Pediatric RESEARCH

REVIEW ARTICLE

Cortical interneuron function in autism spectrum condition

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Cortical interneurons (INs) are a diverse group of neurons that project locally and shape the function of neural networks throughout the brain. Multiple lines of evidence suggest that a proper balance of glutamate and GABA signaling is essential for both the proper function and development of the brain. Dysregulation of this system may lead to neurodevelopmental disorders, including autism spectrum condition (ASC). We evaluate the development and function of INs in rodent and human models and examine how neurodevelopmental dysfunction may produce core symptoms of ASC. Finding common physiological mechanisms that underlie neurodevelopmental disorders may lead to novel pharmacological targets and candidates that could improve the cognitive and emotional symptoms associated with ASC.

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INTRODUCTION

The modulation of cortical excitatory and inhibitory synapses within local circuits is driven by a delicate balance between excitatory glutamatergic pyramidal neurons (PNs) and GABAergic cortical interneurons (INs).[1] PNs specialize in transmitting information within and between cortical regions, and non-cortical structures. INs contribute to network coordination by inhibiting the activity of target cells by hyperpolarizing the postsynaptic membrane, and consequently decreasing the probability that the target neuron will fire.[1–4] Dysfunction in this system can lead to regional and global loss of information within the brain from embryonic development to adulthood. A number of neurodevelopmental disorders, including autism spectrum condition (ASC) may be driven in part by IN dysfunction.[5–7]

Altered balance of excitatory and inhibitory inputs onto neurons (known as E/I imbalance) has emerged as a potential hypothesis for many of the difficulties associated with ASC.[8–10] Altered E/I balance may induce language delays and communication challenges[11] and disrupt sensory processing, problems frequently observed in ASC.[12,13] Impairment or loss of parvalbumin (PV) INs may contribute to E/I balance disruption and consequently desynchronize neuronal oscillations that coordinate the activity of distant brain regions.[14–17] Changes in neuronal activity and oscillations in individuals with ASC are associated with, or even predict, sensory and cognitive symptoms of autism.[11,16] Gamma deficits in the first 3 years of life predict difficulties in language development, cognition, and switching attention, which are implicated in ASC.[18,19] Altered IN function is also linked to epileptic seizures,[20] as INs are essential for preventing hyperexcitability in the brain,[21] and epilepsy is commonly comorbid with ASC.[20]

The first part of this review provides an overview of how INs develop in the medial and caudal ganglionic eminences of the developing cortex. This includes a discussion on the mechanisms of cell fate, neuronal migration, and challenges observed in ASC. In the second part of the review, the structure and function of cortical INs is discussed, along with changes in ASC. Changes in synaptic communication and neural oscillations associated with specific social deficits in rodent models are reviewed. The generation of cortical neurons derived from induced pluripotent stem cells (iPSCs) from individuals with ASC is discussed. Models using three-dimensional (3D) cortical human tissue reproducing and confirming neurophysiological mechanisms associated with ASC from rodent and clinical studies may improve the prospect for translational interventions. Throughout, we demonstrate the importance of INs in neurodevelopmental disorders with an emphasis on autism in general.

CLASSIFICATION OF CORTICAL INS

Cortical INs are highly diverse and differ broadly in terms of morphology, physiology, and molecular characteristics.[22] Nearly all neocortical INs can be classified into three categories based on expression of the calcium (Ca^{2+})-binding protein, parvalbumin (PV), the neuropeptide somatostatin (SST), or the ionotropic serotonin receptor (5HT3aR[23]). PV INs are fast spiking and non-accommodating[24] and can be further subdivided into basket and chandelier cells. Basket cells target the soma and proximal dendrites of PNs and other INs, while chandelier cells target the axon and initial segment of PNs.[23,25] SST-positive INs fire action potentials (AP) in bursts, in response to depolarizing current. The largest subset of SST-positive INs are Martinotti cells; bitufted cells that project axons into layer I of the cortex where they target tuft dendrites of PNs.[26] These axons can project horizontally across layer I to multiple columns and provide cross-columnar inhibition. 5HT3aR INs are a diverse group of INs expressing neither PV nor SST (~30% of INs in the somatosensory cortex).[27] These INs express molecular markers, including vasoactive intestinal peptide (VIP), and reelin, and exhibit differing electrophysiological and morphological characteristics. VIP-expressing cells include double-bouquet cells and bipolar cells, both of which target dendrites. VIP (+) bipolar neurons co-express calretinin (CR), and have low input resistance and an irregular firing pattern.[28] Double-bouquet cells exhibit bitufted morphology along with descending axonal arbors.

