Cortical interneuron dysfunction in epilepsy associated with autism spectrum disorders

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Summary

Autism and epilepsy are two associated disorders which are highly prevalent, share common developmental origins, and demonstrate substantial heritability. In this review, cross-disciplinary data in a rapidly evolving field that bridges neurology and psychiatry are synthesized to identify shared biological mechanisms. The relationship between these debilitating, lifelong conditions is examined at the clinical, genetic and neurophysiological levels in humans and in animal models. Scopus and Pubmed searches were used to identify relevant literature. Clinical observations have prompted speculation about the inter-dependence of autism and epilepsy, but causal relationships have proved difficult to determine. Despite their heritability the genetic basis of ASD and epilepsy has remained largely elusive until the advent of next generation sequencing. This approach has revealed that mutations that are either causal or confer an increased disease risk are found in numerous different genes any one of which accounts for only a small percentage of cases. Conversely, even cases with identical clinical phenotypes are genetically heterogeneous. Candidate gene identification has facilitated the development of mouse genetic models, which in parallel with human studies have implicated shared brain regions and circuits that mediate disease expression. Diverse genetic causes of ASD and epilepsy converge on cortical interneuron circuits as one important mediator of both disorders. Cortical interneurons are amongst the most diverse cell types in the brain and their unique chemical and electrical coupling exert a powerful inhibitory influence on excitatory neurons via the release of the neurotransmitter, gamma amino butyric acid (GABA). These multifaceted approaches have validated theories derived from the field of developmental neurobiology, which propose that the neurological and neuropsychiatric manifestations are due an altered ratio of excitation to inhibition in the cortex.

Clinical Overlap of ASD and epilepsy

Early descriptions of children diagnosed with autism recognised the increased risk of seizures in later life that was not associated with dysmorphic physical characteristics [1; 2]. Similar neuropsychiatric traits have been described in children who have clear physical abnormalities [3]. The latter 'syndromic' forms of autism have prompted a

broadening of the original diagnostic criteria and the adoption of an umbrella term, Autism Spectrum Disorder (ASD) (Table 1). Across multiple studies, all seizure types can be observed in ASD [4]. The incidence of epilepsy in ASD correlates broadly with age, resulting in two peaks of age distribution, one before five years of age, and the second in adolescence [4]. The prevalence of epilepsy in children who present with an ASD is approximately 30% [5], and this figure rises to 90% in syndromic disorders such as Rett syndrome, which is an X-linked disorder associated with severe intellectual deficit and ASD [6]. Co-morbid intellectual disability (ID) accounts for the bulk of this association but even in the absence of ID the risk of epilepsy in ASD is 5 – 8% which is eight times higher than in the general population [7]. Conversely, 4 – 5% of children with epilepsy also have an ASD [7]. In particular ASD features prominently in certain childhood epilepsy syndromes, in which seizures typically precede ASD behaviours. Based on EEG criteria a subset of childhood-onset electroclinical syndromes termed 'epileptic encephalopathies' (EE) underscore the link between ASD, ID and epilepsy (Table 2). Moreover, in ASD subjects a correlation between the severity of ID and the risk of epilepsy can be identified despite the heterogeneous study populations, and varying definitions of the comorbidities [8]. By using common definitions of ASD, ID and epilepsy in nearly six thousand cases a recent large study attempted to better define the relationship between epilepsy and ASD [5]. ASD was diagnosed using standardized diagnostic instruments or parent reports, ID was defined on the basis of an intelligence quotient (IQ) score less than 70, and a diagnosis of epilepsy was made on the basis of parent report. The conclusion was that ASD subjects with epilepsy had a greater risk of low IQ, poor language skills and developmental regression [5].

