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Cortical interneurons in autism

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Abstract

The mechanistic underpinnings of autism remain a subject of debate and controversy. Why do individuals with autism share an overlapping set of atypical behaviors and symptoms, despite having different genetic and environmental risk factors? A major challenge in developing new therapies for autism has been the inability to identify convergent neural phenotypes that could explain the common set of symptoms that result in the diagnosis. Although no striking macroscopic neuropathological changes have been identified in autism, there is growing evidence that inhibitory interneurons (INs) play an important role in its neural basis. In this Review, we evaluate and interpret this evidence, focusing on recent findings showing reduced density and activity of the parvalbumin class of INs. We discuss the need for additional studies that investigate how genes and the environment interact to change the developmental trajectory of INs, permanently altering their numbers, connectivity and circuit engagement.

The study of the neurobiological mechanisms of autism has involved parallel efforts: one in clinical research aiming to characterize the range of atypical behaviors and symptoms that are diagnosed as autistic spectrum disorder (ASD), and the other in basic science investigating the underlying disruptions in the fundamental mechanisms of brain function[1]. An existential challenge has been that describing autism as a single entity at any level of analysis, from genetics to behavior, is clearly difficult[2] considering that some individuals with ASD display formidable talents and abilities, while others suffer from devastating cognitive, emotional and behavioral symptoms. On the one hand, the heterogeneity of symptoms in ASD—which can include anxiety, social withdrawal, sensory over-reactivity, learning disability, language delay, motor stereotypies, attention deficits, seizures and sleep disturbances—suggests that many different brain regions are involved. On the other hand,

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the fact that individuals with ASD exhibit substantial similarities in the repertoire of atypical behaviors, despite vastly different genetic and environmental causes, points to a common underlying pathophysiology across brain regions. If this is the case, it should be possible to treat many autism symptoms by first identifying the underlying core circuit difference, and then nudging it closer to a neurotypical state. Unfortunately, finding that common change in brain function as a unifying model in autism has proved challenging. Unlike neurodegenerative diseases, there are no clear universal anatomical or neuropathological hallmarks in autism that serve as clues to the underlying mechanism. This has led to speculation that the symptoms of ASD are tied to changes in the emergent properties of brain function that control behavior[3]. The search for a unifying theory of autism will therefore require investigation of the physiology and function of brain circuits. Although several theories of ASD pathogenesis exist (Box 1), here we review and discuss the evidence that IN dysfunction is one key factor in autism.

From the original theory of excitation/inhibition imbalance to the more recent evidence of interneuron dysfunction

One of the first unifying theories of autism, proposed two decades ago, was the notion that symptoms were caused by reduced GABAergic inhibition[4], leading to an increased ratio of excitation to inhibition (E/I), as a functional correlate of delayed cortical maturation[5]. The hypothesized E/I imbalance in favor of excitation was appealing because it could explain the co-occurrence of seizures in autism, even though epilepsy is actually present in only a minority of children with sporadic autism[6,7]. Remarkably, our current understanding of the E/I imbalance in autism (as broadly defined at the level of synapses and circuits), or how it relates to other symptoms (for example, language delay and anxiety) remains quite limited. One problem is that, as intuitive and simple as this model may seem, it has clear limitations. Many different cell types regulate E/I balance, making it difficult to identify a specific target for therapy. In addition, computational studies have demonstrated that the E/I model may be too inflexible to account for all the observed changes in neuronal activity in autism[8]. A more nuanced view of E/I dysfunction in autism, one that considers the roles of different IN subtypes under different behaviorally relevant brain states, would be more informative.

One of the proposed mechanisms for a higher E/I ratio is reduced firing of parvalbumin (PV)-expressing INs. A growing literature suggests that hypofunction of PV neurons is fundamental to the pathogenesis of autism and, therefore, these cells could be a key to developing novel targeted therapies[9-13]. Much of the available data has focused on PV-expressing fast-spiking (FS) INs[14], for which multiple deficits have been cataloged in postmortem examination of brains from people with ASD and in various mouse models (Fig. 1). These include reductions in PV protein expression, numbers of PV-expressing INs, and PV/FS neuron activity in vivo. Interestingly, *PVALB* is among the most downregulated genes across the entire cerebral cortex in ASD[15,16]. Other IN types may also have important roles in the pathophysiology of autism, but have been less broadly investigated, so currently it remains uncertain whether their contribution to autism pathophysiology is as substantial.

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Atypical development of interneurons in autism

Autism is diagnosed in early childhood, usually in toddlers who exhibit atypical developmental trajectories or delayed milestones. Hence, atypical brain function begins long before neurons and their connections are fully mature and before circuits have undergone experience-dependent changes during critical plasticity periods. This begs one of the quintessential questions about autism: when exactly do developmental processes first start to deviate from neurotypical brain development? Understanding the exact timeline will be critical for the discovery and implementation of treatment strategies for ASD (Fig. 2). For instance, if atypical development is already apparent in the third trimester of pregnancy, will treating children after symptoms are first identified be too late to help them? The bulk of research in autism models has thus far focused on adult animals. Developmental studies will be more challenging because they must consider a multitude of developmental periods, including those corresponding to peak neurogenesis, cell migration, axon guidance, establishment of synaptic connectivity and circuit maturation. In this first section of this Review, we summarize what is known about IN development in autism, both in humans and in mouse models.

Lack of pathognomonic autism phenotype from human postmortem and neuroimaging studies

Despite the clear clinical criteria for diagnosing autism, according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders, corresponding neuropathological diagnostic criteria do not exist. While it may be argued that there is still a paucity of postmortem human studies in autism, it is striking that no obvious difference has emerged as a common macroscopic neuroanatomical hallmark in autism since the 1980s[17]. Thus, in ASD, there are no consistent brain pathologies or malformations that stand out at autopsy or in magnetic resonance imaging (MRI) studies, such as infarcts, agenesis of corpus callosum, cortical tubers, lissencephaly, mesial temporal sclerosis or polymicrogyria. The absence of a gross neuropathological signature in ASD suggests that the underlying changes are either microscopic or, more likely, due to differences in functional connectivity. Several functional MRI studies have reported a unique pattern of increased local connectivity and reduced long-range connectivity in both human autism and animal models[18]. At the microscopic level, some neuropathological studies have revealed subtle differences in minicolumnar anatomy in prefrontal cortex and in the temporoparietal auditory area in patients with autism[19], as well as disorganized neocortical architecture (dysplasias) and misplaced clusters of neurons (heterotopias and periventricular nodules), including possibly INs[20,21]. Other studies discovered differences in the density and size of dendritic spines using the Golgi method[21-23]. However, these findings need to be replicated considering the technical problems inherent to human neuropathological studies (for example, postmortem delay and fixation quality). Overall, these changes are presumably consistent with altered neurogenesis, neuronal migration, and maturation, but whether they affect preferentially excitatory neurons or INs is not clear.

