HHS Public Access

Author manuscript

Nat Rev Neurosci. Author manuscript; available in PMC 2022 April 12.

Published in final edited form as:

Nat Rev Neurosci. 2021 May ; 22(5): 290–307. doi:10.1038/s41583-021-00443-x.

The role of GABAergic signalling in neurodevelopmental disorders

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Abstract

GABAergic inhibition shapes the connectivity, activity and plasticity of the brain. A series of exciting new discoveries provide compelling evidence that disruptions in a number of key facets of GABAergic inhibition have critical roles in the etiology of neurodevelopmental disorders (NDDs). These facets include the generation, migration and survival of GABAergic neurons; the formation of GABAergic synapses and circuit connectivity; and the dynamic regulation of the efficacy of GABAergic signalling through neuronal chloride transporters. In this Review, we discuss recent work that elucidates the functions and dysfunctions **[Au:OK?]** of GABAergic signalling in health and disease, that uncovers the contribution of GABAergic neural circuit dysfunction to NDD etiology and that leverages such mechanistic insights to advance precision medicine for the treatment of NDDs.

Introduction

Neurodevelopmental disorders (NDDs) affect one in six children in the US[1] and have a very strong genetic basis. There are many, diverse, NDDs — including fragile X syndrome (FSX), Angelman syndrome, Rett syndrome,and autism spectrum disorders (ASDs). Yet, a considerable portion of clinically identified NDD risk genes **[G]** encode components of the GABAergic inhibition system, including transcription factors **[G]**, GABA receptors, inhibitory synaptic proteins and chloride transporters[2]. Genetic mutations or epigenetic perturbations that disrupt the expression of these NDD risk genes can cause profound dysfunctions in the generation, migration and survival of inhibitory neurons as well as in the connectivity, function and plasticity of inhibitory circuits.

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Author contributions

X.T. researched data for the article. All authors contributed equally to discussion of content, writing and reviewing or editing of the manuscript.

Competing interests

R.J. is a cofounder of Fate Therapeutics, Fulcrum Therapeutics and Omega Therapeutics.

Peer review information

Nature Reviews Neuroscience thanks [Referee name], [Referee name] and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

In this article, we review studies that characterize developmental processes of the GABAergic inhibition system in normal brains, and recent work using preclinical models of NDDs to reveal the genetic, molecular, cellular and circuit bases of NDD-related impairments in GABAergic inhibition. We mostly use genetic forms of autism spectrum disorders (ASDs), such as Rett syndrome, as examples in this Review, to reflect the large volume and extensiveness of literature. We also discuss research that supports or challenges a prevalent hypothesis of NDD — excitation–inhibition (E/I) imbalance **[G]** — and extend this hypothesis to a broader framework of imbalanced circuit homeostasis propagated by deficits in neuronal chloride transporter activity and GABAergic signalling.

Currently, very few therapeutic options are available to treat NDDs. A mechanistic understanding of GABAergic inhibitory dysfunction in NDDs may assist future development of feasible clinical solutions for diagnosis and treatment of NDDs. Here we discuss the genetic, molecular and brain activity markers of NDDs that may enable more accurate diagnosis of disease and a stratification of patients **[G]**. We also discuss exciting emerging work that indicates converging NDD disease mechanisms — such as impairments in the expression of neuronal chloride transporters and the reduction in interneuron numbers — that could potentially be applied broadly to correct neural circuit abnormalities across multiple NDD subtypes and may assist in the development of precision medicine **[G]** therapeutics.

The basics of GABAergic inhibition

During mammalian brain development, GABAergic neurons that are generated in proliferative zones migrate to target brain structures to assemble into the GABAergic signalling system. This system modulates network excitability through the phasic and tonic inhibition modes mediated by GABA receptors, and through the dynamic regulation of transmembrane chloride gradient determined by neuronal chloride transporters. The highly dynamic GABAergic system has pivotal roles in modulating the activity and plasticity of neural networks during development and adulthood.

Building the GABAergic cell infrastructure.—GABAergic interneurons, which constitute approximately 20–30% of the neurons in the cerebral cortex, mostly originate from proliferative zones that produce largely distinctive interneuron populations, including the medial ganglionic eminence (MGE), caudal ganglionic eminence (CGE), lateral ganglionic eminence (LGE) and preoptic area (POA)[3] (Fig. 1a).

The temporal and spatial patterns of the expression of transcription factors drive the cardinal specification of these interneurons. For example, MGE-derived progenitor cells in the developing **[Au:OK?]** mouse brain express *Nkx2.1* during the progenitor stage, followed by sequential expression of the transcription factor genes *Lhx6*, *Sox6*, *Sip1* and *Satb1*. These transcription factors coordinate changes in gene expression that determine the identity of fast-spiking interneurons that express the calcium-binding protein parvalbumin (PV), and non-fast-spiking interneurons that contain the neuropeptide somatostatin (SST)[3]. By contrast, CGE-derived progenitor cells specifically express the transcription factors encoded by *Coup-tf1* and *Coup-tf2* and generate bipolar interneurons that express vasoactive

intestinal peptide (VIP) or multipolar interneurons that express the glycoprotein reelin (RELN)[4]. Interestingly, the *Dlx* gene family members *Dlx1*, *Dlx2*, *Dlx5* and *Dlx6* are expressed in both MGE- and CGE-derived progenitors[4], indicating their indispensable roles in GABAergic neuron fate determination. Recent technological advances such as single-cell RNA-sequencing[5] as well as the directed differentiation of human stem cells[6] and cell fate transdifferentiation[7] are advancing **[Au:OK? Or 'will advance']** our understanding of the gene regulatory logic underlying the establishment and maintenance of GABAergic neuron identity.

Interneurons generated in proliferative zones migrate tangentially to the subcortical and cortical regions, where they then migrate radially into the developing cortical layers[8, 9]. The termination of interneuron migration at a final destination is determined by cellular responses to the ambient levels of GABA in the tissue: owing to low expression of the chloride exporter KCC2 (also known as the K^+-Cl^- cotransporter) and high expression of the chloride importer NKCC1 (the Na^+-K^+-Cl^- cotransporter) in interneurons during this migration, the intracellular chloride concentrations are high in these cells, and ambient GABA elicits membrane depolarization, leading to calcium influx that promotes interneuron motility[10]. As the interneurons enter the cortex, KCC2 expression is upregulated whereas NKCC1 is downregulated, rendering GABA inhibitory and terminating the migration of interneurons in a voltage-sensitive, calcium-dependent manner[10].

After reaching their final destinations in the cortical and subcortical brain tissues, interneurons undergo further diversification that is driven by interactions between the genetic code, epigenetic factors **[G]** and neural activity[11]. The four main classes of cortical interneurons defined by the markers PV, SST, RELN and VIP each send axonal projections to target different cellular compartments of excitatory pyramidal neurons. PV^+ cells directly target the neuronal soma of pyramidal neurons to reduce neuronal firing, whereas SST^+ and $RELN^+$ cells mainly target the distal dendrites to regulate the integration of excitatory synaptic inputs. VIP^+ neurons, by contrast, target both SST^+ and PV^+ inhibitory neurons and thus mediate disinhibition of pyramidal neurons[3, 12]. **[Au:OK?]** These molecularly and functionally defined interneurons integrate into the developing cortex in coordination with glutamatergic neurons to establish circuit-level excitation/inhibition balance[13, 14] (Fig. 1b).

Modes of GABAergic inhibition.—In a fully developed brain, the GABAergic network modulates neural circuit activity through two major modes of inhibition: phasic and tonic. Phasic inhibition is mainly mediated by action potential-induced presynaptic GABA release, which triggers chloride currents passing through ligand-gated ionotropic GABA type A receptors ($GABA_A$Rs) at postsynaptic sites to provide temporally precise inhibition that rises and decays within hundreds of milliseconds (Fig. 1c). The formation of GABAergic synapses follows two general steps. The initial contact between the presynaptic nerve terminal and postsynaptic cell is established by cell adhesion events mediated by presynaptic neurexin[15] and postsynaptic neuroligin 2[16]. Then, as the presynaptic GABA release machinery matures, inhibitory-synapse-specific postsynaptic scaffolding proteins, including gephyrin[17] and collybistin[18], anchor $GABA_A$Rs and other proteins necessary for GABAergic synapses to function to the postsynaptic membrane.

In contrast to the rapid phasic inhibition at GABAergic synapses, tonic GABAergic inhibition is mainly mediated by extrasynaptic $GABA_A$Rs that provide slow and persistent 'background' inhibition[19]. The extrasynaptic $GABA_A$Rs typically contain α5[20] or δ[21] subunits, whose expression levels are downregulated or upregulated during development, respectively[22]. Such receptors can respond to very low concentrations of ambient GABA in the extrasynaptic space, including the GABA that diffuses from the synaptic cleft when not taken back up by GABA transporters[23] and the small amount of GABA released from astrocytes via the bestrophin 1 channel[24]. Although the conductances of individual extrasynaptic GABA receptors are relatively small compared with their synaptic counterparts, tonic GABAergic inhibition plays a substantial role in the modulation of neuronal excitability owing to the wide distribution of extrasynaptic $GABA_A$Rs and the steady integration of currents over long periods of time[25] (Fig. 1c). In addition to the ionotropic mechanisms, activation of the metabotropic GABA type B receptors elicits long-lasting inhibition of presynaptic glutamatergic release and postsynaptic Ca^{2+} signalling[26].

On top of base synaptic and extrasynaptic GABAergic signalling, one fundamental process that determines the polarity and efficacy of GABAergic signalling is the dynamic regulation of the electrochemical gradient of chloride across plasma membranes by neuronal chloride transporters NKCC1 and KCC2[27]. NKCC1 is the main chloride importer in neurons, whereas KCC2 is the only neuronal transporter that can extrude chloride. At the early postnatal stage of mammalian brain development, the expression of KCC2 is low, whereas NKCC1 expression is high, leading to a high intraneuronal chloride concentration. As a result, when $GABA_A$Rs open upon the binding of GABA, negatively charged chloride ions exit the cell and depolarize the membrane[28] (Fig. 1c). Notably, a depolarizing GABA action does not necessarily trigger action potential firing: the large conductance mediated by $GABA_A$R activation may render the membrane 'leaky' and cause shunting inhibition, such that additional excitatory inputs can no longer trigger action potentials[29]. Therefore, the net effect of GABA-induced depolarization on the functioning of a developing brain network is context-dependent.