A fundamental question is whether seizures cause or contribute to the autistic symptomatology [9]. This has proved difficult to address because of the retrospective nature of most studies. One way that seizures could contribute to the behavioural changes seen in ASD might be through recurrent seizure induced excitotoxicity resulting in permanent damage to cortical neuronal networks that control behaviour. For example, the superior temporal sulcus is involved in facial recognition and social skills, which are impaired in ASD [10]. Conceivably, an epileptic focus in this region during a sensitive period in childhood brain maturation could cause the atypical activation of this region in functional magnetic resonance imaging (fMRI) studies in ASD subjects [11]. In tuberous sclerosis, which is a cause of West Syndrome (Table 2),

only temporal lobe tubers associated with epileptiform discharges within an early childhood sensitive period, conferred an increased ASD risk [12]. Furthermore, epileptic disruption of perisylvian language networks in some cases of benign rolandic epilepsy could contribute to the emergence of ASD [13].

Consistent with the notion that epileptic activity is a driver of ASD behaviours, in a small prospective series of patients undergoing treatment for infantile spasms, those who responded to vigabatrin showed improvement in cognitive scores and ASD-associated behavior [14]. Electrical Status Epilepticus in slow-wave Sleep (ESES) can present as the syndrome of continuous spike-wave in slow-wave sleep (CSWS) or as the Landau-Kleffner syndrome (LKS). There are anecdotal reports that treatment of ESES results in behavioural improvements although these appear to be temporary [15]. In Dravet's syndrome, seizures, which are often refractory to medical treatment begin in the first year of life and delayed development is observed usually from two years of age (Table 2). A correlation between cognitive and behavioural change and the frequency of convulsive seizures has been reported in a cohort of twenty prospectively studied cases of Dravet syndrome, suggesting that autistic behaviour could be linked to the excitotoxic effects of seizures [16]. However, the small sample sizes in these studies make it difficult to unequivocally determine whether seizure control improves behavioural outcomes.

An opposing viewpoint is that it is the underlying pathology rather than seizure severity per se that determines the emergence of ASD. In tuberous sclerosis, most children who have seizures in the first year of life will develop an ASD [17]. Furthermore, in a recent study of EE there was no correlation between the severity of ID and the refractoriness of seizures [18]. In a prospective trial of sixty-nine patients with infantile spasms early treatment of seizures did not lower the risk of ASD [19]. The clinical features of ESES also suggest that seizures might not be causally linked to ASD. Seizures are the dominant presenting feature in children with CSWS, whereas language regression, auditory agnosia and ASD behaviors are the hallmarks of LKS [20]. Altogether, a consensus has begun to emerge that ASD and epilepsy represent different manifestations of shared pathology, but that in certain cases the location of the epileptogenic zone and the developmental phase of seizures can adversely affect behavioural outcomes [9].

Altered balance of excitation and inhibition in the cortex in ASD

The attempt to find common mechanisms of epilepsy and ASD has focused on the function of cortical circuits following the proposal that in ASD, as in epilepsy the balance of cortical excitation to inhibition is tipped in favour of excitation [21]. The well-recognized sensory perceptual abnormalities in ASD are thought to reflect this change in cortical function. Altered sensory processing is manifested in multiple sensory domains including auditory, visual and tactile [22]. The investigation of sensory processing in ASD has largely been based on electrophysiologic parameters, termed event-related potentials (ERP) that are time-locked to an external event or stimulus. Individuals with syndromic and non-syndromic forms of ASD who report perceptual abnormalities have been found to display enhanced ERPs in some studies but not in others [22]. In Rett syndrome 'giant' somatosensory evoked potentials (SEPs) emerge at a time when seizures begin and resolve in older Rett syndrome cases when clinical seizure activity wanes [23]. Similarly, in Fragile X syndrome (FXS), increased amplitudes of cortical auditory and visual evoked potentials have been detected [24].

Enhanced cortical excitation has also been proposed to account for reduced fidelity of cortical sensory processing in ASD. In a functional magnetic resonance imaging (fMRI) study cortical ERPs across multiple sensory modalities showed greater trial-by-trial variability in ASD cases than controls [25]. There is also a deficit in cross-modality integration of sensory information in ASD, that is not adequately explained by defective unimodal sensory performance [26]. The insular cortex represents one region where sensory and autonomic inputs are integrated with emotional and cognitive experiences. Abnormal functional connectivity within the insula in ASD has been reported in multiple studies and has recently been explained on the basis of an increased excitation-to-inhibition ratio [27].