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Reduced density of parvalbumin interneurons in human autism and in animal models: evidence and controversy.

One of the first suggestions that the inhibitory neurotransmitter GABA is affected in autism came from autoradiography studies of GABA receptor density[24,25]. More recently, human neuroimaging studies using magnetic resonance spectroscopy have shown reductions in the actions of GABA in the brains of individuals with autism[26]. However, a positron emission tomography neuroimaging study failed to identify differences in $GABA_A$ receptor density in any brain region of adults with ASD or in three animal models of autism[27]. Although this does not rule out impaired GABA signaling or the contribution of different IN types to ASD, these potentially conflicting results certainly illustrate the need for more expanded studies of IN neuropathology in human ASD and animal models.

One of the most notable discoveries to emerge from neuropathological studies is the low IN density in postmortem tissue from individuals with autism (Box 2). Early studies reported on variable changes in the density of GABAergic neurons in the hippocampus and cerebellum in ASD[28,29]. Then, a pioneering study revealed a significant reduction in the numbers of PV INs in the prefrontal cortex of individuals with autism compared to age-matched control participants[30], while the proportion of INs expressing calbindin and calretinin was unaffected. There are two main types of PV neuron, basket cells and chandelier cells (ChCs), which innervate the soma and proximal dendrites and the axon initial segment (AIS) of excitatory pyramidal (Pyr) neurons, respectively[14]. Using markers of perineuronal nets (PNNs), which are extracellular matrix structures rich in proteoglycans that specifically surround basket cells to stabilize synapses, the same group then demonstrated that ChC-PV neurons are selectively vulnerable in autism[31]. Considering that individual ChCs and basket cells can innervate more than 1,000 Pyr neurons[32], their loss (or hypofunction) in autism would be expected to have dramatic consequences on network excitability and function.

The reduction in PV neurons in human tissue is recapitulated in several ASD mouse models, including the valproate and MIA models of sporadic autism[33,34], the *Ube3a*-het model of Angelman syndrome[35], the *Mecp2* duplication model of Rett syndrome[36], the *Fmr1* knockout (KO) model of fragile X syndrome (FXS)[37,38], and *Shank1* and *Shank3* KO mice[39]. However, in the case of the *Cntnap2* models of cortical dysplasia focal epilepsy syndrome, not all studies are in agreement; two studies in mice have reported a reduced density of PV INs[40,41], and even in zebrafish loss of *Cntnap2a* led to decreased density of dlx5a-expressing INs in the forebrain[42], but a third study in mice found that PV IN density was unchanged[43]. For a useful list of the changes in PV cell density or PV protein expression in mouse models of autism, please refer to a previous review[11]. It is important to note that it remains unresolved whether the reduced counts of PV neurons in ASD reflect true loss of INs or merely a decrease in PV protein expression due to hypoactivity (Box 2). Regardless, this phenotype of PV cell loss and/or hypofunction has important implications for our understanding of disease pathogenesis and potentially for the design of future therapies in ASD.

As PV INs are the most prevalent IN subtype in the cortex, it is not surprising that studies in mouse models of autism have focused on the PV class, but this does not exclude the possibility that other IN subtypes are also affected. So far, there is no evidence of reduced

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numbers of other IN classes in human neuropathological studies, and only limited evidence in mouse models[44,45]. Somatostatin (SST) INs might also be affected because, like PV neurons, they are derived from a common precursor in the medial ganglionic eminence (MGE)[46]. In two studies that have reported on the density of SST INs in ASD models so far, one found it was unaffected in L2/3 somatosensory cortex of *Fmr1* KO mice[47], whereas the other found a higher density in the anterior cingulate cortex in MIA mouse models compared to controls[44]. To determine whether specific IN subtypes are selectively vulnerable in autism, and establish when loss of INs first begins, future studies will need to examine different ASD mouse models across development, using the earliest markers for MGE-derived IN precursors.

Developmental changes in early network activity and fate of INs.

Do PV neurons undergo excessive developmental cell death in autism? During neurotypical neocortical development in mice, INs are born in excess, and then approximately 30–40% are eliminated[48,49]. This wave of MGE-derived IN apoptosis in the first postnatal week occurs just days after a similar wave of developmental cell death of Pyr neurons[50]. In recent years, it has been suggested that this process of developmental IN death is finely regulated by intrinsically generated neuronal activity[48,51]. For example, during the first postnatal week in mice, INs and Pyr neurons jointly participate in synchronous bursts of activity[52,53]. The observation that INs and Pyr cells are coactive during these network events[54] is consistent with a functional ‘handshake’ between inhibitory and excitatory neurons that takes place at very early postnatal stages (Fig. 3). Thus, MGE-derived INs are already spontaneously active before they express PV[55]. Artificially manipulating network activity during this time period leads to drastic changes in the survival of these INs[48,50,54,56]. These studies in mice argue for a dynamic network of developing neurons that establish E/I balance by regulating the survival of INs in an activity-dependent manner. Thus, the reduced PV cell density in autism could be caused by disruptions in early cortical network activity, or in their ability to establish functional connections with Pyr neurons. This has not yet been investigated in models of ASD, but one would expect that decreased firing of INs in early development (see below) would prevent them from making appropriate synapses with Pyr cells and from participating with them in synchronous network events. The importance of this handshake between Pyr and PV cells may extend beyond the initial postnatal period in mice. Indeed, deleting *Fmr1* from forebrain Pyr neurons after P14–21 (using CaMK2-Cre-mediated gene deletion), well after PV cells migrate to the cortex and undergo developmental death, results in a reduced density of PV-expressing cells and PV cell hypoactivity (M. Rais, J. W. Lovelace, X. S. Shuai, W. Woodard, S. Bishay et al., unpublished observations). Future studies will need to address this important question with both acute and chronic manipulations of the activity of Pyr neurons and INs, at various developmental stages, including critical periods[57]. The role of brain-derived neurotrophic factor–tropomyosin receptor kinase-B (TrkB) signaling may be especially relevant in light of recent evidence of delayed maturation of INs in *Fmr1* KO mice, which can be rescued by a TrkB receptor agonist[58].

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Interneuron diversity, genetic identity and synaptic specificity

The GABAergic system is remarkably complex, owing in large part to the wide variety of IN subtypes, which differ in their functional properties and in their morphology[59]. The axonal arbors of neocortical INs often exhibit extraordinarily complex patterns of elaboration, such that they can synapse onto excitatory neurons in different compartments (the soma, dendrite and even AIS) and different layers, as well as onto other inhibitory neurons (Fig. 3). This synaptic specificity of INs has particular relevance to autism because differences in how INs make contacts with their target neurons during development could conceivably change circuit function, contributing to symptoms of autism.