With development, the expression of KCC2 substantially increases and NKCC1 expression decreases. As a result, the intraneuronal chloride concentration is reduced, and GABA signalling is functionally 'switched' to hyperpolarize the membrane and reduce neuronal firing[27]. The timing of GABA functional switch **[G]** in the rodent brain is generally between the first and second postnatal week in the cortical and hippocampal regions[28, 30], with a few exceptions: for example, PV^+ cells that regulate hippocampal neurogenesis in the dentate gyrus remain depolarized by GABAergic projection from medial septum even in adult animals[31], and GABAergic inputs onto mouse hippocampal CA3 neurons remain depolarizing throughout development[32]. **[Au:OK?]** By contrast, the GABA functional switch in humans occurs over a longer timeline. Cultured human stem cell-derived cortical excitatory neurons supported by astrocytes take 2–3 months to reach robust KCC2 expression and complete the GABA functional switch[33]. In the human brain, although *KCC2* mRNA is detected in certain brain regions at birth[34], substantial elevation in KCC2 protein expression and GABA functional switch occurs in the cortex during the first postnatal year[35].

NKCC1 indirectly modulates the formation of both excitatory and inhibitory synapses and drives synaptic network maturation through GABA-induced depolarization and the activation of NMDA receptors[36, 37, 38]. By contrast, KCC2 directly interacts with actin cytoskeleton-binding proteins to regulate dendritic spine morphogenesis and the function of excitatory synapses[39, 40, 41, 42]. **[Au:OK?]** In line with this, **[Au:OK?]** proteomics studies reveal that KCC2 has more protein-binding partners at excitatory synapses than it does at inhibitory synapses[43]. Therefore, KCC2 is a 'keystone' molecule that modulates both excitation and inhibition in neurons. **[Au:OK?]** In summary, neuronal chloride transporters provide a 'master switch' to dynamically modulate the efficacy of GABAergic signalling, which, in conjunction with the number and strength of GABAergic synapses, establishes network-level balance between excitation and inhibition.

Balancing neural circuit excitation with inhibition.—Neurons assemble into functional ensembles and further incorporate into neural circuits that form the basis of the complex input–output and plastic properties of the neural network. **[Au:OK?]** GABAergic inhibition is central to shaping these emerging network properties, by providing a homeostatic mechanism **[G]** that maintains network excitability and plasticity at optimal levels to facilitate the gating, processing and storage of information.

At the circuit level, the basic building blocks of cortical inhibitory microcircuitry are primarily mediated by different subtypes of GABAergic neurons: feedforward inhibition by fast-spiking PV^+ basket cells, feedback inhibition by burst-spiking SST^+ Martinotti cells **[G]** and disinhibition by irregular-spiking VIP^+ cells that innervate the dendrites of SST^+ cells[44]. Moreover, late-spiking $RELN^+$ cells provide volume inhibition to the distal dendrites of pyramidal neurons[3]. Although different GABAergic neuronal subtypes target specific cellular compartments, they usually form highly dense synaptic innervation patterns in their projection areas and are largely non-selective for their postsynaptic neuronal partners[45]. Precise stimulation of individual PV^+ or SST^+ neurons with two-photon glutamate uncaging and simultaneous recording of postsynaptic inhibitory currents from nearby pyramidal neurons has revealed dense and nonspecific functional connectivity of these inhibitory cell types in the cortex[46, 47]. By contrast, activation of VIP^+ neurons inhibits SST^+ neurons to "make holes in [the] blanket inhibition"[44]. The complex integration of the various GABAergic inhibition units into brain circuitries determines the computation properties of the network.

In addition to preventing runaway excitation, GABAergic inhibition provides precise control over network synchronicity and generate rhythmic oscillations (Fig. 1d). During prenatal and early postnatal developmental stages in the mammalian brain, GABA is the principal excitatory neurotransmitter that generates oscillatory activity patterns, such as giant depolarizing potentials (GDPs)[48]. GDPs synchronize activity to instruct the wiring of immature cortical circuits and engage long-term potentiation (LTP) mechanisms to enhance hippocampal synaptic efficacy[49]. In the adult brain, hippocampal oscillatory activity patterns, such as gamma oscillations, serve as circuit 'pacemakers' to temporally link the activities of widely distributed cells and facilitate information retrieval and consolidation[50, 51]. Spiking of PV^+ interneurons in the adult mouse brain preferentially correlates with gamma oscillations (30–80 Hz), whereas spiking of SST^+ interneurons

correlates with beta oscillations (15–30 Hz)[52]. At postnatal day 8–10 in rats (the approximate timing of the GABA functional switch in these animals), sustained gamma oscillations emerge in the hippocampus and entrain the activity pattern of the prefrontal cortex[53]. Taken together, oscillatory network activities sculpted by GABAergic inhibition at different stages of brain development may underlie the refinement and maturation of circuitry and facilitate the proper gating and storage of information[54].

Homeostatic mechanisms at the synaptic and circuit levels are crucial for fine-tuning the network excitability across space and time. Comparative neuroanatomy studies show that the ratio of excitatory to inhibitory synapses is fairly constant across different brain regions in different species[55, 56]. In response to high neural activity, the strength of GABAergic synapses is homeostatically enhanced by the accumulation of $GABA_A$Rs in the adult mouse brain[57]. Neural activity also regulates the functional development of GABAergic interneurons. Ambient glutamate released during high neural activity preferentially activates NMDA receptor subunit 2C (NR2C)-containing and NR2D-containing NMDA receptors on inhibitory cells[58]. Transient blockade of these receptors during the first week of mouse brain development leads to a long-term reduction in inhibitory synaptic activity and impairs the morphological growth of inhibitory neurons[58]. More than 40% of interneurons die during the first 2 weeks of mouse brain development, apparently due to intrinsic cell-autonomous mechanisms[59]. However, recent work suggests that interneuron survival in mice during early postnatal development depends on the activity of pyramidal cells, and thus interneuron death represents an activity-dependent mechanism to reduce inhibitory cells to achieve homeostasis[60]. Future investigation of the effects of interactions between intrinsic factors and synaptic input on the survival rate of interneurons may reconcile these findings.

Experience-dependent plasticity during early brain development and in adulthood is crucial for animal health and fitness to environmental challenges. Multiple studies indicate a pivotal role of GABA in plasticity during the critical period **[G]** of development (Fig. 1d). Activity-dependent refinement of functional connections in the developing visual cortex is abolished in *Gad2*-knockout mice, which demonstrate impaired GABA signalling[61]. **[Au:OK?]** Similarly, blocking $GABA_A$Rs containing the α1 subunit, but not the α2 subunit, impairs critical period plasticity in mice **[Au:OK?]** [62]. Brief inhibition of NKCC1 with bumetanide during early development prolongs the plasticity window in rat **[Au:OK?]** visual cortex, indicating the fundamental role of depolarizing GABAergic transmission in defining the window of such plasticity[63]. **[Au:OK?]** In the adult rat **[Au:OK?]** brain, activity-dependent reductions in GABA release at or near active synapses allows for the expression of LTP[64], and GABAergic **[Au:OK?]** control of synaptic plasticity underlies hippocampal memory encoding and retrieval[65]. Remarkably, critical period plasticity can be rapidly induced by enhancing tonic inhibition in the mouse visual cortex[66], and transplantation of MGE interneuron progenitors in the brains of adult mice re-opens the plasticity window[67]. Intriguingly, reduction of GABAergic transmission in the adult rat visual cortex could also partially reactivate ocular dominance plasticity **[G]** [68]. These results suggest extensive and complex involvement of GABAergic inhibition in determining the onset and closure of developmental critical periods, as well as in adult brain plasticity in the context of learning and memory.

GABAergic dysfunction in NDDs

Driven by the clinical observation that a substantial proportion of individuals with NDDs also have comorbid seizures, the E/I imbalance hypothesis, first proposed in 2003, states that dysfunctional GABA action in brain circuits is central to NDD pathogenesis[69]. E/I imbalance can arise from perturbations at multiple levels. Within the diverse population of individuals with NDDs, different abnormalities in genetics, molecular activity or neural circuitry may cause cascading alterations that unify in the manifestation of E/I imbalance and NDD symptomology (Fig. 2). In this section, we summarize recent work that comprehensively investigates the E/I imbalance hypothesis as a potentially unifying framework to understand various subtypes of NDD that may generate mechanistic insight that can inform translational studies and advance research for NDD diagnosis and treatment.

Genetic, epigenetic and environmental risk factors.—NDDs have strong genetic risk components. Analysis of different cohorts **[Au:OK?]** suggests that genetic factors consistently contribute more than do environmental factors to the risk of developing ASD[70]. **[Au:OK?]** Cumulatively, results from human genetic studies provide strong support for the E/I imbalance hypothesis of NDD[2]. Many NDD risk genes encode proteins that are key components of the GABAergic inhibition system, including $GABA_AR$ proteins such as the GABAR subunit-β3 (encoded by *GABRB3*)[71], and the presynaptic cell-adhesion molecules neurexin 1 (NRXN1)[72], NRXN2[73] and NRXN3[74]. Schizophrenia-associated mutations in the gene encoding inhibitory synapse-specific postsynaptic cell-adhesion molecule neuroligin 2 cause impairments in NDD-relevant behaviours in mice, including social learning and memory[75]. Copy number variations and exonic deletions in the *GPHN* gene, which encodes the synaptic scaffold protein gephyrin required for $GABA_AR$ clustering and inhibitory synapse formation, have been identified in individuals with ASD, although more recent ASD genetic studies show inconsistencies relating to this association[76, 77],. Loss-of-function mutations in the gene encoding collybistin, a GDP-GTP exchange factor that binds with gephyrin and regulates $GABA_AR$ clustering, may cause severe encephalopathy involving X-linked intellectual disability **[G]** and epilepsy[78]. Pathogenic variants of *SLC6A1*, which encodes the voltage-dependent GABA transporter 1 (GAT1) primarily expressed in GABAergic neurons and astrocytes, have recently been described in patients with myoclonic atonic epilepsy and intellectual disability[79]. Moreover, rare variations in the regulatory domain of *SLC12A5*, which encodes KCC2, have also been identified in individuals with ASD or schizophrenia[80].

Many NDD risk genes, including *CHD2*[81], *FOXP1*[82] and *TCF4*[83], encode transcription factors and chromatin modifiers that may regulate the generation of GABAergic neurons, formation of inhibitory synapses and maintenance of E/I balance. Leveraging the human genetic data to identify the molecular pathways in which multiple NDD risk genes converge may shed light on common disease etiologies.

For example, several ASD risk genes that are associated with high incidences of intellectual disability and seizures, including *MTOR, TSC1* and *TSC2*, *PTEN* and *NF1*, are involved in the phosphatidylinositol 3-kinase–mammalian target of rapamycin (PI3K–mTOR) pathway, which **[Au:OK?]** has a central role in integrating metabolic and growth factor

signalling pathways to regulate cellular metabolism, growth and survival[84]. Collybistin and gephyrin also bind with mTORC1 and inhibit its signalling, potentially representing a mechanism involving GABAergic synapse and mTOR signalling that contributes to NDD pathogenesis[85]. The WNT (wingless) signalling pathway represents another 'hub' at which multiple NDD risk genes converge. Genes that encode proteins involved the canonical WNT pathway, including WNT1, WNT2, WNT3 and WNT9B ligands, and signalling molecules such as the adenomatous polyposis coli protein (APC) and β-catenin, and the transcription factor TCF4, that mediates the transcriptional output of the WNT signalling, have also been implicated in NDD pathogenesis[86]. These results warrant further preclinical studies to investigate the roles of, and the potential for targeting, perturbed signalling pathways in the etiology of NDDs.