Abnormal patterns of electrical oscillations in the cortex of individuals with ASD also support the notion of an altered ratio of cortical excitation to inhibition. Cortical oscillations reflect rhythmic changes in cortical excitability and are generated by synchronized rhythmic patterns of neural activity in many neurons locally or across cortical regions. Consistent reductions of cortical oscillations at high (gamma) frequencies during sensory processing in multiple sensory domains have been found

in children with ASD [28]. In epilepsy, pathological oscillations at the upper end of the frequency spectrum, which overlap gamma frequency bands are well-described [29]. The correlation between behavior deficits in ASD and oscillatory states of the brain, and the detection of pathological oscillations in epilepsy has led to the proposal that gamma oscillations could serve as a potential biomarker of both disorders [28; 29]. This has stimulated the search for the neural substrates of gamma frequency oscillations, in particular.

Interneurons in ASD and epilepsy

Defective cortical interneuron function has long been established as a core mechanism of epileptogenesis [30]. Moreover, interneuron dysfunction principally in the cortex is an emerging theme from studies of brain circuitry that underpin ASD. These cells are remarkably diverse in their morphology, physiological properties and connectivity, but share in common their expression of the inhibitory neurotransmitter, gamma amino-butyric acid (GABA). Despite the diversity of interneuron subtypes cortical interneurons can be classified into just a handful of cardinal groups. One classification scheme is based on differential expression of the molecular markers parvalbumin (PV), somatostatin (SST), vasointestinal peptide (VIP), cholecystokinin, neuropeptide Y (NPY), nitric oxide synthase (NOS), reelin and calretinin [31]. At approximately 40% of the GABA cell population, PV neurons represent the largest class of cortical inhibitory interneurons. PV neurons are fast-spiking cells that depend on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor mediated excitatory post-synaptic potentials (EPSPs) for their fast kinetics [32]. They are chemically and electrically coupled by GABAergic synapses and gap junctions, respectively [33]. This type of interconnectivity permits powerful distributed inhibition of excitatory glutamatergic neurons through synchronized PV neuronal activity.

A substantial body of evidence implicates PV interneurons in the generation of gamma oscillations [34]. Rhythmic action potential spike discharges by PV interneurons occurred more consistently than firing by other cell types during gamma oscillation cycles. Conditional ablation of AMPA receptors in PV interneurons of transgenic mice reduced the power of gamma oscillations as did genetic blockade of gap junction integrity in these cells. Furthermore, optogenetic control of the activity of specific cortical PV interneurons with light pulses elicited LFP oscillations in vivo at gamma

frequencies. Conversely, optogenetic inhibition of PV interneuron firing attenuated gamma oscillations. Finally, modulation of excitation-inhibition balance by optogenetic control of PV inhibitory tone reversed ASD-associated deficits in a freely moving mouse model expressing an inducible depolarizing opsin in PV interneurons or pyramidal cells [35]. Through their contribution to the generation of gamma frequency and other oscillations, PV and other cortical interneurons have been proposed to transform the input-output activity of pyramidal neurons, permitting computational tasks which implement behavioral functions [36].

Shared genetic risks for epilepsy and ASD

The participation of cortical interneurons in cortical circuitry depends on cell type specific transcriptional programs in these neurons and in other interconnected cortical subtypes. Many of the genes expressed in the cortex have been found to harbor mutations in human genetic studies of ASD in particular, which were prompted by twin studies showing substantial heritability of ASD in the order of 70% [37]. Three main classes of mutations have been described in large cohorts of ASD cases that also confer an increased seizure risk in many instances. De novo copy number variants (CNV) are thought to explain 6-8% of ASDs and include highly penetrant chromosomal abnormalities, for example the 16p11.2 deletion-duplication [38; 39]. A further 10-12% contribution to the risk of ASD is derived from de novo single nucleotide variants (SNV) [40-45]. Finally, rare autosomal and X-linked recessive mutations account for a further 5% of cases [46; 47]. Overall, several hundred genes with diverse functions appear to be implicated.