Specificity of synaptic connectivity by cortical INs.

Investigating the molecular determinants of IN synapse specificity will be critical to understanding circuit modifications in autism. Whereas the signaling pathways that govern synapse specificity for excitatory neurons (for example, neurexins and neuroligins) have been well characterized[60], less is known for INs[61,62]. It is important to realize that INs come in different flavors and that even a broad class defined by expression of a single neurochemical marker (for example, PV, SST or vasoactive intestinal peptide (VIP)) can comprise different subtypes. Hence, circuit dysfunction in autism may not necessarily arise from deficits in an entire class of INs but could stem from more subtle changes in a particular subtype within that class, including differences in their ability to establish specialized synapses.

Recent discoveries about synaptic organizer molecules that preferentially promote IN synapses in specific compartments of cortical Pyr neurons may provide insight into how the connectivity of INs is affected in autism (for example, dendritic tuft, soma and AIS). For instance, CBLN4 has been identified as a key determinant of how SST neurons target the apical dendrites of Pyr neurons[63]. CBLN4 is released presynaptically and acts as a bridge between neurexins in order for SST neurons to establish functional synapses at the appropriate dendritic targets. Forcing expression of CBLN4 in PV basket cells promotes the ectopic formation of synapses onto dendrites of Pyr neurons, making them act like SST neurons, while other synaptic organizers (for example, LGI2) are likely to have analogous roles for PV basket cells and ChCs[63] (Fig. 3). In addition, neuregulin-ERBB4 signaling controls the development of inhibitory circuits in the cerebral cortex by regulating the connectivity of INs with Pyr neurons[64,65]. Future studies may establish links between these or other signaling pathways and autism, by identifying when synaptic targeting deficits of INs first begin in mouse models of autism and the role of genetic or environmental risk factors in this process. Even the most intricate aspects of this early physical handshake between inhibitory and excitatory neurons may be critical to the functional changes that underlie the pathogenesis of autism.

Genetic susceptibility and transcriptomic profiling of interneurons in autism.

Single-gene neurodevelopmental disorders associated with autism offer a unique window into the role that those mutations play in altering the trajectories of circuit assembly in the brain. Even in sporadic autism, genetic susceptibilities conspire with deleterious

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environmental factors to disrupt those developmental trajectories (Fig. 1). To better understand the role of specific environmental influences (pollutants, medications, infectious agents and social or sensory deprivation) in the pathogenesis of ASD, additional large epidemiological studies will be needed. Despite an abundance of animal models of sporadic ASD that attempt to reproduce environmental triggers, the face and construct validity of these models is not always compelling. By contrast, ASD is highly heritable (50–70% heritability) and the genetic contributions have been characterized in formidable detail[66]. The genetic architecture of autism is enormously complex, with hundreds of risk genes or loci currently known to be associated with increased risk of ASD (although most variants are yet to be elucidated).

Perhaps not surprisingly, ASD-risk genes are enriched in mid-fetal development, corresponding to the time window when excitatory and inhibitory neurons are born and migrating[66]. Single-cell mRNA expression studies have shown that many genes that are differentially expressed in human ASD are IN-specific genes, including *PVALB, SST* and *VIP* (which are all downregulated in ASD)[67,68]. However, data from these and other studies[16] indicate that the most differentially expressed genes in human ASD are found in excitatory neurons of upper cortical layers. It is also noteworthy that gene-network analyses have revealed upregulation of networks in astrocytes and microglia (consistent with inflammation), while networks in neurons were downregulated. Another interesting finding is that *PVALB* expression levels progressively decrease with age in people with ASD, even throughout adulthood[69]. Considering that symptom severity can lessen over time in a subset of individuals with autism[70,71], it will be interesting to determine whether this reflects compensatory plasticity of the network, and also to explore the relationship between PV levels and symptom severity across the life span.

Taking into account the potential role of INs in autism pathogenesis, and the fact that common transcriptomic signatures have been identified in brain tissue from individuals with ASD, it will be important to characterize the transcriptome of INs during typical development. We have entered a new era of IN classification that integrates their functional and morphological characteristics with their gene expression profiles. This new system, which differentiates between more cohesive ‘met’ (morphology, electrophysiology and transcriptome) types[72], has equipped us to test why specific aspects of IN development and function are selectively vulnerable in autism. For example, clarifying how the developmental trajectories of different met types of INs are influenced by specific gene variants will probably lead to better stratification of autism, a critical step for eventual personalized medicine efforts.

One wonders, incidentally, whether the same IN diversity that enabled complex social behaviors, theory of mind and higher cognition in humans may also have predisposed them to autism. Transcriptomic studies are beginning to provide a more granular appreciation of how different IN types evolved in mammals[73], which could address that question. Interestingly, there are more differentially expressed genes in GABAergic neurons than in excitatory neurons[74]. Furthermore, among autism risk genes that are meaningfully expressed in INs, there is much higher correspondence between pairs of primates (for example, humans to macaques or humans to marmosets) than between human and mouse, and this

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exceeds the similarity for non-risk genes (F. Krienen, personal communication). Although virtually all different IN subtypes are conserved from mice to marmosets, at least one new group of INs (Tac3-expressing cells) was identified in the striatum of marmosets and humans that was not found in mice or ferrets[73]. It is fascinating to consider the possibility that human-specific IN subtypes could be particularly affected in ASD.

Interneurons and circuit dysfunction in autism

Whatever the degree of transcriptomic convergence in ASD, it pales in comparison to the phenotypic convergence that is observed at the level of circuit dysfunction, in particular as it relates to changes in INs. Symptoms of autism are ultimately linked to disruptions in the properties that emerge when neurons interact in the brain, so an understanding of how circuit activity is affected in different brain regions in autism will be necessary to develop effective symptomatic therapies.

Network hypersynchrony and neuronal hyperexcitability.

One of the earliest discoveries of altered network dynamics in autism was hypersynchrony, which has been described in multiple *Mecp2* mouse models of Rett syndrome[75], and is particularly evident in neonatal and early postnatal *Fmr1* KO mice, which exhibit a significant delay in the desynchronization of cortical network activity at roughly postnatal day (P) 12 (refs.[76-78]). It is possible that this early hypersynchrony could be due to changes in the expression of certain GABA receptor subunits[79,80], a delayed GABA polarity switch[81,82], changes in the intrinsic properties of INs[58] or loss of PV INs[38].