In addition to germline mutations, spontaneous mutations that occur during cell division give rise to somatic mosaic mutations **[G]** that occur at a much higher rate than germline mutations[87] and that are hard to detect by sequencing blood-derived DNA samples. Depending on the developmental timing of somatic mutation acquisition, mutant cells could distribute widely in the human brain, presenting a possible mechanism for focal epileptic brain malformations associated with a subset of NDD cases[88]. Somatic mosaic mutations in NDD risk genes such as *CHD2* and *SCN2A* have been identified in blood samples from individuals with ASD[89]. Moreover, somatic copy number variants have been found in NDD risk genes such as *TCF4* and *NRX1*[90], and somatic activation of *AKT3* through copy number variants or activating mutations cause large hemispheric brain malformations[91], indicating that somatic disruption of the PI3K–mTOR pathway may promote growth and survival of mutant cells. Whether somatic mutations affect GABAergic interneurons, and how such perturbations contribute to NDD pathogenesis, are key questions for future studies.

Epigenetic markers, including DNA methylation and histone modifications, as well as chromatin topological structural features such as chromatin looping **[G]**, affect the expression of genes involved in cell identity determination, gene expression and responses to physiological stimuli, and these may be disrupted in NDDs. For example, mutations in *DNMT3A*, which encodes the epigenetic modulator DNA (cytosine 5)-methyltransferase 3A, may disrupt gene methylation patterns, increasing risk of autism[92]. Knocking out *Fmr1* gene from neurons in mice results in excessive translation of epigenetic regulators and considerable epigenetic misregulation[93]. In line with such a mechanism, epigenetic silencing of the expression of $GABA_AR$ subunit genes *GABRA1* and *GABRB3*[94] has been observed in individuals with ASD who do not carry any mutations in these genes. Furthermore, brain tissue samples from individuals with sporadic ASD show reduced expression of glutamic acid decarboxylase 65 and 67 kDa proteins (GAD65 and GAD67), the rate-limiting enzymes in GABA synthesis[95].

Notably, epigenetic mechanisms have human-specific features[96]. For example, a CGG trinucleotide repeat expansion mutation at the 5' UTR of the *FMR1* gene, which encodes synaptic functional regulator (FMRP), induces hypermethylation and silencing of this gene. The protein-synthesis function of FMRP can be restored in human iPSC models of FXS through CRISPR-based targeted demethylation of the CGG repeats[97]. By contrast, insertion of similar size CGG repeats into the mouse *Fmr1* locus does not result in DNA

hypermethylation or repression of *Fmr1* expression, and the resulting gene-edited mice do not show obvious FXS-related phenotypes[98].

Genes constantly interact with the environment throughout the lifespan. Perinatal exposures to certain environmental risk factors, such as infectious agents, medications, substances of abuse and toxins, may perturb gene-expression profiles to increase the risk of NDDs. During the dynamic and vulnerable fetal stage of human brain development, maternal immune activation (MIA) caused by severe infections during pregnancy are associated with increased frequency of NDDs in children[99]. A mouse model of MIA produces offspring that have an abnormal cortical phenotype as well as impairments in communication and social interaction[100]. Optogenetic reduction of over-excitation of pyramidal neurons in primary somatosensory cortex ameliorated the behavioural abnormalities in MIA-affected offspring, suggesting a potential role of GABAergic inhibition in MIA[101]. Moreover, in two distinct rodent models of environmental factor-induced NDD — prenatal exposure to the antiepileptic drug valproic acid (VPA)[102, 103, 104], and postnatal exposure to the environmental toxin bisphenol A (BPA)[105] — the expression of KCC2 was considerably reduced. Therefore, understanding the interactions of genetics with epigenetic and environmental factors may help explain the variable severity of disease phenotypes of individuals with shared or related genetic NDD diagnoses.

Convergence of NDD mechanisms.—A mechanistic understanding of the shared and distinct pathophysiological pathways in different types of NDD may uncover convergent molecular pathways to be targeted for intervention.

As discussed above, there is strong human genetics evidence for the convergence of NDD mechanisms involving perturbations in the mTOR and WNT pathways. Moreover, disruptions in the mTOR and WNT signalling have been found in various NDD subtypes. *MECP2*-edited human stem cell-derived neuron models of Rett syndrome show reduced mTOR activity and global reductions in RNA and protein synthesis[106]. Furthermore, brain-derived neurotrophic factor (BDNF) and insulin-like growth factor 1 (IGF1), which stimulate the mTOR pathway, ameliorate disease-related phenotypes at the molecular, synaptic and behavioural levels in both male and female mice lacking the X-chromosome-linked *Mecp2* (the gene mutated in individuals with Rett syndrome)[106, 107]. Extracellular signal-regulated kinase (ERK) and WNT signalling are also disrupted in a mouse model of Rett syndrome[108]. Conditional knockout of the gene encoding β-catenin, a critical component of the canonical WNT pathway, specifically from PV^+ GABAergic neurons, causes ASD-like behaviour in mice[109], whereas *Apc*-knockout mice show increased β-catenin levels but also show ASD-like behaviours[110]. In the $Pten^{+/-}$ mouse model of ASD, β-catenin expression is elevated and contributes to abnormalities in cell division and brain development[111]. The ASD risk gene *CHD8*, which encodes chromodomain helicase DNA binding protein 8, regulates WNT signalling in a cell type-dependent manner[112]. Together, these results indicate that stringent regulation of the mTOR and WNT pathways at optimal levels may be necessary for normal brain function. **[Au:OK?]**

The many syndromic forms of NDD **[G]** have distinct etiologies but display partially overlapping clinical features, including E/I imbalance. Mouse models of Rett syndrome

carrying a mutant allele of *Mecp2* show reduced inhibitory and excitatory conductances in cortical neurons and increased E/I ratio[113], and increased occurrence of epilepsy and breathing apnea[114], suggesting disrupted GABAergic inhibition. Down syndrome is caused by trisomy of chromosome 21 and is characterized by intellectual disability and hippocampal dysfunction. Altered GABAergic neurotransmission impairs LTP in a mouse models of Down syndrome[115, 116, 117], which is rescued both by $GABA_AR$ antagonism and by restoring physiological inhibitory transmission with bumetanide[117, 118]. *Fmr1*-knockout mouse models of FXS exhibit reduced tonic inhibition[119, 120], network hyperexcitability[121] and abnormal striatal GABA transmission[122]. In mouse models of Angelman syndrome, a NDD whose symptoms include developmental delay, language disorder, movement disorder and seizures, imprinted silencing of *UBE3A* causes hyperexcitability in the brain, partly owing to a disruption in tonic inhibition[123]. CDKL5 syndrome is a severe neurodevelopmental disorder caused by mutations in the *CDKL5* gene and involves early-life seizures, autistic behaviours and intellectual disability. Knocking out *Cdkl5* in mice alters inhibitory transmission in the cerebellum[124], whereas restoring the expression of the GABA synapse regulator neuronal PAS domain-containing protein 4 (NPAS4) in the prefrontal cortex reverses synaptic and behavioral deficits in a 16p11.2-duplication mouse model[125]. Together, these observations suggest convergence of NDD mechanisms on perturbations in GABAergic signalling.

When determining potential target genes for NDD treatment, the ‘survivor’s bias’ in human genetic studies should be fully considered: that is, congenital mutations in genes essential for survival are rarely identified by DNA sequencing in individuals with NDDs. For example, in mice, congenital loss of *Slc12a5*, which encodes KCC2, is incompatible with life[126]. Therefore, protein-disrupting mutations in the coding region of *SLC12A5* are extremely rare in humans, and few regulatory-domain variations of *SLC12A5* have been identified in individuals with ASD or schizophrenia[80]. Nevertheless, mounting evidence demonstrates impaired expression or function of KCC2 in many mouse models of NDD, including those of Rett syndrome[33] or FXS[127], as well as *Chd8*-mutant mice[128] and VPA-induced ASD models[102, 103, 104]. In a mouse model of Down syndrome, a substantial increase in NKCC1 but no alteration in KCC2 was reported[117]. Similarly, increased NKCC1 and reduced KCC2 expression were reported in human tuberous sclerosis complex (TSC) and focal cortical dysplasia (FCD) brain specimens[129]. Therefore, various NDD subtypes may share a common mechanism of misregulated KCC2 and/or NKCC1 expression, which may result in excessive intraneuronal chloride levels that cause runaway excitation, seizure activity and overall E/I imbalance in neural circuits. **[Au:OK?]**

Clinical research of NDDs further support the E/I imbalance hypothesis. For example, the correlation between GABA levels and the strength of GABAergic inhibition, as measured by binocular rivalry **[G]**, a behavioural biomarker that relies on E/I balance, is disrupted in the brains of individuals with ASD[130]. Moreover, the typical correlation between GABA concentrations and gamma-band coherence, another network property reliant on E/I balance, is not observed in individuals with ASD[131]. An electroencephalography (EEG)-based study reveals larger functional E/I variability in children with ASD than in typically developing children[132]. A recent work demonstrates altered visual discrimination in FXS patients that

is paralleled by similar changes caused by altered PV^+ neuron activity in a mouse model of FXS, indicating a potentially translatable biomarker for investigating E/I balance in NDD patients[133]. **[Au:OK?]**

A common dysfunction underlying NDD is a reduction in the number of GABAergic interneurons in the brain. In postmortem brain tissue from individuals with ASD, the number of PV^+ neurons was decreased and the abundance of PV-encoding mRNA downregulated[134]. A similar phenotype of reduced interneuron cell number has been recapitulated in several mouse models of ASD. A mouse model carrying mutations in the presynaptic neurexin family gene that encodes contactin-associated protein-like 2 (*CNTNAP2*), exhibits reduced interneuron cell density, as well as epilepsy and ASD-like behaviours in all three core domains relevant to the disorder: stereotypic behaviours, reduced sociability and impaired communication[135]. Mice carrying mutations in NDD risk genes, including those encoding the postsynaptic scaffold proteins *Shank3* and *Shank1*[136], the transcription factors *Mecp2*[137] and *Arid1b*[138], and the phosphatase *Pten*[139], also exhibit decreased interneuron cell number and/or reduced PV expression. Similar decreases in PV^+ neuron density were also reported in prenatal VPA-exposed mice, which also show dysfunction in PV^+ neuron-related neural circuits in the cortex and striatum[140]. Interestingly, higher-than-normal numbers of interneurons has been reported in the somatosensory cortex of th3e Ts65Dn mouse model of Down syndrome[141]. Another study of postmortem tissue samples from children with ASD reports cortical disorganization and abnormal expression of glutamatergic neuronal markers, but mild to no changes in GABAergic markers[142]. A systematic analysis of interneuron density in different NDD subtypes may promote the development and application of therapies that normalize interneuron number.