By studying the pathophysiological consequences of these genetic mutations in animal models deleterious effects on PV interneuron function in particular have been revealed. Of these major advances two important disease classes are discussed here because of the insight they provide into the pathophysiology of ASD-epilepsy comorbidity. First, the syndromic forms of ASD and epilepsy, which are monogenic in their inheritance, but whose phenotype is typically distinct from the ‘classic’ forms of ASD identified in twin studies. Second, EE in which de novo mutations make a large contribution to disease causation (Table 3). The genes are referred to by their abbreviated names below and full gene names are provided in Table 3.

The genetic basis of three important syndromic forms of ASD which are associated with epilepsy have been elucidated. Fragile X syndrome is caused by an expansion of a CGG repeat in the 5' untranslated portion of *FMR1* which results in its hypermethylation and transcriptional silencing [48]. Absence of FMRP deregulates the expression of its target mRNAs. At the genetic level *FMR1* appears to be implicated more broadly in ASD. Three independent studies have found that ASD-linked genes are enriched for FMRP targets [49]. Mutations in genes that encode two interacting proteins, TSC1 and TSC2, which regulate the mammalian target of rapamycin (mTOR) signaling pathway were identified as the cause of tuberous sclerosis, an important cause of West syndrome [50]. Rett syndrome has been linked to mutations in the X-linked gene, *MECP2*, which encodes a protein involved in regulating gene expression through histone modification [51].

Recently, the genetic origins of less common syndromic forms of ASD and epilepsy have been revealed. A recessive mutation in *CNTNAP2* was shown to cause cortical dysplasia-focal epilepsy syndrome (CDFE) [52]. *CNTNAP2* encodes CASPR2, which clusters voltage-gated potassium channels ($K_v1.1$) at the nodes of Ranvier [53]. CDFE was originally characterized in a restricted population of Old Order Amish children, who displayed simple partial or complex partial seizure semiology associated with fronto-temporal epileptiform EEG abnormalities [52]. However, copy number variants involving *CNTNAP2* have also been detected in LKS and CSWS providing an example of phenotypic heterogeneity in a monogenic disorder [54]. The latter observation is reinforced by the finding that a second gene, *SHANK3* a well characterised ASD gene that encodes a synaptic scaffolding protein is also linked to LKS/CSWS [55]. *SHANK3* maps to the critical region of 22q13.3 that is deleted in Phelan-McDermid syndrome, a disorder associated with epilepsy, ID, hypotonia, autistic behavior and language impairment (Table 3). Seizures, which are not correlated with deletion size, typically develop around puberty and include absence, generalized tonic-clonic, myoclonic and focal subtypes [56]. Interestingly, *SHANK3* duplication is also associated with ASD and epilepsy, indicating that the correct gene dosage is required for normal neuropsychiatric function [57].

Next-generation sequencing has greatly facilitated the genotyping of large patient cohorts with neurodevelopmental phenotypes encompassing ASD and EE. De novo

mutations have been identified in genes encoding receptors, ion channels, cytoskeletal proteins and transcription regulators but recurrent disruptive mutations occur at greater frequency in a restricted set of genes. Across multiple studies recurrent de novo mutations are observed in several genes including *SCN1A, GABRB3* and *DNM1* that are predicted to compromise the function of one of the parental copies of the gene. (Table 3) [45; 58; 59]. In the majority of instances the remaining normal gene product is not produced in sufficient quantities resulting in a disease state (haploinsufficiency). Heterozygous loss-of-function mutations in *SCN1A*, which encodes the pore-forming α subunit of the sodium channel $Na_v1.1$ are responsible for causing the majority of cases of Dravet's syndrome [60], but other studies also implicate mutations in the same gene in other EE [18], in non-EE epilepsy syndromes and in ASD without EE [58] (Table 3). GABA receptors are subdivided into ionotropic $GABA_A$, metabotropic $GABA_B$ and $GABA_C$ subtypes. *GABRB3* encodes the β3 subunit of the $GABA_A$ receptor, which is also known to contribute to the epilepsy risk in Angelman syndrome, a disorder that has substantial phenotypic overlap with ASD [61]. *DNM1* encodes a GTPase that is required for neurotransmitter release through its role in regulating endocytic vesicle formation [62]. The function of other genes in this category is poorly understood (Table 3).