A related phenotype, neuronal hyperexcitability, has also been repeatedly described in autism models, including *Fmr1* KO mice[83-85], *Shank3* KO mice[86,87] and *Ube3a* mice[88]. Both hyperexcitability and hypersynchrony could explain why some children with autism are predisposed to seizures and sensory hypersensitivity[89]. One might expect that hyperexcitability would lead to exaggerated sensory-evoked responses in Pyr neurons of sensory cortices. However, the data are mixed, and some studies have revealed reduced stimulus-evoked responses in models of autism. For example, whisker-evoked responses are suppressed in L2/3 neurons of somatosensory cortex in *Fmr1* KO mice, *Cntnap2* KO mice, *16p11.2*$^{del/+}$ mice[90] and *Syngap1* mice[91]. Of course, this could reflect homeostatic compensation by other IN subtypes within L2/3, whereas deeper layers (which have not been extensively studied in autism models) might show the expected hyperexcitability. This dichotomy between internal constructs in L2/3 and cortical output by L5/6 would be consistent with the notion of independent circuits and attractors operating with different dynamics[92]. Sensory hypersensitivity may instead be caused by a failure of neurons to adapt to ongoing stimulation, as observed in *Fmr1* KO mice[93,94] and in humans with autism[95]. This lack of neuronal adaptation may prevent the individual from tuning out certain irrelevant stimuli (for example, when they are non-threatening or not behaviorally salient). It would be worth exploring whether similar defects in adaptation occur in other ASD mouse models and whether they arise from changes in the activity of certain INs.

More generally, the concepts of hyperexcitability and hypersynchrony alone are not particularly satisfying when aiming to understand symptoms in autism. Even though

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hyperexcitability and hypersynchrony are likely to disrupt various aspects of brain function, it is not immediately obvious how they contribute to more subtle sensory processing symptoms in autism (tactile defensiveness and impaired discrimination), or to social and learning deficits. Instead, changes in how IN activity is modulated in different brain regions, under certain behavioral states or tasks, would be a better framework for approaching specific symptoms of autism. Over the last decade, several studies that recorded neuronal activity in vivo have depicted how different IN subtypes are engaged in behavior in previously unrecognized ways. For example, PV neurons are important for modulating the tuning of Pyr neurons in primary visual cortex (V1) to improve visual discrimination[96], whereas VIP neurons are involved in a disinhibitory circuit that provides an error signal critical for reinforcement learning[97,98]. Studies are now beginning to evaluate the role of INs in specific behavioral assays in autism mouse models[87,99].

Gamma oscillations and other brain rhythms.

Another way in which INs modulate network activity and behavior is through the generation of brain oscillations. Rhythmic oscillatory activity underlies many processes from sensory integration to higher-order cognitive functions[100]. Using electroencephalography, a number of deficits in brain rhythms have been described in children and adults diagnosed with ASD, as well as in animal models. Increased power of resting-state gamma band (>30 Hz) oscillatory activity is associated with sensory processing and communication deficits in individuals with ASD[101-103] and FXS[104]. PV INs are critical for generating gamma rhythms[105,106], although SST INs also modulate gamma oscillations[107]. A recent study revealed that PV hypofunction (after PV cell-specific deletion of the NR1 subunit of NMDA-type glutamate receptors) results in increased broadband gamma power through decreased synchronicity[108]. There is also remarkable cross-species convergence in this phenotype, with electroencephalography alterations in the gamma-frequency band shown in humans with FXS and in *Fmr1* KO mice, including similar inverse power coupling of gamma and theta oscillations[104,109-113]. Gamma rhythms are also disrupted in the mouse model of 22q11.2 deletion syndrome[114], as well as in the MIA[34] and valproate models of sporadic or environmental autism[81,115]. Therefore, although the circuit mechanisms for these oscillatory phenotypes are still not clear, changes in gamma band activity in ASD can be viewed as further evidence of IN dysfunction.

PV cell hypoactivity.

A growing number of studies have demonstrated that PV neurons are hypoactive in several genetic models of autism, including those related to mutations in *Fmr1, Mecp2, Shank3, Syngap1* and *Cntnap2* (Table 1). Interestingly, PV cell hypoactivity coincides with other phenotypes related to PV INs in multiple ASD models (Fig. 4). The evidence of IN hypoactivity comes from multiple laboratories using different techniques in various cortical regions. In the *Fmr1* KO model, in vivo experiments with either calcium imaging or cell-attached recordings in mice, or silicon probes in rats, have all shown that FS/PV INs are hypoactive in both primary visual and somatosensory cortices[90,99,116]. The decrease in the activity of PV INs has been demonstrated to be causally related to the behavioral deficits because artificially raising the activity of PV neurons using chemogenetics rescues alterations in orientation selectivity in V1 and perceptual learning in *Fmr1* KO mice[99].

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The same strategy also reverses CA1 network dynamics and deficits in contextual fear conditioning and social memory in 22q11.2del (*Lgdel*$^{+/-}$) mice[114,117]. Similarly, optogenetic activation of PV INs corrects social impairments in *Cntnap2* mice, where PV cells in the prefrontal cortex fail to modulate their firing during social interactions in the way they do in wild-type mice[118]. Decreased firing of PV INs could also be responsible for the observed developmental delay in the desynchronization of cortical network activity in *Fmr1* KO models[76,78], and for the differences in theta/gamma power coupling in auditory cortex[111,119].

If PV IN hypofunction is a major contributor to the pathophysiology of autism, then it should be present in early brain development, given that changes in behavior are already apparent in toddlers. Although gamma oscillations are nearly absent in V1 of *Fmr1* KO rats as early as P9–11 (ref. [116]), the earliest documented ages of reduced activity of PV cells so far are P18–22 in *Fmr1* KO mice[90] and P19–24 in *Fmr1* KO rats[116]. However, our own recent observations suggest that in *Fmr1* KO mice, PV neurons are already hypoactive at P15 (N. Kourdougli and C.P.-C., unpublished data). It remains to be determined whether cortical IN precursors that will eventually become PV cells are already hypoactive in the first postnatal days, although this could explain the low numbers of PV neurons observed in *Fmr1* KO mice and other models of autism. Indeed, INs that are hypoactive and do not establish the appropriate handshake with excitatory neurons could be destined to die.

Interneurons in autism: friend or foe?