GABA signalling in NDD-related circuit and behaviour abnormalities.—Driven by the genetic- and molecular-level dissection of NDD etiology, outstanding questions emerge regarding how converging genetic and molecular deficits affect the function of different cell populations, and how neural circuit-level perturbations give rise to behavioural symptoms (Fig. 2). Risk-associated mutations are typically present in many cell types in the brain. It is therefore crucial to generate a systematic understanding of the NDD-related circuit abnormalities and to further dissect the respective roles of different cell types in mediating brain circuit dysfunction.

Dravet syndrome, a form of intractable childhood-onset epilepsy that is associated with sleep disorder, cognitive deficits, autistic-like behaviors, intellectual disability and sometimes sudden unexpected death in epilepsy (SUDEP), provides an example to dissect the pleiotropic roles of disease risk genes in different cell types. Dravet syndrome is caused primarily by heterozygous mutations in the sodium channel gene *SCN1A* or, in some cases, in its paralogue *SCN2A*. *SCN1A* encodes the α-subunit of $Na_V1.1$, the primary voltage-gated Na^+ channel in several classes of GABAergic interneurons. As such, in $Scn1a^{-/-}$ or $Scn1a^{+/-}$ mice, the sodium current density is selectively and substantially reduced in inhibitory interneurons, but not excitatory pyramidal neurons[143]. Similarly, specific deletion of *Scn1a* from inhibitory interneurons in mice is sufficient to cause seizures and premature death[144]. By contrast, *SCN2A*, which encodes the α-subunit of the $Na_V1.2$ channel, is expressed mainly in excitatory neurons[145], and $Na_V1.2$ haplodeficiency

in excitatory neurons is sufficient to trigger epilepsies in mice[146]. A shared pathological feature of depolarizing GABA reversal potential was detected in the neocortical and hippocampal pyramidal neurons of two mouse models of Dravet syndrome: one involving haploinsufficiency of *Scn1a*, and another in which both copies of *Scn1b*, which encodes the β-subunit of $Na_V1.1$, are deleted[147]. Whether the depolarizing effect of GABA is caused by haploinsufficiency of $Na_V1.1$ or $Na_V1.2$ in pyramidal neurons, or arises to compensate for the deficits in GABAergic inhibition, remains to be elucidated.

Another example of congenital mutations that cause brain region- and cell type-specific abnormalities is the NDD risk gene encoding patched domain containing 1 protein (*PTCHD1*), which is implicated in about 1% of individuals with intellectual disability and ASD[148]. In mice, *Ptchd1* is selectively expressed in the thalamic reticular nucleus (TRN), which consists mostly of PV^+ GABAergic neurons and gates the information flow between the cortex and thalamus[149]. *Ptchd1*$^{y/-}$ mice show attention-deficit hyperactivity disorder (ADHD)-like symptoms and impairments in attention, locomotion and learning[149]. GABAergic neurons in the TRN of wild-type mice express very low levels of KCC2 and show depolarizing GABA responses, contributing to the generation of burst firing in the TRN[150]; however, such GABA-mediated TRN burst firing is impaired in *Ptchd1*$^{y/-}$ mice, leading to reductions in sleep spindle activity and highly fragmented sleep[149]. Sleep disorder is a common comorbidity of NDDs, present in sporadic ASD[151], Rett syndrome[152] and Dravet syndrome[153]. Therapeutic strategies that target striatal GABAergic circuitry might ameliorate sleep disruptions in individuals with NDDs.

A mechanistic understanding of the roles of NDD risk genes in specific cell populations should yield biological insights that facilitate the development of targeted therapy. Modern conditional and intersectional genetic manipulation tools enable targeting of such genes in genetically defined subsets of neurons[154]. For example, although *MECP2* is expressed throughout the mammalian brain, targeted knockout of *Mecp2* from GABAergic neurons or a subset of forebrain GABAergic neurons in mice recapitulates many Rett syndrome and autistic features, including repetitive behaviours, motor deficits and breathing apnea[155]. By contrast, restoring *Mecp2* specifically in GABAergic neurons is sufficient to rescue multiple disease features in male *Mecp2*$^{y/-}$ mice or female *Mecp2*$^{+/-}$ mice[156]. Knocking out *Ube3a* from $GAD2^+$ cells in mice causes Angelman syndrome-like EEG abnormalities and enhances seizure susceptibility[157], whereas knocking out *Shank3* in a subset of inhibitory neurons causes abnormal social and locomotor behaviours[158]. Knocking out the gene encoding the $GABA_AR$ δ-subunit specifically from cerebellar granule cells causes anxiety-like and altered social behaviours without affecting motor performance[159], suggesting that disruptions of non-motor functions of the cerebellum that are mediated by tonic GABAergic inhibition may contribute to certain NDD symptoms.

Optogenetics and chemogenetics enable modulation of specific cell population activities to illuminate the roles of neural circuits in behaviour. In support of the E/I imbalance hypothesis of ASD, optogenetic activation of excitatory neurons in the medial prefrontal cortex induced high-frequency rhythmicity and impaired social behavior in mice[160]. Moreover, optogenetic activation of GABAergic neurons in the medial amygdala enhanced social behaviour, whereas activation of glutamatergic neurons in the same region increased

repetitive behaviour[161]. Furthermore, chemogenetic activation of PV neurons in a mouse model of FXS reversed impairments in perceptual learning[133]. Taken together, these preclinical mechanistic studies highlight the contribution of GABAergic neurons to multiple core NDD-related symptoms.

The brain is highly plastic at the synaptic and circuit levels, and constantly undergoes homeostatic adjustments. NDD risk genes play a major part in regulating neural circuit plasticity. Visual cortex plasticity is impaired in mouse models of Rett syndrome[162] and Angelman syndrome[163], whereas the barrel cortex plasticity window is delayed in a mouse model of FXS[164]. Inhibiting NKCC1 during a developmental critical period restores somatosensory cortex synaptic plasticity in FXS-model mice[165], suggesting that disruptions of chloride homeostasis may underlie cortical plasticity impairments in NDD.

NDD risk genes also modulate homeostasis in the brain. Perturbations that exceed the correcting ability of homeostatic plasticity may lead to NDD symptoms, whereas over-compensation may render homeostatic mechanisms maladaptive and cause secondary disruptions to brain function[166]. *Shank3* is crucial for homeostatic compensation in mouse visual cortical circuits to recover from perturbations to sensory drive[167]. Similarly, abnormal sensory-evoked responses have been reported in individuals with ASD or FXS[168], and in mouse models of Rett syndrome and tuberous sclerosis complex[169]. Surprisingly, a recent work reported that in four mouse models of ASD (*Fmr1*, *Cntnap2* and *Tsc2* mutants, and mice with 16p11.2 deletion), **[Au:OK?]** the sensory-evoked firing rates of somatosensory cortex neurons are largely normal, despite a shared decrease in inhibitory conductance[170]. The authors of the study concluded that the E/I imbalance observed in these mouse models may be a homeostatic response to normalize synaptic drive. Further work that takes into consideration the potential alterations in the polarity of the effects of GABA in these NDD models may shed light on whether homeostatic mechanisms cause or correlate with sensory response abnormalities.

Targeting inhibition to treat NDDs

The prevailing view is that changes to brain function in individuals with NDDs are irreversible. However, landmark studies using mouse models of Rett syndrome show that restoring *Mecp2* in *Mecp2*-null adult mice can reverse most disease-related deficits even after disease onset[171]. Similarly, phenotypic rescue through gene restoration in adult animals have been achieved in mouse models of NDD carrying mutations in *Shank3*[172] or *Fmr1*[173]. These results suggest that NDD brains are not ‘broken’ beyond repair, but perhaps are just ‘out of tune’ — and that appropriate therapeutic interventions could largely reverse symptoms even after disease onset.

DNA- and RNA-targeting therapies.—Gene-replacement and gene-editing therapies are promising therapeutic avenues that may correct the genetic root cause of the monogenic subtypes of NDD. Gene-replacement therapy typically uses a viral vector to transfer genes of interest to the target cells to compensate for the faulty endogenous genes (Fig. 3a). In mouse models of Rett syndrome or FXS, viral deliveries of *MECP2* or *FMR1*, respectively, are well-tolerated and confer promising phenotypical reversal[173, 174]. Gene-editing therapy

that aims to correct disease-causing mutations at their original loci using CRISPR or transcription activator-like effector nucleases **[G]** (TALENs) (Fig. 3b) has the advantages of maintaining existing spatial and temporal patterns of endogenous gene regulation. Clinical trials of CRISPR-mediated gene-repair therapy targeting the mutant *CEP290* gene have been initiated for the treatment of the retinopathy type 10 Leber congenital amaurosis (ClinicalTrials.gov Identifier: NCT03872479).

For NDDs caused by aberrant gene expression, rather than protein-disrupting mutations, one approach is to repurpose catalytically inactive CRISPR variants as programmable epigenome-editing tools that recruit epigenetic modifiers to the gene of interest and modulate its transcriptional output without changing the DNA sequence[175, 176] (Fig. 3c). Targeted demethylation of the *FMR1* gene in a human embryonic stem cell (ESC)-derived neuronal model of FXS leads to gene reactivation and functional rescue[97].

Currently, the application of gene therapies is limited by the inefficiency of viral vectors in targeting the brain, and by the potential for over- or under-expression of dose-sensitive target genes. Substantial improvements to viral or non-viral gene delivery systems are needed to deliver the right amount of gene to the right places at the right time and thus unleash the full potential of gene therapy. Another approach is to use conveniently administrable pharmacological modulation of epigenetic mechanisms to alleviate disease symptoms, as demonstrated by bromodomain-containing protein 4 (BRD4) inhibition in FXS neurons[93], and histone deacetylase (HDAC) inhibition in *Shank3*-deficient mice[177].

RNA molecules can be encapsulated in lipid nanoparticles and delivered into cells with relative ease, compared with DNA molecules. Delivery of mRNA transiently increases target protein translation[178] (Fig. 3d), whereas delivery of antisense interference RNA (RNAi) temporarily degrades specific RNA transcripts, silencing gene expression[179]. Moreover, antisense-oligonucleotides (ASOs), short modified DNA sequences that hybridize and silence target mRNA transcripts, have remarkable in vivo stability (Fig. 3e). Brain delivery of ASOs to knock down *MECP2* transcripts reversed disease phenotypes in a mouse model of *MECP2*-duplication syndrome[180]. Remarkably, a customized ASO treatment of one individual with Batten disease, a disorder caused by misspliced RNA transcripts, demonstrated good safety and efficacy that improved behavioural symptoms and reduced epileptic seizures[181]. These promising, life-changing results highlight a novel avenue of developing precision molecular medicine for NDDs.

