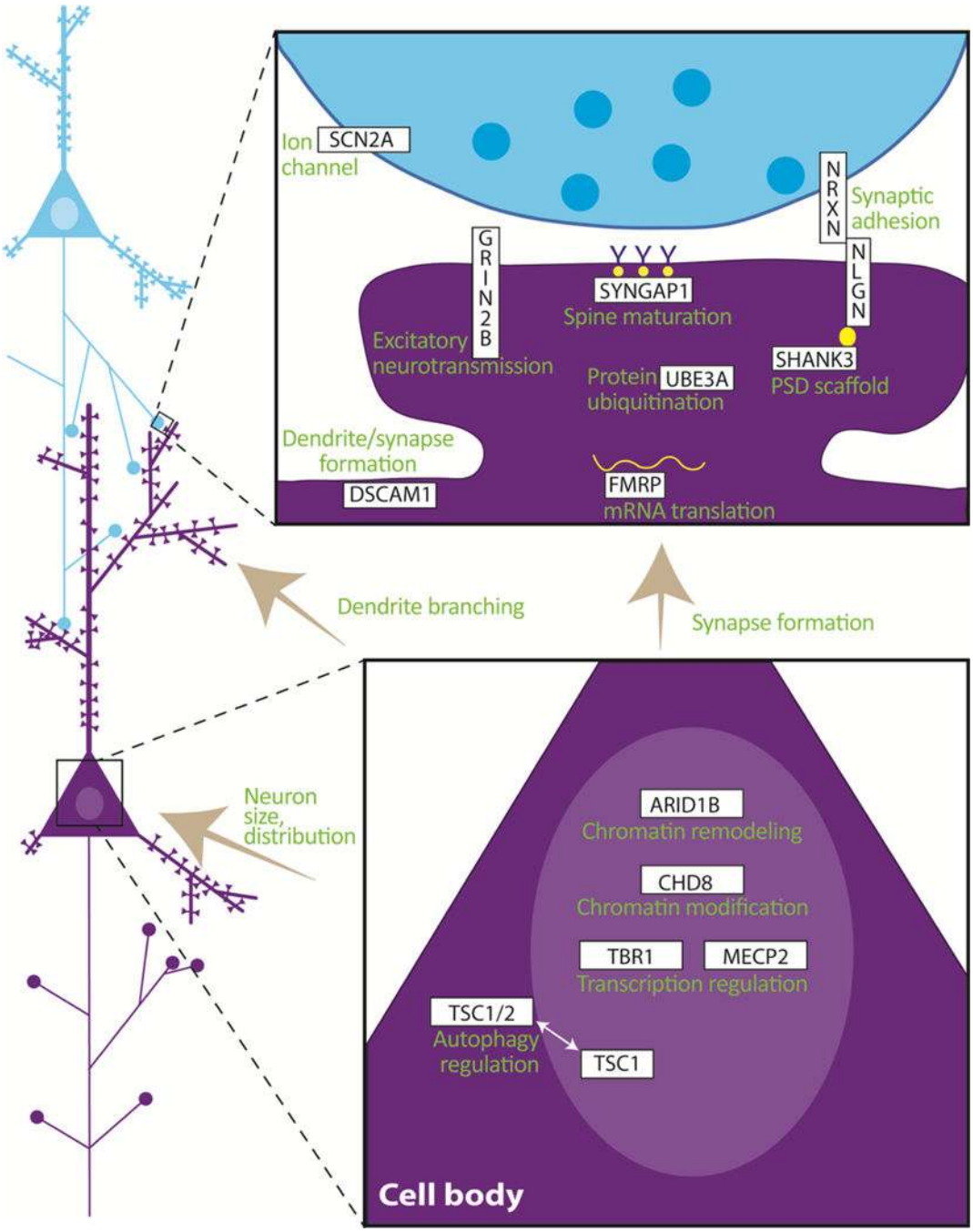

Fig. 6. Schematic representation of synaptic function of genes studied in models of ASD

Findings from different studies converge at the synapse, pointing to a deficit in the function of one or more synaptic proteins necessary for neural transmission and activity-dependent changes in spine dynamics. The cells in green and purple represent excitatory neurons and the orange cell is an inhibitory neuron. The proteins implicated in synaptic changes seen in ASD are represented in boxes, with their respective functions indicated in red text. SCN2A = sodium channel, voltage-gated, type 2 alpha subunit; NRXN = neurexin; NLGN = neuroligin; SHANK3 = SH3 and multiple ankyrin repeat domains protein 3, shown bound to

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glutamate receptors (blue Ys) and neuroligin via interacting proteins (brown dots); UBE3A = E6AP-E3 Ubiquitin Protein Ligase; FMRP = fragile X mental retardation protein, shown bound to an mRNA; TSC1/2 = tuberous sclerosis 1 or 2; MECP2 = Methyl-CpG-binding protein 2; CHD8 = Chromodomain helicase DNA-binding protein 8; SYNGAP1 = Synaptic GTPase activating protein 1; ARID1B = AT-rich interactive domain containing protein 1B; GRIN2B = Glutamate receptor ionotropic, NMDA 2B; DSCAM = Down syndrome cell adhesion molecule; TBR1 = T-brain-1.