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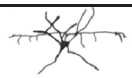
Table 1 List of IN categories discussed in the article and their characteristics

Type	Subtype	Morphology	Staining	Characteristics	References
Parvalbumin	General		PV	Fast Spiking	(4)
	Basket Cells		PV	Very fast spiking	(4)
	Chandelier Cells		PV	Regulate excitatory activity	(21,65)
Somatostatin	General		SST	Fire APs in bursts, antiepileptic, released during high frequency firing	(133)
	Martinotti Cells		SST	Regular spiking pattern	(133)
5-HT_{3A}	General		5-HT_{3A}		(24)
	Vasoactive Intestinal Peptide (VIP)	Bipolar Cell  Double bouquet cells 	5-HT_{3A}	Weakly inhibit PV networks, but strongly inhibit SST networks	(133)
	Reelin		5-HT_{3A}	Polarizes neurons towards cortical plate in development.	(28,134,135)
	Neuropeptide Y		5-HT_{3A}	May regulate monoamine activity, eg: serotonin, norepinephrine, dopamine	(24,136–138)
Pyramidal Cells	PN			Excitatory, Glutamatergic	(101,139–141)

Neurogliaform cells express reelin and have a distinct morphology characterized by numerous short dendrites that form a spherical dendritic field. These neurons are late spiking and also target dendrites[29] (Table 1, Fig. 1).

In contrast with PNs that migrate radially from the ventricular zone of the dorsal forebrain, cortical INs originate from the subpallium or ventral forebrain, particularly the medial and caudal ganglionic eminences (MGE and CGE) and the preoptic area (POA), and migrate tangentially to the cortex[30–32] (Fig. 2). In utero fate mapping and transplant studies show that a majority of PV and SST INs derive from the MGE in a defined spatiotemporal manner.[33,34] SST INs are generated from the dorsal MGE, while PV INs are generated from both the dorsal and ventral MGE. Temporal dynamics also play a role in the type of IN produced. Production of SST INs peaks earlier compared to PV INs, and a higher percentage of SST INs are produced earlier in development.[33] Interestingly, MGE-derived INs migrate to the cortex in an inside-out configuration, similar to that of PNs, with older cells occupying lower layers.[35] The CGE, formed by the fusion of the posterior aspects of the MGE and lateral ganglionic eminence (LGE), generates 5HT3aR INs expressing VIP, reelin, or neuropeptide Y (NPY). Generation of neurons from the CGE starts and peaks later in development compared to the MGE, and in contrast does not exhibit the same inside-out cortical integration.[36] A small but diverse population of INs originates from the POA, located ventral to the MGE. These INs mainly occupy superficial cortical layers, and include neurons expressing NPY, PV, and SST.[37]

Fate determination of INs is regulated by several factors (reviewed in refs.[38,39]). First, the ganglionic eminences are specified by transcription factors Ascl1 and Dlx1 and 2. Mice

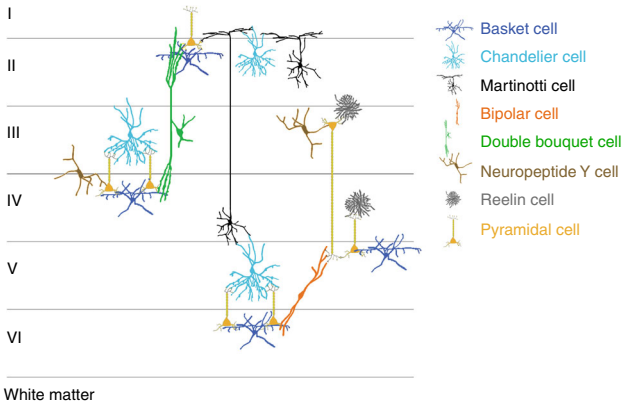


Fig. 1 Cellular connections within the cortex. PV BCs are PV INs found in layers II–VI where they regulate PN activity through synaptic connections with PN dendrites.[25,29] CCs are another class of PV INs, and range from the border of layers I to III, and layer V.[25,29] CCs regulate PN activity by synapses on axons.[25,29] Martinotti cells are SST INs found in layers II–VI, and inhibit the tuft dendrites of PNs.[25,29] Bipolar cells are 5-HT_{3A} that can be inhibitory through GABA release, or excitatory through VIP release.[25,29] Bipolar cells can be found in layers II–VI, but their axons extend through all layers, because they mainly synapse basal dendrites of PNs.[25,29] Double-bouquet cells are also 5-HT_{3A} and VIP positive, target dendrites, and are found in layers II–V.[25,29] NPY neurons also express 5-HT_{3A} (although some may express SST as well) and can be found throughout the cortex, but are most often found in II–III.[25,29,133] Reelin cells are developmentally involved in radial layer formation, but in mature tissues regulate synaptic plasticity in PN dendrites[25,29,134,135]