Protein-targeting therapies.—For NDD subtypes of polygenic or sporadic origin, therapies designed to target a particular gene are not a treatment option. A strategy to overcome this limitation is to develop drugs that bypass the upstream genetic or environmental causes, to directly target the proteins that perform much cellular signalling and function. Uncovering common NDD disease mechanisms and develop therapies that rectify common perturbations in protein expression or function may induce symptomatic relief for NDDs, regardless of how complex or uncertain the cause may be, analogous to how a pair of glasses can provide symptomatic relief to individuals with myopia, regardless of the root causes.

Most small-molecule drugs, including variants of benzodiazepine that are positive allosteric modulators of $GABA_A$Rs[182] and variants of baclofen that are $GABA_B$R agonists, bind to target proteins to change their structure and function (Fig. 4a). R-baclofen treatment effectively reversed synaptic, cognitive, and social deficits in mouse models of FXS[183] and 16p11.2-deletion syndrome[184]. However, clinical trials of R-baclofen in FXS syndrome patients were unable to demonstrate any improvement in behaviour end points, potentially due to the advanced age of the participants and the limited duration of treatment[185]. Drugs that reduce NKCC1 activity or increase KCC2 activity should enhance the efficacy of GABAergic inhibition and may confer benefits to NDD patients, and bumetanide and VU0463271 have been identified as specific blockers of NKCC1[186] and KCC2[187], respectively. A newly developed compound, ARN23746, shows selective inhibition of NKCC1 versus NKCC2 and KCC2[104]. However, so far no drug has been discovered that can facilitate the complex transporter function of KCC2 protein. CLP257, a recently identified compound that enhances KCC2 membrane trafficking, alleviates hypersensitivity in a rat model of neuropathic pain[188], although controversy have arisen over the mechanism of action of this compound[189, 190]. An alternative approach to modulate the activities of KCC2 and NKCC1 is through altering their post-translational modifications; for example, by targeting with-no-lysine kinase (WNK), which phosphorylates KCC2 and NKCC1 to inhibit or activate their function, respectively[191, 192]. **[Au:OK?]** A knock-in mouse model expressing a dephosphomimetic version of KCC2 (KCC2-T906A/T1007A) shows reduced susceptibility to chemoconvulsant-induced epileptiform activity[193]. Whether WNK inhibitors are safe and efficacious in promoting **[Au:OK?]** KCC2 activity and treating NDDs is a topic of considerable interest for both basic research and clinical investigations.

Pharmacological modulation of common molecular pathways perturbed in many NDD subtypes, such as the mTOR and WNT pathways, could confer broadly applicable therapeutic benefits (Fig. 4b). IGF1, which stimulates the AKT–mTOR pathway, shows promising results in preclinical models of Rett syndrome, and treatment with new specific inhibitors of glycogen synthase kinase-α (GSK3α), a key WNT pathway component, corrects pathophysiological abnormalities in a mouse model of FXS[194]. However, a randomized clinical trial that treated girls with Rett syndrome with recombinant human IGF1 (rhIGF1, also called mecasermin) did not reveal statistically significant improvement in clinical symptoms[195]. Nevertheless, a tripeptide segment of IGF1, which is effective in mouse model of Rett syndrome[162], has exhibited efficacy (via a compound called trofinetide) in phase 1 and 2 trials in Rett syndrome, and is now in phase 3 trials[196, 197]. These results highlight the importance of further investigating the pharmacokinetics and bioavailability of pathway-modulating drugs to facilitate clinical translation.

Complex protein–protein interactions are crucial in cellular processes such as gene transcription, intracellular signalling and synapse formation. Characterization of the ‘interactome’ of proteins that bind strongly to NDD-relevant proteins such as KCC2 may identify protein-interaction partners that may be targeted therapeutically[43]. Moreover, weak interactions between the intrinsic disordered regions of member proteins mediate dynamic partition of phase-separated protein condensates in cells[198, 199]. NDD-relevant proteins such as MeCP2 and FMRP form intracellular condensates that may contribute to

their functions[200]. Protein condensates localized at glutamatergic and GABAergic synapses show high affinity and selectivity for their respective component proteins[201]. Drugs that either preferentially partition into, or mediate the assembly or dissipation of, target protein condensates specifically in GABAergic neurons or GABAergic synapses may exhibit improved therapeutic efficacy and reduced off-target effects (Fig. 4c).

ASD risk genes *UBE3A* and *CUL3* are involved in protein degradation via ubiquitin ligase and proteosome[202], suggesting that disrupted protein homeostasis may contribute to NDD pathogenesis. An emerging drug development approach, proteolysis-targeting chimeras **[G]** (PROTACs), uses small-molecule compounds to recruit endogenous destruction complex machinery to degrade target proteins, potentially compensating for impaired proteostasis in certain NDDs[203] (Fig. 4d).

Leveraging human stem cell and genome-editing technologies, sophisticated cell-based disease models and high-throughput screening assays can be developed to investigate human genes and protein networks in their native cellular contexts, opening up possibilities to screen for potential therapeutics to modulate the transcriptional and translational regulatory programmes of target genes (Fig. 4e). For example, *KCC2* is an appealing therapeutic target gene that is silenced in multiple subtypes of NDD. Although KCC2 is selectively expressed in neurons, previous KCC2 drug-screening efforts relying on non-neuronal cell lines failed to identify compounds that increase KCC2 levels, possibly because these cells lack the cellular and genomic context that enables **[Au:OK?]** KCC2 expression[204]. To overcome this hurdle, a recent study used CRISPR to insert a luciferase reporter gene into the *KCC2* locus to create a convenient readout of the *KCC2* gene expression level in human neurons. Unbiased screening of the effects of a library including many existing safety-approved compounds on such reporter neurons enabled discovery of the first group of KCC2 expression-enhancing compounds (KEECs) and revealed previously uncharacterized molecular pathways that regulate *KCC2* expression in neurons[205]. Repurposing already approved drugs or drug-like molecules for the treatment of NDDs facilitates the translation from bench to bedside.

Neural circuit-targeting therapies.—At the neural network level, drugs that alter the functional activity or expression level of target proteins exert cascading effects on both the cells expressing the target proteins and other connected cell populations. Studies in the *Scn1a*$^{+/-}$ mouse model of Dravet syndrome[206] and the BTBR mouse model of idiopathic ASD[207] have demonstrated that low doses of benzodiazepine variants such as clonazepam and L-838417 can normalize social and cognitive behaviours in these animals, potentially through potentiating GABAergic inhibition (Fig. 5a). However, pharmacological potentiation of GABA signalling in the neonatal rat brain exacerbates seizure, owing to NKCC1-mediated chloride accumulation[35]. Treatment of mouse models of FXS[103, 165] or Down syndrome[117] and or mice with VPA-induced ASD-like phenotype[103], **[Au:OK?]** with the NKCC1 blocker bumetanide ameliorated electrophysiological and behavioral phenotypes. In clinical trials, off-label use of bumetanide conferred symptomatic benefits on both individuals with sporadic ASD and individuals with FXS[208, 209], although a recent study has shown the efficacy of bumetanide in reducing repetitive behavior in only a small subset of ASD patients[210]. These findings support the notion that pharmacologically

restoring chloride homeostasis can provide symptomatic relief for various NDDs (Fig. 5b). However, bumetanide triggers increased diuresis and electrolyte imbalance in adult patients, mainly due to nonspecific inhibition of NKCC2 in the kidney[211]. A recently developed NKCC1-selective inhibitor may reduce such safety concerns and enable long-term clinical application of chloride-normalizing drugs[104].

Unlike NKCC1 and NKCC2, KCC2 has a brain-specific and neuron-restricted expression pattern[212]. Thus, targeting KCC2 has reduced risk for off-target effects throughout the body. A recently identified group of small-molecule KEECs rescued electrophysiological and behavioural phenotypes in human ESC-derived neuron and mouse models of Rett syndrome[205]. Treating wild-type neurons with a KEEC substantially increased KCC2 expression but did not change neuronal morphology or function, which is relevant for Rett syndrome and other X-linked NDDs in which both wild-type and mutant cells are present owing to random X-chromosome inactivation. Moreover, viral overexpression of a rat *Kcc2* transgene in a mouse model of spinal cord injury showed beneficial effects on functional recovery[213]. Together, these results suggest that enhancing KCC2 expression could be a safe approach for NDD treatment.

Engaging endogenous neuromodulatory signalling mechanisms is a potentially efficacious therapeutic avenue to treat NDDs. Oxytocin is a neuromodulator that is highly relevant to NDD: social deficits caused by reduced oxytocin signalling[214] are largely mediated by downregulation of KCC2 expression and activity[103, 215]. Several reports suggest that oxytocin released during child delivery protects fetal neurons from hypoxia and reduces the risk for NDDs by inducing a temporary upregulation of KCC2[103, 216]. Intranasal delivery of oxytocin shows promising results in treating small groups of individuals with ASD[217, 218], but the short half-life of exogenously applied oxytocin limits its effectiveness. An alternative approach is to drive endogenous oxytocinergic signalling in the brain to rescue NDD phenotypes. Indeed, a proof-of-concept study shows that optogenetic stimulation of the oxytocinergic neurons in the paraventricular nucleus of the hypothalamus (PVN) improves social behaviour in mouse models of ASD[219]. Moreover, recently, treatment of various mouse models of NDD with the probiotic bacterial species *Lactobacillus reuteri* has been shown to alter gut microbiota composition, thereby stimulating vagus nerve afferents innervating the PVN, releasing oxytocin and ameliorating ASD phenotypes[220, 221] (Fig. 5c).

Optogenetics and chemogenetics represent novel groups of experimental therapeutic approaches to directly modulate neural circuit activities (Fig. 5d). Optogenetic activation of GABAergic neurons in the medial amygdala enhances social behavior in mice, whereas activation of glutamatergic neurons in the same brain region increases repetitive behaviour[161]. To obviate the need to implant an optic fibre into the brain and facilitate the application of optogenetics technology for future clinical trials, an ultra-sensitive step-function opsin that can be turned on and off via transcranial optical stimulation was recently developed[222]. Moreover, chemogenetic activation of PV^+ interneurons in the visual cortex was sufficient to rescue deficits in sensory processing in *Fmr1*$^{-/-}$ mice[133], whereas activation of glutamatergic neurons in the prefrontal cortex ameliorated cognitive and social impairments in both *16p11*$^{+/-}$ mice **[Au:OK?]** and *Shank3*-deficient mice[223, 224]. These

brain-stimulating approaches empower mechanistic investigation of the role of GABAergic neurons in NDD pathogenesis and provide promising avenues to develop potential therapies for NDDs that modulate neural circuit activities.