This Review has highlighted much evidence that implicates, directly or indirectly, IN hypofunction in autism in humans and in animal models of ASD. By comparison, Pyr cells in ASD models do not exhibit such marked changes in their numbers or spiking activity as INs, even though they have been studied much more closely. But whether IN hypofunction is directly involved in causing ASD symptoms is not yet settled. At least for now, the puzzle pieces do not fit perfectly together to definitively conclude that IN hypofunction is a primary etiology in autism. Many studies have also investigated the impact of conditionally deleting (or re-expressing) ASD-risk genes in specific IN populations (Box 3), and there is some debate as to whether the observed changes in IN activity in autism are primary or secondary. For instance, although synaptic E/I imbalance is apparent in several autism models, it is not always correlated with increases in the firing rates of excitatory Pyr neurons in vivo; it has therefore been argued that changes in feed-forward inhibition may instead reflect a compensatory attempt to rebalance a primary decrease in excitation[90]. But some may find the idea that INs are capable of compensation in the autistic brain surprising and unlikely. After all, how could INs within circuits that are disrupted during development be healthy enough to mediate adaptive plasticity that restores E/I balance? Furthermore, why would the compensatory process be so drastic as to involve eliminating a large fraction of cortical PV INs?

There are certainly more compelling reasons to believe that PV cell hypofunction is a primary driver of circuit dysfunction in autism. First, mice deficient in PV (*Pvalb* KO mice) exhibit certain behaviors also seen in autism, including impaired social behaviors and increased susceptibility to seizures[120]. Second, blocking synaptic transmission of

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PV neurons in the ventral hippocampus can impair social memory discrimination in mice[121]. Third, PV cell changes in mouse models of autism are detected very early in postnatal development (before one would expect compensatory changes to occur)[58]. Fourth, and perhaps the most convincing evidence for PV cell hypofunction being a primary phenomenon, is that various strategies to directly raise the activity of these INs can restore network activity and rescue behavioral deficits in mouse models[99,117,118,122]. Clearly, more studies will be needed to determine whether acute and chronic treatments that directly affect the function of PV INs provide similar benefits. Settling this debate seems like one of the most urgent priorities for autism research, given the therapeutic implications for ASD. The stakes could not be higher. If it turns out that decreased activity of PV INs is actually a compensatory phenomenon, then therapies that restore IN hypofunction would aggravate symptoms of autism, not treat them.

Outstanding questions

With the emerging protagonist role of INs in autism, many questions remain unanswered. First, when during development do INs first demonstrate changes in autism and what are the principal manifestations of their dysfunction? Second, are all INs involved in autism pathogenesis to some degree, or does one class have a larger impact? Third, can we integrate the findings from molecular and genetic analyses in autism with the physiological and functional changes observed in inhibitory circuits in affected humans and model organisms? Fourth, how exactly does IN dysfunction affect networks and lead to symptoms in autism? Answering these questions may ultimately help to resolve the key issues regarding the primary etiology of autism and whether targeting INs has potential for the development of therapies. We have argued that IN changes in autism are a primary cause of symptoms, but more research is needed to demonstrate that they are not secondary incidental findings or even a compensatory effort to restore homeostasis.

We conclude this Review by suggesting future directions for research that should attempt to answer these questions and further clarify the role of INs in the pathogenesis of autism. First, we need increased sampling of postmortem tissue from humans with autism to assess INs across different brain regions and developmental time points, including embryonic stages, while making correlations with the severity of symptoms. However, identifying fetal tissue would be quite challenging (considering the postnatal diagnosis of ASD) and only possible for single-gene ASDs. Together with human neuroimaging studies, postmortem analyses should also help to stratify autism into categories that would be more useful for clinical trials. Second, developmental studies in mice should investigate the maturation of INs in different models of autism (including their origins, migration, cell death and functional integration into circuits), not just in the neocortex but also in other brain regions. In contrast, investigations in adult animals may be less productive because those mature circuits have had time to adapt and compensate; therefore, the differences observed may not point to viable treatment options. Cerebral organoids derived from human induced pluripotent stem cells and assembloids (of inhibitory and excitatory neurons, plus glia) will offer a unique opportunity to investigate how autism risk genes and environmental insults perturb these developmental trajectories[123]. Interestingly, a recent study uncovered a lower density of GABA-expressing neurons in organoids derived from human induced

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pluripotent stem cells from patients with FXS[124]. It is also worth noting that the number of autism mouse models in which INs have been extensively studied remains fairly small. The field would benefit from additional surveys of IN density and function in other models and in other species. More data on GABAergic impairments, particularly in non-syndromic models, might provide considerable insight into the role of early inhibitory disruption in ASD. Third, using IN cell-specific mouse lines, we can now manipulate and record from different IN subtypes, aiming to determine whether any changes in their activity are primary deficits that precede behavioral deficits or instead represent homeostatic compensation. It will be particularly important to assess the role of INs in specific behavioral changes associated with autism. Fourth, we need to understand when transcriptional dysregulation first begins in INs and how the functional handshake between Pyr neurons and INs shapes their responses. Fifth, because of evolutionary differences across species, parallel studies will be needed in nonhuman primates, including genetic marmoset models of autism, in which more sophisticated social behaviors and learning paradigms can be pursued. Finally, there is already sufficient evidence about IN dysfunction in autism to expand preclinical studies in ASD models to modulate the activity of INs in behaviorally relevant settings. The notion that PV neurons could be harnessed to treat autism is not far-fetched. Besides chemogenetic and pharmacological approaches, another potential therapeutic strategy in ASD would be to use IN precursor transplants, which show promise in animal models[125], although the disadvantage is that they are more invasive and cannot be turned off.

This research will also be relevant for schizophrenia, which shares many of the IN deficits we have described for autism[9] (Fig. 4). Despite the obvious differences in clinical symptoms (for example, auditory hallucinations occur in schizophrenia, while age at symptom onset is younger for ASD), this should not be too surprising given the overlap in underlying genetic risk factors for these conditions. There is also substantial overlap in many symptoms (social withdrawal, poor eye contact, atypical sensory processing and egocentricity). However, a shared deficit in PV INs does not necessarily mean similar changes at the level of circuits. In contrast to ASD, the paucity of good animal models for schizophrenia (especially genetic mouse models) has meant that in vivo studies of network activity are still lagging behind. Thus, we do not yet know whether there is convergence at the level of circuit dysfunction for these two conditions. The hope is that further research into how changes in INs relate to specific neuropsychiatric symptoms will ultimately inform our ability to design new treatments to restore circuit function at the appropriate developmental time windows.