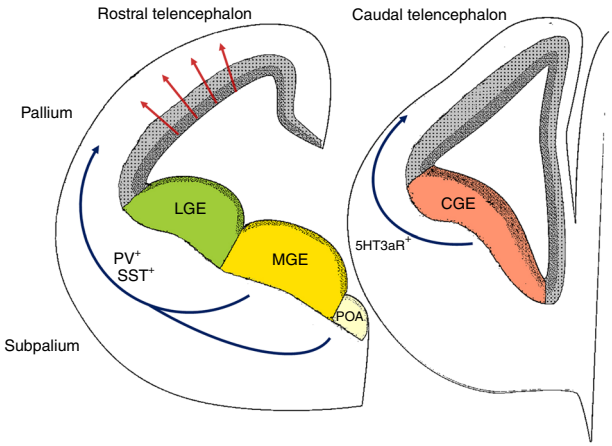


Fig. 2 Migration of cortical neurons in the rodent cortex. Excitatory neurons migrate radially from the ventricular zone and populate the cortical layers (green arrows). GABAergic INs originate from the subpallium from the ganglionic eminences and the preoptic area and migrate tangentially toward the cortex (orange), adapted from[48,49]

lacking Ascl1 or Dlx1/2 lose 50–80% of cortical INs. Dlx1 and Dlx2 also promote the expression of glutamic acid decarboxylase Gad67, vesicular GABA transporter VGAT, and the differentiation and migration transcription factor Arx,[40] making them essential for IN differentiation and migration.

In the MGE and POA, the transcription factor Nkx2.1 acts as a master regulator that drives differentiation of PV and SST INs. The expression of Nkx2.1 is induced by sonic hedgehog (SHH). Within the MGE, higher levels of SHH in the dorsal region induce development of SST INs, while lower levels in the ventromedial region preferentially give rise to PV INs.[41] Loss of Nkx2.1 results in reduction of INs—possibly associated with ventral to dorsal transformation of the basal telencephalon.[42] Evidence also suggests that loss of Nkx2.1 at E12.5 in mice induces a switch in cell fate from PV and SST INs of an MGE transcriptional identity to a CGE-like identity.[43] Nkx2.1 induces the expression of Lhx6, which is expressed in MGE-derived INs beginning from when the cells exit the cell cycle[44] and functions in tangential migration.[45]

Less is known about fate determination in the CGE, but studies suggest the involvement of Prox1, CoupTF2, and Gsx2. Prox1 serves as a molecular marker for INs derived from the CGE and LGE,[46] and is required for the acquisition of properties of CGE-derived INs.[47] CoupTF2 is an orphan nuclear receptor that is enriched in the CGE and is involved in tangential migration.[39] Gsx2 acts upstream of Ascl1 and is required for the specification of the CGE and LGE (Fig. 3).[48,49]

Recent studies by Ma et al.[50] and Hansen et al.[31] show that the molecular mechanisms and developmental programs involved in IN fate determination are well conserved between rodents and primates, and that the majority of cortical INs are generated in the ganglionic eminences. However, their observations indicate that MGE-derived PV and SST INs only account for 20% and 25%, respectively, of the total INs in the primate cortex. This is accompanied by a relative increase in the proportion of CGE-derived INs,[31] particularly CR+INs.[50]

CORTICAL INTERNEURON MIGRATION

From the subpallium, IN precursors migrate tangentially to the pallium and then migrate radially to populate different cortical layers. This process is controlled by interaction with guidance factors and motogens (reviewed in ref. [51]). Initial migration away from the subpallial proliferative zone is mediated by chemorepulsive cues expressed in this region. Chemorepulsion from the MGE and the POA is facilitated by Eph/ephrin signaling.[52,53] The chemorepulsive ligand Slit is also expressed in the VZ, where it interacts with Robo receptors in INs, and facilitates exiting from the proliferative zone.[54] Repulsion from the striatum during tangential migration is also facilitated by Robo,[54] as well as semaphorin and its receptor neuropilin in INs.[55] Several key motogens stimulate migration to the pallium, including hepatocyte growth factor/scatter factor (HGF/SF), brain-derived neurotrophic factor (BDNF), neurotrophin 4 (NT4), and glial cell line-derived neurotrophic factor (GDNF).[35,51] Migration into and within the cortex is also facilitated by chemoattractant pathways such as neuregulin-1/ErbB4, stromal-derived factor 1 (SDF-1)/CXC chemokine receptor 4 (CxCR4), and netrin. Different isoforms of neuregulin-1 are expressed in the developing cortex and serve as a short- and long-range attractant for a subpopulation of INs expressing the receptor ErbB4. Consequently, the loss of this signaling reduces the number of cortical INs.[2] In contrast, loss of SDF-1/CxCR4 seems to selectively affect later-born neurons, and induces the reduction of INs in superficial cortical layers and ectopic migration to deep layers.[56] The diffusible factor netrin also plays a role in chemoattraction during migration—especially in the upper cortical plate.[57] Finally, evidence suggests that the switch of the Na–K–Cl cotransporters NKCC1 to KCC2 during early postnatal development is an important stop signal for IN migration. During early development, both GABA and glutamate facilitate neuronal migration by depolarizing the membrane and generating Ca^{2+} currents. The switch to KCC2 makes GABA hyperpolarizing, which restricts cellular motility.[51,58]