To mitigate the loss of GABAergic neurons in NDD subtypes associated with mutations in genes such as *CNTNAP2*, *ARID1B, MECP2* or *PTEN*, a cell therapy approach is to transplant inhibitory neurons generated in vitro into the brain (Fig. 5e). Mouse MGE-lineage interneurons transplanted into the mouse brain can integrate into the circuit for a long time after transplantation[225], re-open the plasticity window of the host brain[67] and, in a pilocarpine-treated mouse model of epilepsy, reduce seizure severity[226]. Furthermore, GABAergic neurons to be transplanted can be genetically modified to express transgenes that enhance their inhibitory function[227] or to express optogenetic and chemogenetic responsive elements that enable on-demand GABAergic inhibition in the brain. To translate these encouraging approaches for testing into clinical trials, a robust pipeline to produce clinical-grade human cells that closely resemble MGE-derived interneuron progenitor cells would needs to be developed, and the interneuron transplantation procedure would need to be optimized.

Individual-level diagnosis and therapies.—A comprehensive framework to understand NDD pathogenesis is to consider both genetic and environmental perturbations that cause molecular abnormalities in cells. Such deficits further propagate to affect circuit function, ultimately leading to behavioural symptoms. Following the same logic, therapeutic intervention at the genetic, molecular or circuit level may propagate to positively influence behaviour. Thus, it is crucial to evaluate the progression of NDDs and assess the therapeutic efficacy of interventions at the organism level.

The ability to make early and accurate clinical assessments is the foundation for effective management and treatment of NDDs. Key behavioural metrics such as motor coordination, stereotypical behaviour, eye contact and sleep patterns can be used for diagnosing NDDs and assessing NDD severity (Fig. 6a). A panel of objective biomarkers, such as EEG, MRI, functional MRI (fMRI), MR spectroscopy and whole-genome or exon sequencing, as well as body fluid cytokine, antibody and metabolite biomarkers, can be used in conjunction with behavioural assays to assist diagnosis and assessment of disease severity (Fig. 6b). One study used the Autism Diagnostic Observation Schedule **[G]** (ADOS) and other behavioural assays, together with MR spectroscopy measurement of brain GABA concentrations, to reveal that the typical link between GABA levels and the efficacy of inhibition is specifically absent from individuals with ASD[130]. Impairments in gamma frequency oscillations[228] and the disrupted coherence of auditory-evoked gamma-band responses[131] are other neurophysiological biomarkers found in people with ASD and that rely on differences in GABA signalling. Stratification of individuals with NDDs, based on symptomatic diagnosis and various biomarker metrics, could drive evidence-based precision medicine, and provide objective measurements with which to assess treatment outcomes (Fig. 6c).

Deep brain stimulation (DBS) has been applied clinically to treat various brain disorders (Fig. 6d). A study reported that DBS applied at 100 Hz to the internal segment of

the globus pallidus inhibits local neuronal firing in a $GABA_AR$-dependent manner in monkeys[229], indicating a potential role of reducing GABA signalling in mediating the beneficial effect of DBS, although the exact cellular and synaptic mechanisms remain unclear. One study demonstrated successful rescue of hippocampal memory with forniceal DBS in a mouse model of Rett syndrome[230], suggesting potential application of DBS in the treatment of NDDs. Similarly, it may also worth testing other non-invasive brain stimulation methods, including transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), in treating NDDs. Cognitive and behavioural therapy (CBT) tailored to the needs of patients is already commonly used in the clinic to manage NDD symptoms (Fig. 6e). Multi-modal treatment approaches that combine medication with organism-level treatment may generate synergistic effects to manage and improve symptoms in individuals with NDDs.

Conclusion and perspectives

GABA plays pivotal roles in brain development, physiology and pathophysiology. In this Review, we have discussed GABAergic function and dysfunction in a practical framework comprising the genetic, molecular, circuit and behaviour levels. Gene activities determine the molecular make-up of cells, which assemble into neural circuits that give rise to behavioural phenotypes. Perturbations to this intricate system, such as genetic mutations or aberrant cellular responses to GABA, may propagate throughout the cascade, triggering abnormalities in neural circuit function and causing behavioural symptoms. We have reviewed findings from basic and translational research that support the pivotal role of GABAergic inhibition in pathogenic mechanisms that converge across NDDs, prompting the extension of the E/I imbalance theory of NDD to include the dynamic changes in neuronal chloride homeostasis. We have also highlighted various treatment modalities that could potentially target GABA signalling in NDDs, including small-molecule drugs, gene and cell therapies, and brain stimulation.

Looking ahead, technological innovations and the joint effort of the global scientific community may help to illuminate a number of fundamental questions regarding the causes of and treatments for NDDs that remain largely unanswered (Box 1). Approaching these fundamental questions from the angles of basic, translational, and clinical research will help deepen our understanding of the marvelous complexity of the brain and provide much-needed treatments for people with NDDs.

Acknowledgements

This work is funded by a Bridge to Independence Award from the Simons Foundation Autism Research Initiative awarded to X.T.; US National Institutes of Health (NIH) grant R01 MH104610 awarded to R.J.; and by NIH grant R01 MH085802 and a grant to the Simons Center for the Social Brain from the Simons Foundation Autism Research Initiative awarded to M.S. The authors also thank C. Clairmont for his substantial help with revising the manuscript, and X. Jing, M. Enriquez, D. Tomasello, E. Wogram and E. Majercak for useful comments and discussions.

Glossary definition list

NDD risk genes

Candidate genes identified from clinically-diagnosed NDD patient populations, in which mutations are associated with increased risk of developing NDD symptoms

Transcription factors
Proteins that bind to specific DNA sequence motifs and regulate the transcriptional levels of target genes to determine cellular identity and function

Excitation/inhibition (E/I) imbalance
Disruption in the balance between excitatory and inhibitory drives which causes either overexcitation or underexcitation of neural circuits observed in various NDD subtypes

Stratification of patients
Rational partitioning of patients into subgroups, based on their behavioral metrics, biomarkers, and genetic information, to facilitate precise diagnosis and targeted treatment

Precision medicine
An approach to medicine that considers the individual biological variabilities of each patient, such as sex, genetics, and other biomarkers, for devising personalized treatment regimens

Epigenetic factors
Modifications to the chromosome, including methylation, histone modification, and non-coding RNAs, which regulate the expression level of genes without altering the primary DNA sequence

GABA functional switch
A developmental process during which GABA action switches from excitatory to inhibitory, mainly driven by altered expression and function of chloride transporters NKCC1 and KCC2

Homeostatic mechanism
Regulatory feedback signals involving changes in synapse number, synaptic strength, and GABA signaling efficacy that stabilize neuronal and neural network excitability and function

Martinotti cells
Small multipolar interneurons, with short branching dendrites, that send projections to layer-I and provide dendritic inhibition mainly for layer 5 pyramidal neurons to facilitate feedback inhibition

Critical period
A developmental stage, often perturbed in NDD, during which connectivity of the nervous system is especially susceptible to long-term alterations by environmental stimuli

Ocular dominance plasticity
Changes in relative responses of visual cortex neurons to stimulation of the two eyes due to visual deprivation. This plasticity is triggered by changes in GABA-ergic inhibition

X-linked intellectual disability
A subset of male-biased intellectual disability cases that are associated with inheritance of mutant genes on the X chromosome

Somatic mosaic mutations
Genetic mutations that are absent in the zygote stage but only present in the progenies of mutant cells that occur during the developmental process

Chromatin looping
Dynamic process in which multiple distal genomic regions are brought into proximity through DNA-protein interactions to provide a structural basis for long-range gene transcription regulation

Syndromic forms of NDD
A clinical classification of NDD characterized by patterned neurobehavioral phenotypes and defined genetic causes, including chromosomal aberrations, copy number variations, and single gene mutations

Binocular rivalry
A visual phenomenon regulated by GABAergic inhibition, in which different images presented to each eye compete for awareness, resulting in alternating perception

Transcription activator-like effector nucleases (TALENs)
Artificial DNAase engineered through fusing a TAL effector DNA-binding domain to a DNA cleavage domain for the purpose of cutting and editing specific DNA sequences

Proteolysis-targeting chimeras (PROTACs)
Engineered small molecules composed of two distinct domain classes, one that engages E3 ubiquitin ligase and the other that binds to target proteins for degradation

Autism Diagnostic Observation Schedule (ADOS)
A standardized assessment tool that clinicians may use for diagnosing ASD in patients through the use of semi-structured plays or interview sessions

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Box 1 |

Questions for future research

Here we outline some of the main outstanding questions in the NDD research field, and discuss potential approaches to tackle these questions in order to illuminate the cause of NDD and guide the development of effective treatments.

Biological information flows from the genome and epigenome to the RNA and protein molecules in various cell types. How does such information assimilate into large-scale emergent properties that determine neural circuit activities and animal behavior? Studies that systematically investigate the causal links between molecular information, circuit function, and animal behavior may establish a framework to interpret how biological information is transmitted between these levels.

NDDs often manifest deficits at the molecular, circuit and behaviour levels. How do disease-relevant perturbations cascade through the flow of biological information and lead to NDD symptoms? Investigating how NDD-causing genetic and molecular perturbations cause disease-relevant circuit and behavioural abnormalities may provide opportunities to understand the function and dysfunction of the brain.

Certain aspects of the primate and human brain are fundamentally different from their rodent counterparts. How do primate- and human-specific features of GABAergic inhibition contribute to circuit function and NDD-related dysfunction? A systematic comparison of the brains of different species at the levels of single-cell gene expression and large-scale connectivity may lead us closer to the answers.

NDDs pathogenesis have a strong male sex bias. Do potential sex-specific features in the development and function of the GABAergic inhibition system contribute to such bias? Investigating the effect of both intrinsic genetic factors and extrinsic hormonal cues on GABAergic signalling system may yield mechanistic insights.

Glial cells regulate many aspects of neuronal function and connectivity. Do interactions between GABAergic neurons and glial cells contribute to NDD pathogenesis? Cell type-specific perturbations and a systematic analysis may reveal the crosstalk between them.

It has been demonstrated that symptoms of some preclinical models of NDDs **[Au:OK?]** can be reversed by replacing disease-causing genes. To what extent could restoring GABAergic inhibition deficits reverse NDD disease symptoms after their onset? Pharmacological modulation of the GABAergic signalling may provide broadly applicable therapeutics to ameliorate NDDs.