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Box 1 |

Maternal immune/inflammatory and gut microbiome theories of autism

Although this Review focuses on the growing evidence that INs are involved in the pathogenesis of ASD, alternative mechanisms and hypotheses have also been proposed to explain the heterogeneity of the disorder. Because these other theories have no clear links to INs (at least not yet), we do not discuss them in detail. A particularly evocative hypothesis is centered on interactions between the gut microbiome, the immune system, and brain development. The maturation of neural circuits during both prenatal and postnatal human development is strongly influenced by signals from the external world. Both the maternal gut and vaginal microbiomes, as well as the microbiomes of children, have been proposed to play a role in neural development[132]. The most compelling work in this area has been performed in mice, where studies have suggested that dysbiosis of gut microbiota is associated with autism-like behaviors[133,134]. Furthermore, associations between gut microbiota and the immune system in the mother are thought to be at the heart of altered neurodevelopment in autism[135]. In addition to clear correlations between maternal inflammatory states and the incidence of ASD in human epidemiological studies[136], maternal immune activation (MIA) in rodents causes autism-like behavioral and neuropathological effects in offspring[137]. Links between MIA and inhibitory circuit dysfunction are beginning to emerge[138] and, notably, maternal inflammation affects the earliest steps of cortical IN development, including the proliferation, migration and maturation of INs[139]. Similar links between IN hypofunction and the gut microbiome have not yet been established. Instead, research has focused on the role of microglia, and on links between the gut microbiome and elevated oxidative stress in the context of ASD. Of note, some transcriptomic studies have shown that the differentially expressed genes relating to the molecular state of microglia are among those preferentially affected in autism[68]. Further work in these areas of research is providing insight into how systemic disruptions from the periphery and environment can shape neurodevelopmental trajectories and underlie some subclasses of ASD.

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Box 2 |

Excessive parvalbumin cell death or parvalbumin protein downregulation?

One of the most compelling discoveries in ASD research has been the reduced density of PV neurons in the cerebral cortex, because of remarkable convergence between human and animal studies. But there is a caveat in the interpretation of these findings when they rely solely on immunohistochemical analysis, because a downregulation of PV protein expression could be misinterpreted as a lower density of these INs[39]. Interestingly, PV immunoreactivity correlates with the activity of PV INs, with less active neurons expressing lower levels of PV protein[140]. This is intriguing in light of a number of recent studies showing that PV INs are hypoactive in various mouse models of autism[90,99] (see 'Interneurons and circuit dysfunction in autism'). Therefore, PV expression may be reduced in autism simply because of the hypoactivity of PV cells. Additional studies will be needed to distinguish between the possibility that a subset of PV cells exists in a hypofunctional and not fully mature state (perhaps not even recognizable as PV INs) and the possibility that some of these PV cells in fact die, as a result of this hypoactivity or their inability to establish stable connections with Pyr neurons. Indeed, FS INs are known to exist along a spectrum of cell types whose relative abundance is constantly fine-tuned by neuronal activity throughout the life span of the animal[141]. Human studies are more consistent with a bona fide reduced density of PV INs because there is also decreased staining for PNNs and reduced ChC cartridge counts[31]. If some PV cells are truly absent, it remains to be determined whether this results from excessive apoptosis, reduced neurogenesis or defective migration[142-144]. The most general interpretation of all of the studies so far is that the trajectory of IN development (especially that of PV cells) is disrupted in autism, leading to the premature death of a substantial proportion of PV cells, as well as hypoactivity in others (which is associated with reduced PV protein expression), even though their birth and migration appear to proceed relatively uninterrupted. Incidentally, it has been proposed that PV neurons may be particularly susceptible to genetic and environmental insults, based in part on the high metabolic demands of FS cells (which is why they express high levels of the calcium buffering protein PV) and on the enormous excitatory inputs they receive[145]. However, PV INs do not adopt their FS properties or express PV until the second postnatal week[146], which is after the wave of developmental cell death. Therefore, if PV cells do in fact die, it is probably not because they are hypermetabolic.

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Box 3 |

Parvalbumin cell-specific deletion of autistic spectrum disorder-associated genes

Gene reactivation studies are underway in ASD. To ensure their success and the optimal time windows, it will also be important to determine the consequences of cell-type-specific deletion of ASD-risk genes at the circuit and behavioral levels[147]. Recent studies in mice have investigated whether the conditional deletions of these genes in Pyr cells or in different classes of INs can mimic the behavioral deficits seen with germline mutations. For example, genetic deletion of *Mecp2* in all INs or only VIP cells leads to circuit dysfunction and replicates several behavioral deficits seen in the full *Mecp2* KO mouse, as well as a significant decrease in high-frequency local field potential activity[148]. By contrast, deletion of *Mecp2* in PV-Cre or SST-Cre mice caused no obvious local field potential deficits, and only the SST-specific KO mice had a reduced life span. However, *Mecp2* was probably not deleted from PV neurons until the second postnatal week. It is likely that earlier *Mecp2* knockdown in PV neuron precursors (for example, using a promoter with earlier expression, such as *Nkx2.1* or *Lhx6*) would have resulted in robust circuit and behavioral deficits (if indeed *Mecp2* is critical for PV neuron development). Another study showed that *Shank3* deletion in INs (using the *Dlx5/6* promoter) leads to enhanced spontaneous and stimulus-evoked activity of Pyr neurons, as well as sensory hypersensitivity to relatively weak tactile stimulation, similarly to global *Shank3* KO in mice[87]. There are similar examples of behavioral deficits and circuit dysfunction triggered by IN-specific deletion of other ASD-associated risk genes (for example, *Syngap1, Erbb4, Mecp2* and *Shank1*) and other important synaptic genes (for example, *Grin1* and *Grm5*) This is not so surprising and probably reflects that these genes are essential for IN function, and further supports the argument that IN dysfunction is central to ASD pathogenesis. But while functional alterations in specific cells (such as PV INs) and inhibitory circuits could underlie ASD-associated phenotypes, the gene deletion site may not coincide with the site at which dysfunction is observed. For example, mice in which *Fmr1* is deleted solely in forebrain excitatory neurons exhibit a lower density of PNNs and PV-expressing cells[149]. Considering that many of the genes implicated in human ASD are related to synaptic function (and are not necessarily specific to INs), it seems important to prioritize the investigation of how these mutations disrupt the handshake between Pyr neurons and INs in the corresponding mouse models. More specifically, conditional ASD gene deletion or rescue at different developmental stages will be essential to determine the exact timeline of when and how these genes orchestrate circuit assembly and function.