FUNCTION OF CORTICAL INTERNEURONS

Microcircuits are critical for the function of the cortex. Glutamatergic PNs specialize in transmitting information both within and between cortical areas, as well as to other parts of the brain. Inhibitory GABAergic INs regulate the activity of these PNs. They are involved in the regulation of gating in spiking, temporal, and

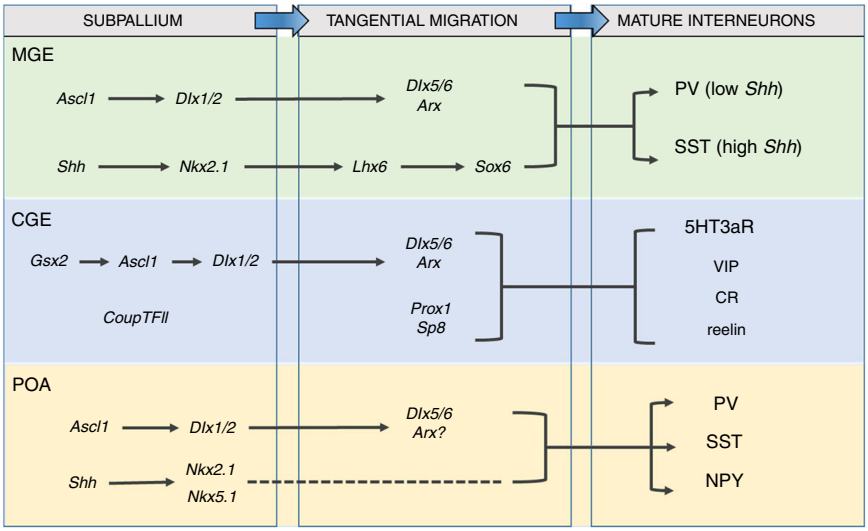


Fig. 3 Gene expression patterns that drive differentiation and maturation of INs in the subpallium. The three major areas in the developing cortex that generate INs are the MGE, the CGE, and the POA.[48,49] The expression of regulatory transcription factors combined with spatiotemporal gradients of growth factors drives the expression of IN subtypes[48,49]

spatial network dynamics,[59,60] as well as aspects of the waking brain state including attention, arousal, and the regulation of pupil diameter in response to brain-state changes (see ref. [61] for a review). In particular, INs regulate the E/I balance which has been suggested to be dysregulated in ASC.[6]

PV-expressing basket cells (BCs), a subtype of cortical IN, have multiple unique properties allowing them to quickly fire APs and release GABA at a lower threshold than other neurons.[62] The resting membrane potential of BCs is generally lower than in other neurons with a reduced dendrite diameter and an extensive axonal arborization that acts as a current sink accelerating the decay of somatic excitatory postsynaptic potentials (EPSPs). Together, these physiological attributes contribute to fast EPSP propagation.[63] The BCs axons have a unique sodium (Na^+)-channel density gradient, with a nearly 18-fold increase in the number of channels per micron in the distal axon compared to the soma, leading to a 2.4-fold faster Na^+ inactivation time compared to the soma.[64] The axon terminals of BCs require fewer open Ca^{2+} channels to release GABA compared to other IN cell types.[65] These differences contribute to the BC fast-spiking phenotype. The increased speed of BC synaptic and spike properties allows them to be superb integrators of information coming from distal PN glutamatergic inputs. This fast integration is required for adequate local E/I balance in micronetworks in the cortex.

Chandelier cells (CC) form axon terminals in vertically oriented clusters, giving them a chandelier-like appearance. They target the axon initial segments of PNs, which receive multiple synaptic boutons each from multiple chandelier neurons.[66] CC synapses express high levels of GAT1 and the $GABA_{A\alpha 2}$ subunit.[67,68] Although CCs are not as well characterized as BCs, it is known that seizures can lead to decreases in nerve terminals,[69,70] and loss of CC.[71] This has led to the hypothesis that chandelier neurons play a role in regulating excess excitatory activity and E/I balance.[21] Other interneuron types are found through the six layers of the neocortex (Fig. 1).