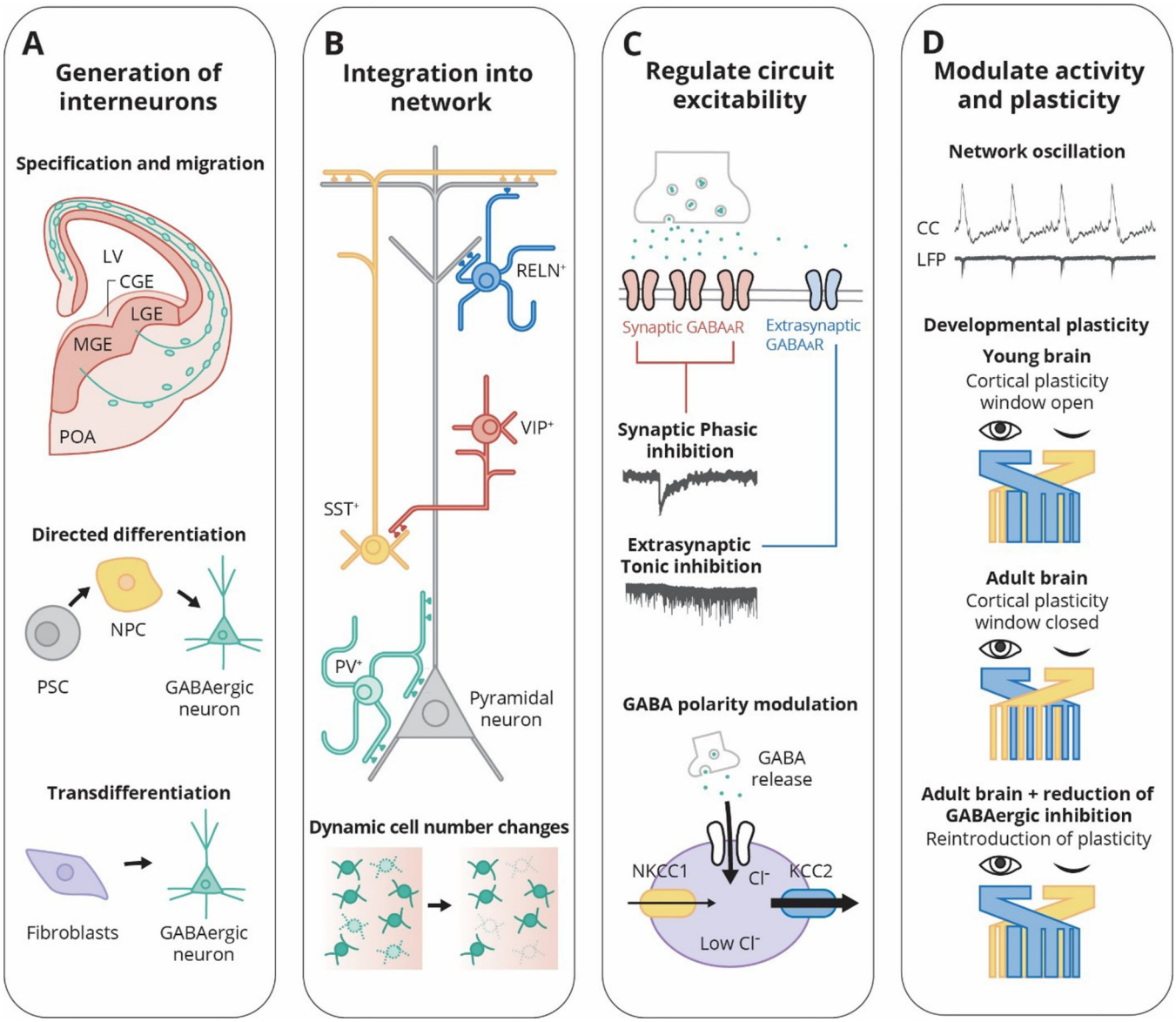

Fig. 1 |. Development of the GABAergic signalling system.

a | In the mammalian brain, GABAergic interneurons are generated in proliferative regions including the medial ganglionic eminence (MGE), lateral ganglionic eminence (LGE), caudal ganglionic eminence (CGE) and the preoptic area (POA), and then migrate around the lateral ventricle (LV) to the subcortical and cortical destination areas, where they undergo further specification[8, 9]. Recent advances in stem cell biology enable the generation of GABAergic neurons in vitro using directed differentiation from pluripotent stem cells (PSCs) to neural progenitor cells (NPCs), then to functional human GABAergic neurons[6]. Also, somatic cells such as fibroblasts can be transdifferentiated into GABAergic neurons through ectopic expression of transcription factors[7]. **b** | A simplified diagram that shows the connectivity of the main subtypes of interneurons, including parvalbumin-expressing (PV^+), somatostatin-expressing (SST^+), vasoactive intestinal peptide-expressing (VIP^+) and reelin-expressing ($RELN^+$) neurons[3]. The number of interneurons also undergoes dynamic changes

during development as the neurons integrate into brain networks[13].**c |** GABAergic signalling regulates neural network excitability through three main mechanisms: fast phasic inhibition mediated by synaptic $GABA_A$ receptors ($GABA_ARs$); slow tonic inhibition mediated by extrasynaptic $GABA_ARs$[19]; and the dynamic regulation of the intracellular chloride concentration by the chloride transporters NKCC1 and KCC2, which determines the polarity and efficacy of GABAergic inhibition[27]. **d |** GABAergic inhibition has major roles in modulating neural network oscillations[54] and brain circuit plasticity during development and in adults. The developmental plasticity window is closed in adult animals such that monocular deprivation no longer changes the eye-specific projection pattern to the visual cortex, indicated by the differently coloured stripes. Blocking GABAergic inhibition in adult animals reactivates visual cortex plasticity[54]. CC, current clamp; LFP, local field potential.

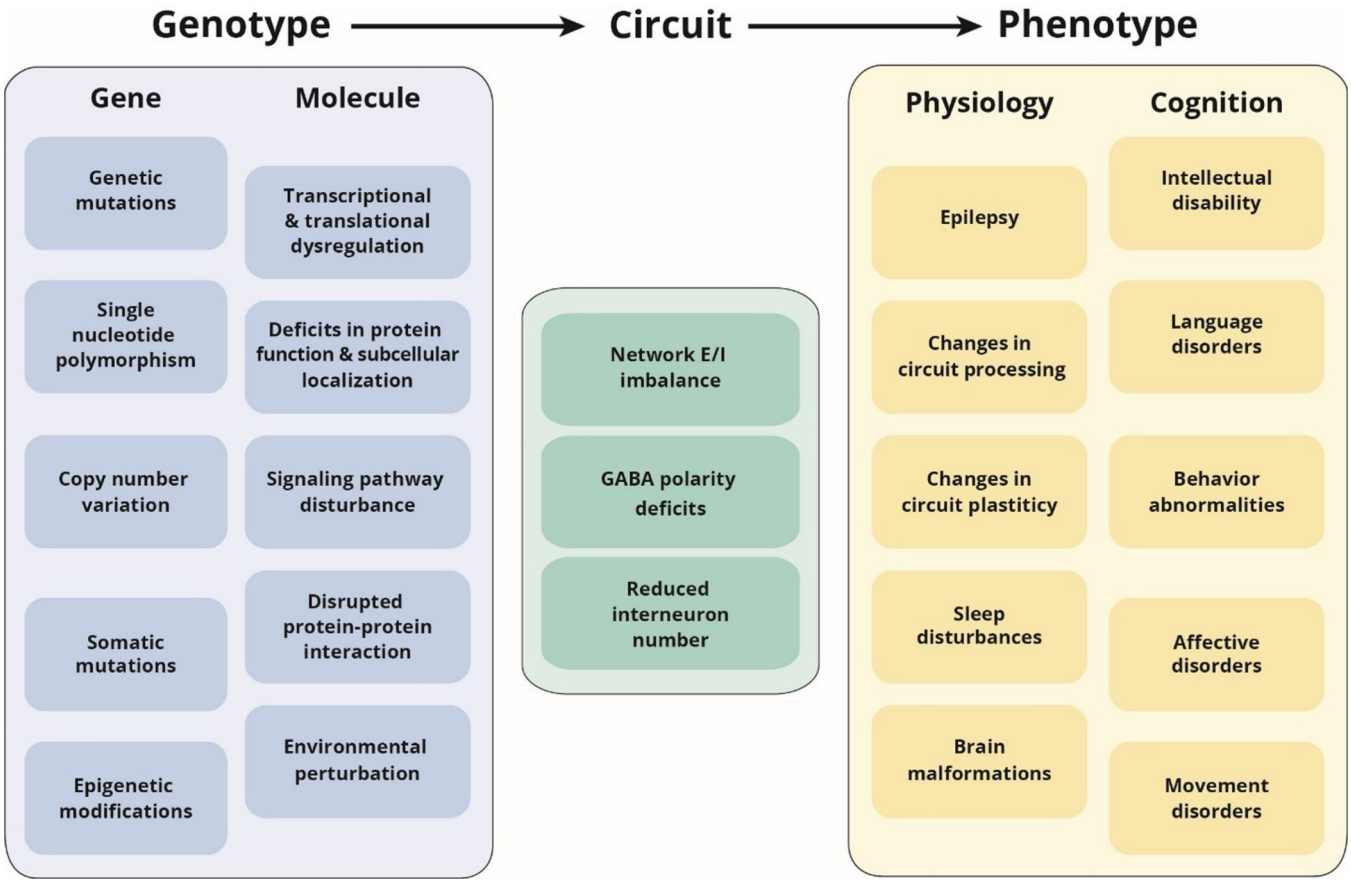

Fig. 2 |. Pathogenic mechanisms underlying neurodevelopmental disorders.

A large fraction of NDDs are caused by genetic mutations and epigenetic aberrances occurring at the whole-organism or somatic levels that cause deficits in the expression level, localization and interaction pattern of the RNA and protein molecules. Such a plethora of perturbations engage a number of GABA-related disease mechanisms at the circuit level, which lead to physiological and cognitive impairments present in different subtypes of NDD.

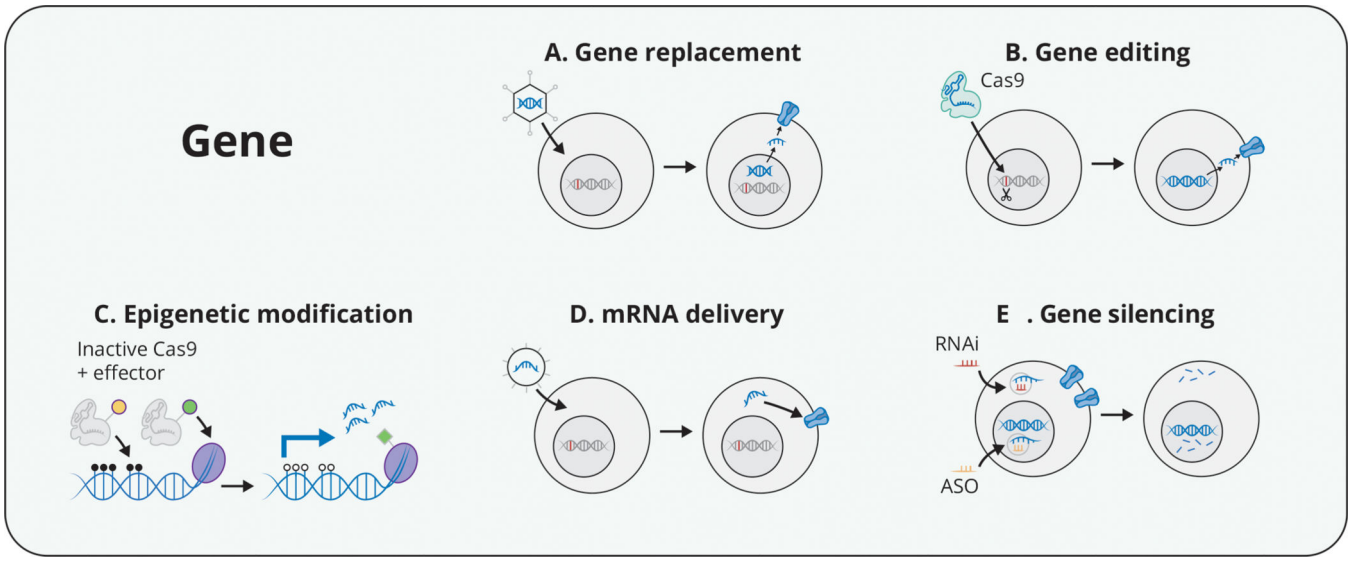

Fig. 3 |. Therapeutic opportunities at the genetic level for managing NDDs.