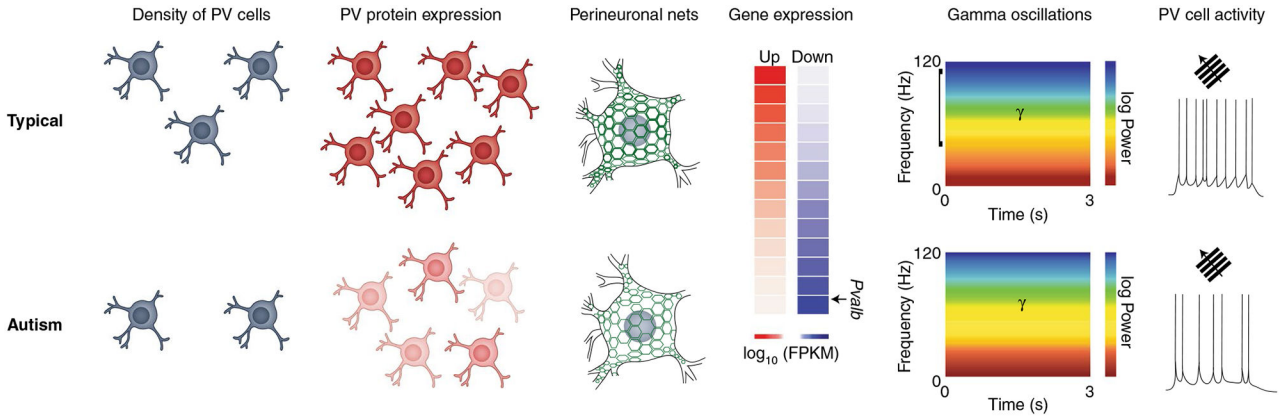

Fig. 1 |. Evidence of parvalbumin-expressing cell hypofunction in autism.

There are several lines of converging evidence from human and animal studies that support a role of PV IN hypofunction in the pathogenesis of autism: (1) the density of PV INs is reduced; (2) the expression levels of PV protein are lower; (3) the density of PNNs around INs is decreased; (4) the power of baseline gamma oscillations (regulated by PV and SST INs) is increased; and (5) the activity of PV INs is decreased (for example, decreased visually evoked activity). FPKM, fragments per kilobase of transcript per million mapped reads.

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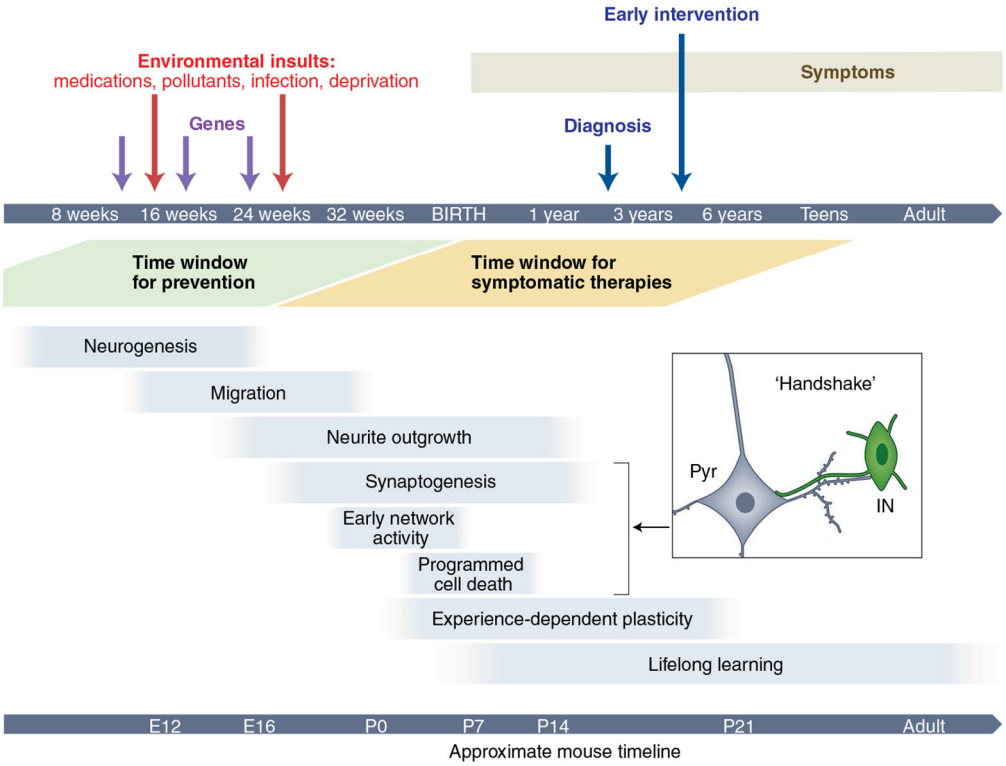

Fig. 2 |. Genes and environment affect milestones of brain development in autism.

During human gestation, environmental insults and genetic risk factors can affect typical brain development at specific milestones, eventually leading to the symptoms of autism. These stressors could affect the birth and migration of neurons, their ability to form synaptic connections or generate the earliest forms of spontaneous network activity, and programmed cell death, as well as experience-dependent plasticity and learning. We propose that these changes occur at a critical stage when excitatory and inhibitory neurons participate in a structural and functional handshake (bracket and arrow). By the time symptoms of autism are recognized in toddlers, the circuit alterations may be irreversible, such that a complete restoration of typical network function (even with genetic rescue in single-gene autism disorders) may not be possible. However, it may be feasible to restore circuit function to a more typical regime by interventions after diagnosis. An approximate timeline of the equivalent stages of circuit formation in mice is also shown for comparison.

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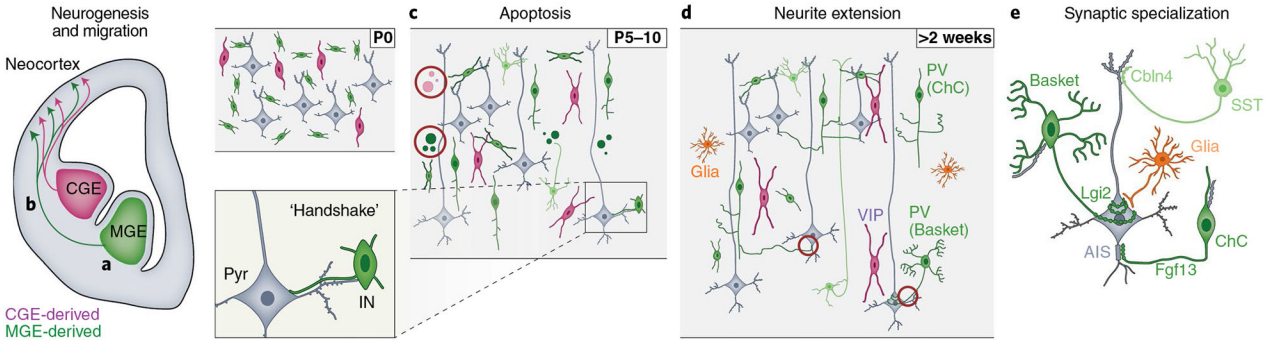

Fig. 3 |. Birth, migration and fate of cortical interneurons.