DYSREGULATION OF INTERNEURONS IN NEURODEVELOPMENTAL DISORDERS

Altered IN function has been documented in several developmental disorders including schizophrenia, intellectual disability, and autism. Existing studies have focused on synaptic deficits in cortical INs. However, disruption of genes involved in IN neurogenesis and migration may also play a significant role. Genetic variants of IN developmental genes have been linked to neurodevelopmental disorders. Polymorphisms in Dlx1/2 have been linked to autism susceptibility.[72,73] The Dlx target Arx is one of the most frequently mutated genes in X-linked intellectual disability. Mutations of Arx both in rodents and humans also result in infantile spasms, possibly due to reduced inhibition.[74] Lastly, mutations in ErbB4 are linked to schizophrenia.[1] These highlight the importance of early developmental events in phenotypes associated with neurodevelopmental disorders, and suggest that the tight balance of E/I is important for normal brain function.

The consequences of disrupted IN function during development have been difficult to study due to embryonic lethality conferred by mutations in genes linked to these phenotypes. Several conditional knockout (KO) models have been successful in demonstrating that specific deletions in INs can mimic behaviors associated with neurodevelopmental disorders. For example, deletion of HGF/SF in mice causes embryonic lethality, but deletion of the urokinase plasminogen activator receptor which activates HGF/SF reduces HGF/SF activity and results in viable mice with selective reduction of calbindin (+) INs in the frontal and parietal cortex. These mice exhibit behavioral abnormalities including seizure susceptibility, anxiety-like behavior, and reduced sociability.[75,76]

IN dysfunction has also been demonstrated in schizophrenia.[77] Several studies have shown that targeted deletion of ErbB4 using MGE-derived INs (Lhx6-Cre[78]) or in PV INs (PV-Cre[79]) results in hyperactivity, impaired working memory and fear conditioning, and decreased pre-pulse inhibition. Mutation of schizophrenia-associated genes (DSC1) or loci (22q11.2) in mice also results in the alteration of PV IN number or distribution.[1,35]

Mouse models of ASC also display disruption of IN function and development. Mutation of MeCP2 is implicated in about 90% of cases of Rett syndrome. In mice, targeted deletion of MeCP2 in GABAergic neurons results in autism-related phenotypes, including repetitive behaviors, as well as decreased GABA transmission.[80] CNTNAP2, a member of the neurexin family, is also linked to autism. CNTNAP2-KO mice exhibit autism-related behaviors, hyperactivity, and epileptic seizures. Immunolabeling shows abnormal cortical neuron distribution, indicating migration deficits, and a marked reduction in IN numbers, particularly in PV INs.[81]

INTERNEURONS, E/I BALANCE IN THE CORTEX, AND AUTISM

ASC encompasses challenges in processing social and emotional information, and executive function, which are governed in part by connections between sensory, motor, and dopaminergic pathways to the prefrontal cortex (PFC). These pathways are highly implicated in autism. Multiple studies implicate the PFC in psychological resilience and coping.[14,82,83] The physiological reduction of the stress response based on the subjective sense of control of the stressor is governed by the neuronal connection between the medial PFC (mPFC) and the serotonergic dorsal raphe nucleus.[84] PFC deficits in ASC may affect executive function, particularly executive function deficits from dopaminergic basal ganglia circuits.[85–89] Thus, the PFC and the cortex in general may be key physiological substrates contributing to ASC psychological challenges.

Neuronal oscillations support inter-regional communication, and require INs for proper regulation.[16,17] It has been hypothesized that environmental stimuli, field potentials, and spiking activity may be best detected in the postsynaptic neuron at an optimum time window between 10 and 30 ms, corresponding approximately to a gamma cycle.[16] The emergence of gamma oscillations in the cortex has been hypothesized to require mutually connected INs, a time constant provided by the $GABA_A$ receptor, and sufficient activity to induce spikes in the INs (see ref. [16] for a review). Oscillations (including other frequencies in addition to gamma) have been proposed to provide the framework for straightforward communication between neurons and brain areas, as opposed to stochastic patterns of spikes, and are involved in the regulation of E/I balance.[17,90] Sleep-dependent oscillations may assist with linking memory and judgment between the PFC and hippocampus, respectively.[90] Aberrant gamma activity in early life may predict difficulties in the development of language, cognition, and attention; all of which have been implicated in ASC.[18,19] This collectively highlights multiple roles that INs play in neural oscillations and E/I balance.