Gene replacement therapies utilize virus to introduce a functional transgene copy to restore mRNA and protein production (A), whereas gene editing therapies employ technologies such as CRISPR/Cas9 to correct mutant genes at their endogenous loci (B). Catalytically-inactive Cas9 can be repurposed to modify the epigenetic status of specific genes to activate or silence their expression without changing the primary DNA sequence (C). RNA-based therapies modulate gene expression levels in the target cells by delivering mRNA, which can be further translated into protein products (D); or by delivering RNA interference (RNAi) and antisense oligonucleotides (ASO) to knock down particular RNA transcripts (E).

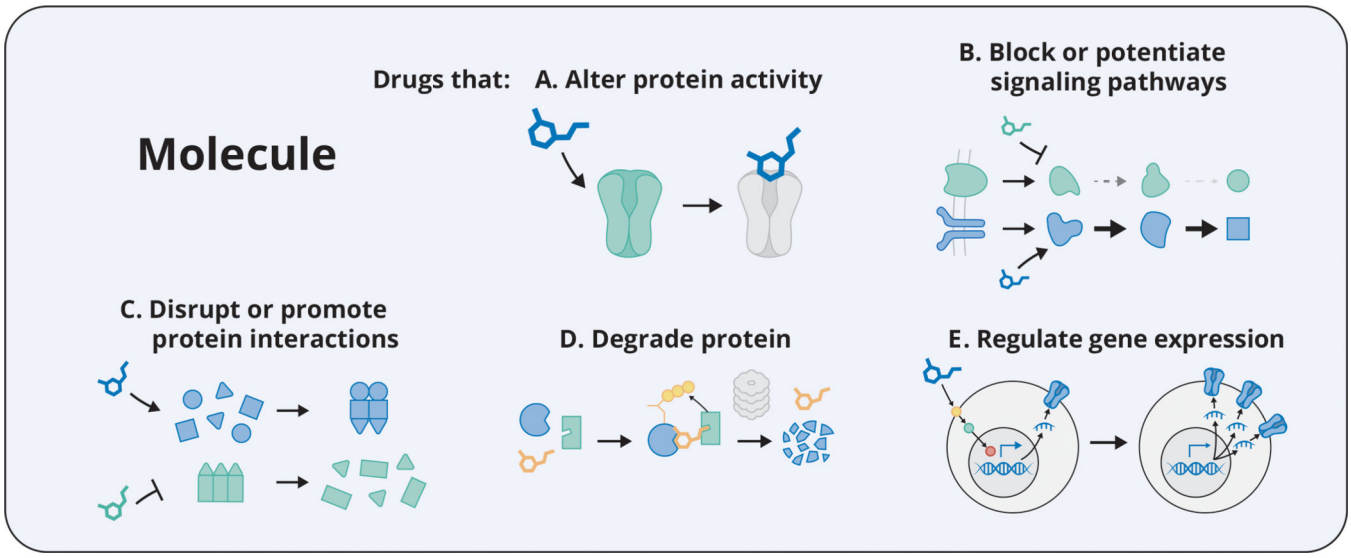

Fig. 4 |. Therapeutic opportunities at the molecular level for managing NDDs.

Various drugs have been developed to modulate the molecular processes in the cells through: binding with the target proteins to alter their biological activities (A), modulating the activities of molecular signalling pathways (B), altering the interactions between the target proteins and other proteins (C), facilitating the degradation of target proteins (D). Moreover, novel therapeutics have been developed to modulate the expression levels of the genes that encode the target proteins (E).

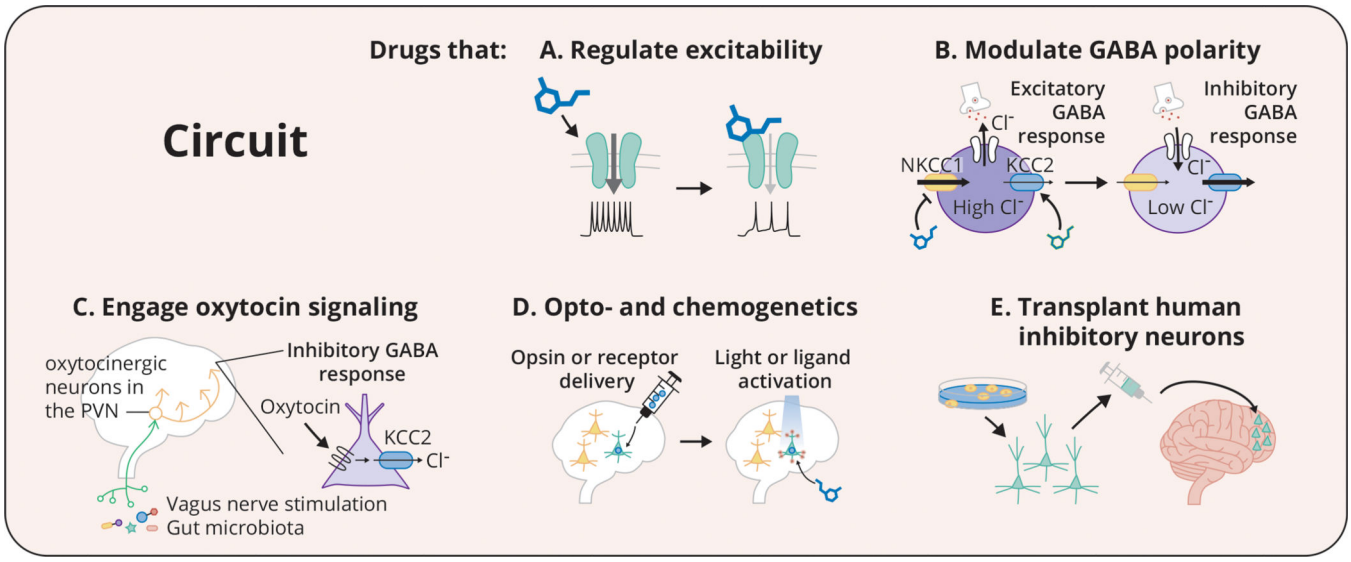

Fig. 5 |. Therapeutic opportunities at the circuit levels for managing NDDs.

At the circuit level, drugs have been developed to regulate the excitability of neurons through modulating the activity or expression of ion channels and receptors (A). Neuronal chloride transporters are master regulators of the polarity and efficacy of GABAergic signaling, therefore presents a good target for therapeutic development (B). Experimental therapeutic modalities are also under development to engage endogenous neuromodulatory mechanisms such as oxytocin (C), to directly modulate the activity of target neuronal population with optogenetics or chemogenetics (D), and to restore the density of inhibitory neurons through transplantation of *in vitro* differentiated GABAergic interneurons (E).

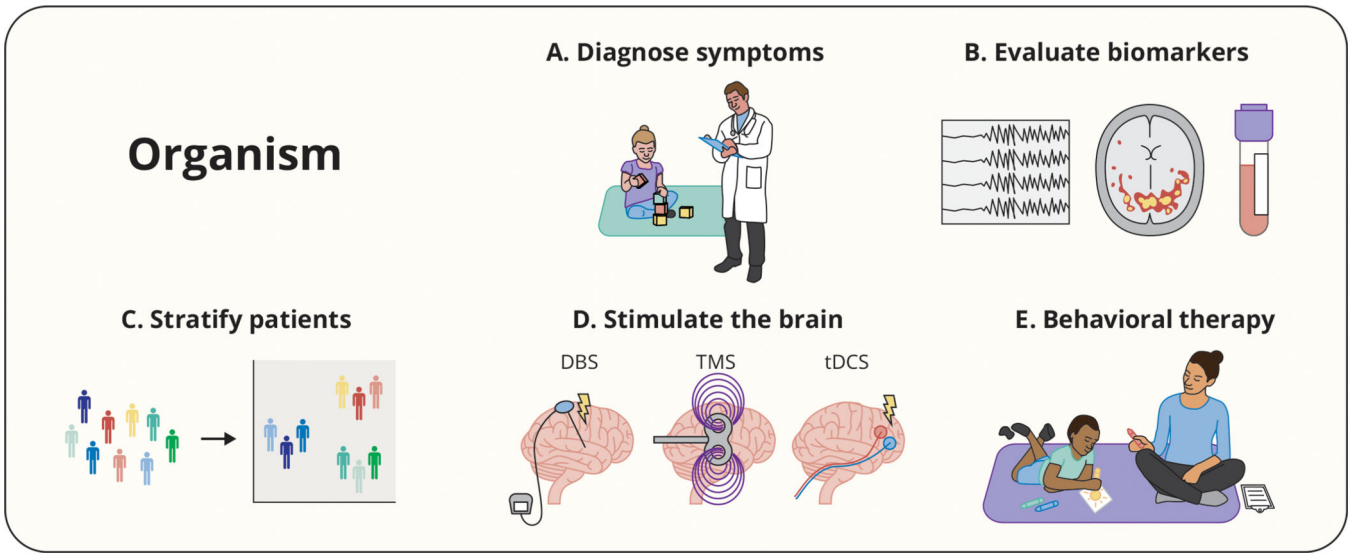

Fig. 6 |. Therapeutic and diagnostic opportunities at the individual level for NDDs.

Symptomatic diagnosis of disease-related phenotypes (A), combined with clinically relevant biomarkers such as EEG, fMRI, blood and CSF biomarkers (B), assist the diagnosis and stratification of patients to receive precision medicine treatment (C). Moreover, various brain stimulation methods including deep brain stimulation (DBS), transcranial direct current stimulation (tDCS), transcranial magnetic stimulation (TMS) (D) and behavior therapies (E) may also systematically ameliorate NDD symptoms.