Cartoon of the typical trajectory of cortical IN development highlighting five critical stages of maturation that could proceed differently in autism. Many of the phenotypes observed in autism, whether reduced density of MGE-derived INs, mis-targeting of axons by PV neurons, network hypersynchrony or PV hypoactivity, could be traced back to an important checkpoint of postnatal development, when Pyr neurons and INs are first establishing functional connections (the 'handshake'). Whether one of these changes triggers the others and is the ultimate culprit of circuit dysfunction is not yet clear. **a**, Neurogenesis: birth of IN precursors at the MGE or caudal ganglionic eminence (CGE). Future PV and SST INs are generated in the CGE and express markers such as NKX2.1 and LHX6. **b**, Migration: MGE-derived and CGE-derived IN precursors migrate first tangentially and then radially into the cerebral cortex. Although cortical dysplasias described in some humans with autism could be explained by slower neuronal migration, changes in the migration of INs specifically have not been documented in autism. **c**, Apoptosis: a wave of developmental cell death in the second postnatal week is responsible for the loss of around 30% of cortical INs. Sensory-evoked and spontaneously generated cortical activity is responsible for this refinement in IN population density. Decreases in the intrinsic activity of INs (for example, due to immaturity) could lead to excessive death of INs in autism and explain the reduced density of PV neurons. **d**, Neurite extension: INs extend dendrites and axons in search of appropriate synaptic partners. IN hypoactivity could interfere with their ability to interact with Pyr neurons during an early acquaintance handshake or embrace between them. Immaturity of PV dendritic arbors has been reported in some mouse models of autism. **e**, Synaptic specialization: cortical INs eventually adopt their mature morphology and establish specialized synapses that target specific compartments of Pyr cells and other INs. This cell-type-specific structural connectivity is regulated by synaptic organizer proteins. It will be important to investigate whether differences exist in the expression of these genes, or in synaptic specificity, between autism models and controls.

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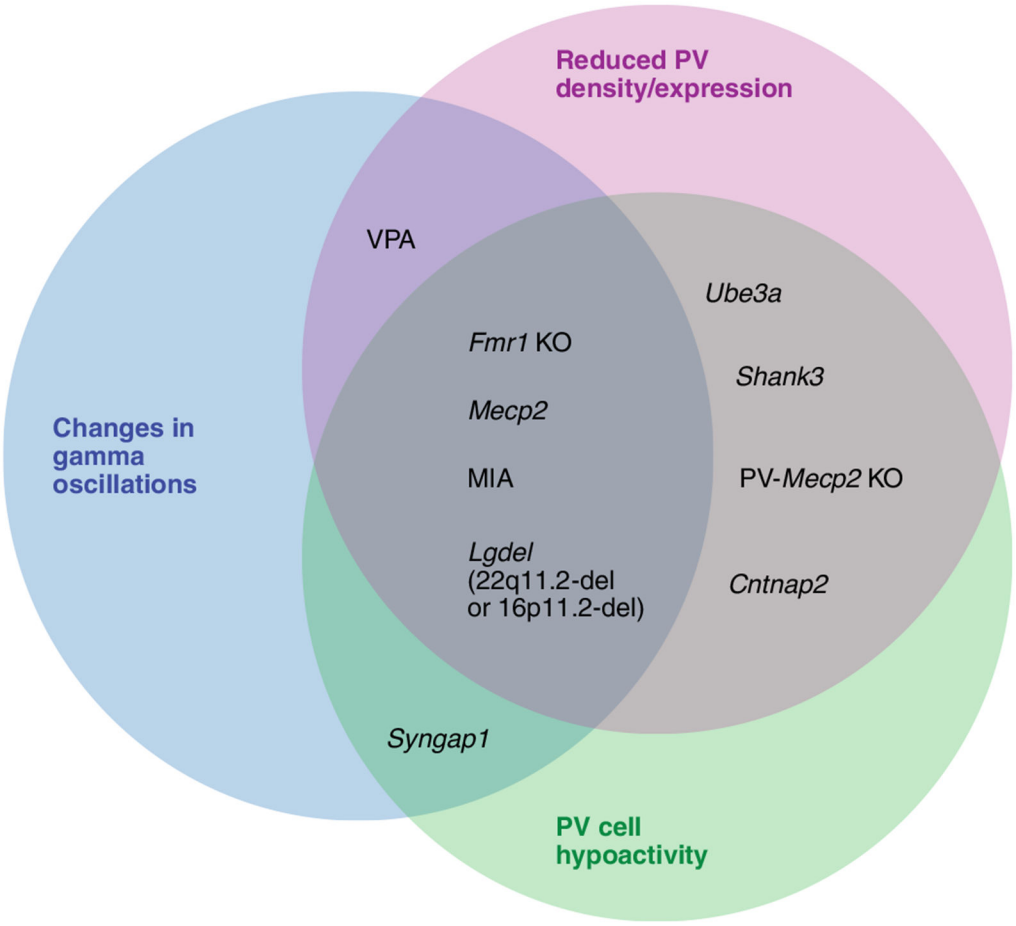

Fig. 4 |. Shared phenotypes of parvalbumin cell hypofunction in rodent models of ASD.
This Venn diagram represents the shared phenotypes related to PV IN hypoactivity in rodent models of autism. Note that some of the models (for example, *Lgdel* and MIA) have also been linked to schizophrenia. VPA, valproic acid.

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Table 1 |

Parvalbumin cell hypoactivity across autistic spectrum disorder mouse models

Autism model	Disease	Brain region	Age	Ref.
MIA poly(I:C)	Sporadic autism of environmental cause	Medial prefrontal cortex and ventral hippocampus	Adult (P90–110)	34
Mecp2	Rett syndrome	Somatosensory cortex	Adult (P42–92)	90
		Visual cortex	P60+	126,127
Cntnap2	Cortical dysplasia, focal epilepsy syndrome	Somatosensory cortex	Adult (P42–92)	90
		Medial prefrontal cortex	Adult	118,128
Fmr1 KO	FXS	Visual cortex	Adult (2–3 months)	99
		Somatosensory cortex	Adult (P42–92)	90
		Visual cortex (rat)	P19–24	116
Ube3a	Angelman syndrome	Visual cortex slices	P25–adult	88
		Medial prefrontal cortex slices	Adult (20–30 weeks)	129
Shank1/3	*SHANK*-related autism	Somatosensory cortex	Adult (4–6 months)	87
		Hippocampal CA1 slices	3–5 weeks	130
Syngap1	*SYNGAP1*-related autism	Somatosensory cortex (GAD2 neurons)	>6 weeks	91
		Neocortical organotypic slices	<2 weeks	131
16p11.2$^{del/+}$ or *LgDel*$^{+/-}$	22q11.2 deletion syndrome	Somatosensory cortex	Adult (P42–92)	90
		Hippocampal organotypic slices	<2 weeks	117