Rodent studies suggest that dysfunction of the E/I balance in the mPFC may represent a physiological bottleneck for information processing in ASC, potentially driving social deficits. Optogenetic stimulation of PNs has been shown to saturate AP propagation in the postsynaptic neuron, leading to synaptic data loss.[6] Saturation in the mPFC (but not in other cortical areas), is associated with decreased social preference, social exploration, and inhibited fear conditioning, which are rescued by optogenetic activation of INs.[6] Knock-in (KI) mice overexpressing neuroligin 3 (an ASC-associated mutation) exhibit reduced coupling between low gamma amplitude and the theta phase, but a stronger and wider coupling between high gamma and theta rhythms during social interaction, indicating a dysfunction of temporal information integration in the local circuits.[15] The KI mice also recruit fewer mPFC neurons to lock gamma and theta oscillations during social interactions and have a lower probability of locking in the social state, while exhibiting a higher probability of locking during the quiet state.[15] Using optogenetic techniques, INs stimulated at 40 Hz nested at 8 Hz, show enhanced power and coupling strength of gamma and theta bands.[15] This stimulation enhances social preference within both wild-type (WT) and KI mice, while constant 40 Hz and 8 Hz nested in 20 Hz, has no effect, and a higher frequency of 80 Hz inhibits social preference in both WT and KI mice.[15] Collectively, these studies support the hypothesis that GABAergic INs in the PFC play a crucial role in the regulation of behavior and information processing that are hindered in ASC.

ELECTRICAL ACTIVITY IN INDIVIDUALS WITH ASC

Electroencephalography (EEG) and magnetoencephalography (MEG) allows non-invasive measurements of neural activity in human subjects and has been used to measure gamma oscillations in individuals with ASC. A decrease in gamma power in the left hemisphere following an audio tone was found in children with ASC,[12] and a similar reduction within both parents and children with ASC.[13] Another group showed elevated pre-stimulus activity associated with decreased language scores, and a decreased post-stimulus activity in children with ASC.[11] Only gamma oscillations were related to reduced language scores, with a positive pre-stimulus association between gamma and language deficits in the right hemisphere, along with a negative post-stimulus association between gamma activity and difficulty with language.[11] The authors hypothesized that an inability of GABAergic INs to maintain a neutral tone, or ability to rapidly return to a baseline state before the next stimulus may have contributed to an increased signal to noise deficit in individuals with ASC, leading to audio processing challenges.[11]

Evidence suggests that social and or psychotherapeutic interventions may potentially ameliorate gamma activity deficits. Relative left hemisphere dominance is associated with both positive affect and increased social motivation, while relative right hemisphere dominance is characterized by social withdrawal, negative emotions, a poorer outcome, and spherical dominance is present from infancy to adulthood.[91] ASC teens taking a 5-month Program for the Education and Enrichment of Relational Skills (PEERS) class showed a significant shift from right to left hemisphere dominant gamma activity when measured before and after.[91] Furthermore the students with the most social contacts, increased understanding of PEERS concepts, and decrease in ASC symptoms as rated by their parents showed the greatest shift in gamma activity.[91] Given that PV neurons are needed to initiate and regulate gamma oscillations,[16,17,90] these studies show the importance of cortical INs in ASC and suggest plasticity in gamma and INs related to social learning.

ASC, INTERNEURONS, AND IPSCS

iPSCs are stem cells derived from the mature tissue of individuals with genetic susceptibility to disease, and can be used for in vitro disease modelling.[92] Through treatment with the four Yamanaka factors: OCT3/4, SOX2, c-Myc, and KLF4,[93] fibroblasts,[94] peripheral blood mononuclear cells (PBMCs),[95,96] or dental pulp cells[97] from individuals with ASC can be reprogrammed into iPSCs, retaining the original genetics of the individual from which they were derived.[98] Dual SMAD inhibition can differentiate iPSCs into neural precursor cells (NPCs, Fig. 4).[95,99] Additional protocols allow development toward anterior or posterior fate, deep or superficial neuronal subtypes, glutamate, pyramidal, GABA, PV, and somatostatin neurons.[100–109] iPSCs can be used to generate a 2D monolayer, 3D embryoid bodies (EBs,[110]), or organoids to model cortical development.[111–115] EBs can form rosettes, a 3D model of cortical development (Fig. 5,[109]). Serum-free EBs[110,116] can acquire CGE-like characteristics with CR (+) neurons showing structural and electrophysiological properties similar to GABAergic INs.[109] It is important to note that iPSC organoids develop into neurons at a rate similar to the human embryo, thus these models potentially correspond to first trimester human neurons.[117]

Recent iPSC studies have selected idiopathic cohorts from ASC individuals with macrocephaly,[118,119] as it is associated with poorer clinical outcomes.[120,121] Other studies used EB rosettes to model cortical development finding increases in the GABA IN cell fate marker DLX2,[118,119] Marchetto et al. found increased proliferation measured by increased percentage of Ki67+ cells within the ASC group with decreased cell cycle length.[119] The percentage Ki67+ cells from both ASC and control were significantly correlated with brain volume of the individual from which they were derived.[119] Mariani et al., also found a decrease in cell cycle length, but no change in proliferation or Ki67+.[118] Other differences were either no change PN with a reduction in GABA markers[119] or decreased markers for PN development and synaptic activity.[118]

Monogenic ASC iPSC studies have also found evidence of E/I balance disruption. Rett syndrome-derived EBs showed reduced

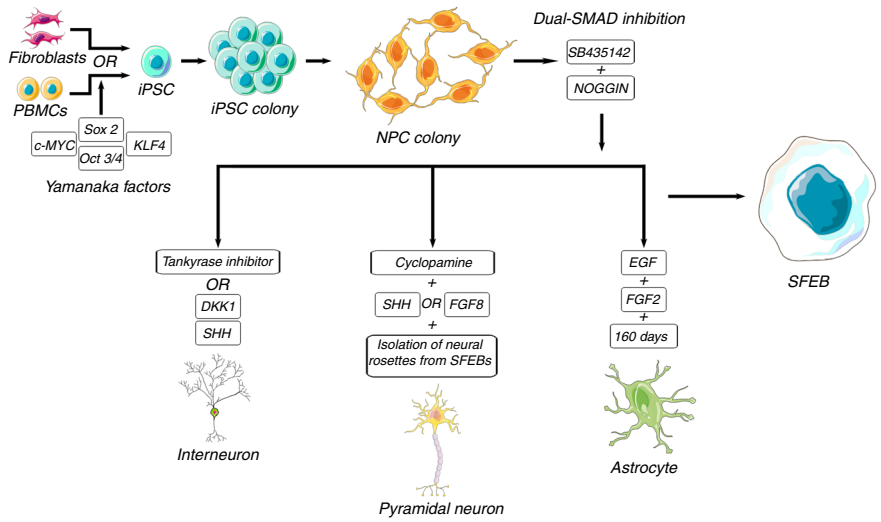


Fig. 4 Outline of the development of neurons from iPSCs. Tissue samples can be induced to form pluripotent stem cells (iPSCs) through the application of Yamanaka factors. Using dual SMAD inhibition combined with different additional protocols, iPSC colonies can be grown into serum-free embryoid bodies (SFEBs) and/or a diverse array of neuronal subtypes. Images adapted with permission from Servier Medical Art (https://smart.servier.com)

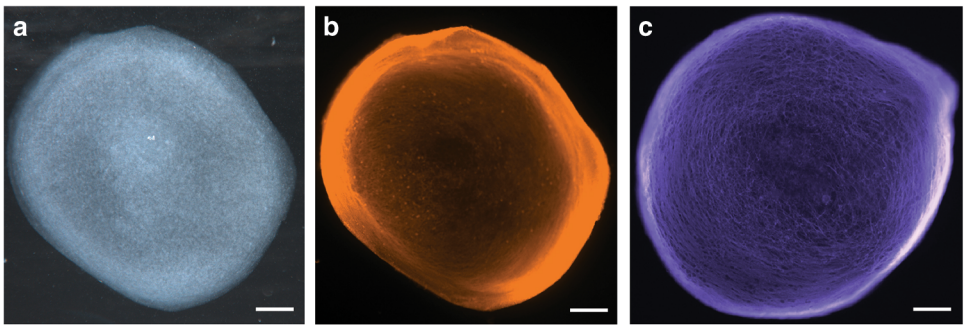


Fig. 5 Examples of EBs made from human iPSCs. **a** EB created on a cell-culture insert in bright-field and **b** stained for the excitatory neuronal marker VGLUT (orange). **c** An example of the ultrastructure of an EB revealed by staining for beta-tubulin III (violet)

glutamate synapse number, and decreased frequency and amplitude of excitatory postsynaptic currents (EPSCs), but no change in frequency or amplitude of inhibitory postsynaptic currents (iPSCs).[122] The insertion of a stop codon into MeCP2 was found to prevent expression of KCC2 which drives GABA to switch from excitation to inhibition.[123] Another monogenic iPSC line is based on a *de novo* mutation in TRPC6 a Ca^{2+}-permeable dendritic ion channel involved in excitatory synapse formation, and not previously linked to ASC.[97] Surprisingly MeCP2 was found to act upstream of TRPC6, effecting glutamate activity and synaptic density similarly to Rett syndrome.[97] Other monogenic gene disorders are associated with ASC including 15q, 16p11.2, and 22q.[124–126] Not all of these monogenic autism subtypes have been turned into iPSC lines and have yet to be sufficiently characterized or modeled with respect to iPSC-derived interneuron function.

ASC THERAPEUTIC TARGETS IN IPSC-DERIVED NEURONAL CULTURES

Evidence suggests the growth factor IGF-1 may be ameliorate physiological deficits associated with ASC. In iPSC models, IGF-I increased KCC2 expression in neurons derived from MeCP2 individuals.[123] IGF-I also restored spiking behavior to idiopathic ASC cells[119] and glutamatergic synapse number in Rett syndrome[122] and following TRPC6 loss.[97] Restoration of gamma oscillations represents a target that may improve information processing, particularly social information processing in the PFC. Optogenetic stimulation of INs in mice enhanced power and coupling strength of gamma and theta bands as well as social preference.[6,15,127] Pharmacological compounds capable of promoting gamma synchronization through the stimulation or changing gene expression for receptors involved in this process would represent a novel and useful treatment strategy.

Although the iPSC field is in early development, the potential to model aspects of ASC using human neurons is promising.[128] The ability to develop human cortical tissue in vitro presents scientists with multiple new options to design experiments that integrate results from both rodent and clinical studies that will result in greater clinical translatability.

CONCLUSION

Cortical networks are necessary to transmit information across the brain, and dysfunction of this network is associated with many ASC phenotypes. GABAergic INs regulate the E/I balance, including temporal and spatial network dynamics that may govern the processing of sensory, social/emotional, and cognitive information. Deficits in gamma and theta activity, which are controlled by interneurons, result in perceptual and social deficits in both individuals with ASC and animal models.[6,12,13,15,91] This suggests that cortical IN dysfunction may contribute to many phenotypes associated with ASC (Fig. 6).

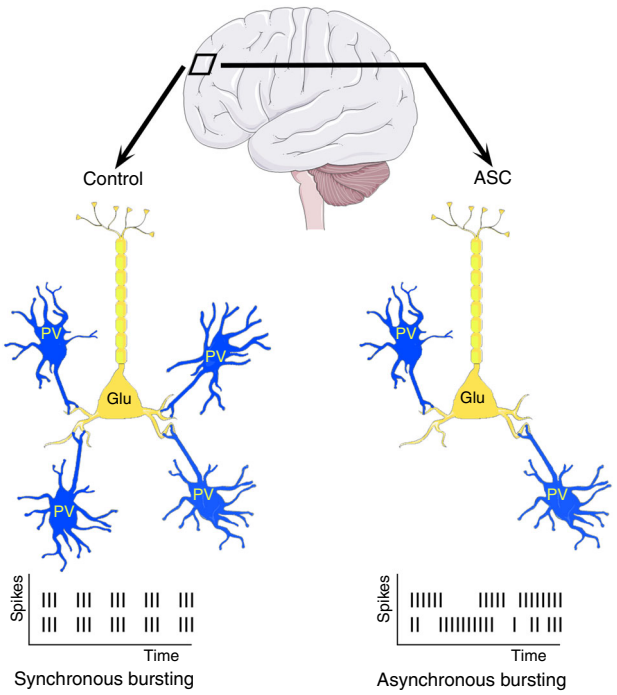


Fig. 6 Cortical IN contribution to physiological and behavioral ASC phenotypes. Both postmortem studies from individuals with ASC as well as mouse models have shown decreases in cortical interneurons.[7,73,74] Individuals with ASC also show deficits in gamma oscillations that have been correlated with sensory and social deficits.[12,13,91] Animal models have shown that excessive activity of glu PN neurons can lead to data loss at the synapse.[6] Genetic mouse models of ASC have shown deficits of gamma and theta phase locking during social, and excessive locking during non-social activities.[15] Artificial induction of gamma and theta coupling rescued social activity in these mice.[15] Collectively these data support the hypothesis that dysfunction of cortical interneurons is capable of disrupting the connectivity to the cortex and other brain areas through modulation of excitatory/inhibitory balance

Given the complexity and dynamic nature of the brain microcircuitry and the many unknowns, caution must be exercised to avoid overly simplifying the E/I relationship with disease phenotype. As different regions have different microcircuit profiles,[129,130] conclusions gleaned from measurements in one region may not be applicable to others. This is underscored by work showing that despite high variation in evoked excitatory and inhibitory inputs to visual PNs, the overall E/I input ratio is constant.[131] Nevertheless, the potential of using E/I balance as a readout for conditions such as autism or as a guide for developing effective therapy remains powerful, particularly as such tools as high-content assays become more mainstream. In addition, E/I balance could provide insight into the presence and severity of comorbidities such as epilepsy in autism.[74,132] The role of interneurons in the regulation of E/I balance should be regarded as a contributing factor to observed phenotypes in neurodevelopmental conditions, and deserves further intensive study.

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