Together, these large scale genetic sequencing studies reveal the importance of gene dosage for normal brain function and the heterogeneity of genetic causes underlying homogeneous clinical presentations. A single genetic locus can be associated with a variety of clinical manifestations, encompassing diverse electrographic abnormalities and seizure semiologies. Conversely, even for well-characterized syndromes such as EE there is striking heterogeneity of genetic loci [63]. Moreover, genes implicated in EE also appear to be linked to non-EE subtypes of epilepsy (Table 3). Nevertheless, the diverse array of genes though seemingly functionally distinct from one another are interconnected in common cellular processes. In silico analyses have attempted to establish the 'relatedness' of different gene loci through network modeling approaches using empirical data from high throughput gene co-expression and protein-protein interaction assays [49; 64]. These approaches attempt to link genes into functionally-related clusters without consideration of the specific cell type or time points of analysis. Broadly, ASD genes/proteins map to one of four clusters enriched for components of the Wnt signaling pathway, FMRP regulated genes, synaptic

function, and chromatin modifiers, which overall substantially overlap the EE gene network [49; 63]. How these diverse set of genes can nevertheless give rise to specific cellular perturbations that lead to ASD behaviours and epilepsy is revealed by empirical studies.

Interneuron dysfunction in animal models of ASD and epilepsy

Mouse genetic models that target orthologues of genes implicated in monogenic forms of ASD in humans, such as *MECP2* and *FMRP* have been essential in identifying the cell types and circuits that are disrupted in ASD and epilepsy. In these studies and in other mouse models of genes more recently identified by large-scale sequencing efforts a deficit of PV interneurons, principally, and/or GABAergic inhibitory signaling function is striking (Table 3). Furthermore, statistical modeling of genome-wide quantitative assays of gene expression in the cortex of genetically heterogeneous ASD cases has led to the identification of a subset of genes enriched in PV interneurons [64]. Conversely, many ASD candidate genes appear to be regulated by SATB1, a transcription factor required for PV and SST interneuron development, based on the presence of SATB1 binding sequences within these genes [65]. Table 3 lists twenty genes, of which nearly 60% result in interneuron dysfunction when mutated.

The Fragile X protein, FMRP regulates the expression of the post-synaptic $GABA_A$ receptor subunit δ mRNA [66]. In *Fmr1* knockout mice the eight GABA receptor subunits are downregulated in the cortex, there is a 20% reduction in somatosensory cortical interneurons that affects the PV subtype, and impaired function of somatostatin-expressing interneurons [67; 68]. Whether these deficits are related to hypermorphic function of metabotropic glutamate receptors (mGluRs) which are believed to mediate many of the features of FXS, and are highly expressed in interneurons, is unclear. In a Drosophila model of Fragile X syndrome rescue of the mutant phenotype by nine compounds included three that act in the GABAergic pathway [69]. Furthermore, in the *Fmr1* knockout mouse, ASD-associated behavior deficits and audiogenic seizures responded to treatment with $GABA_A$ and $GABA_B$ agonists [70; 71]. In humans with FXS, the $GABA_B$ agonist, STX209 (arbaclofen) has shown promise in ameliorating social deficits in a phase II placebo-controlled, double blind, cross-over trial, which has led to the planning of a larger phase III study [72].

Whether arbaclofen had anti-epileptogenic effects in these subjects has not been reported.

Cntnap2 is expressed in the embryonic ganglionic eminences and its expression perdures in migrating interneurons [73]. In a mouse model of CDFE, homozygous knockout of *Cntnap2* resulted in core ASD behavioural deficits and epilepsy, mimicking the autosomal recessive mode of inheritance in humans with this disorder [73]. The brains of these mice showed a deficit of PV neurons in particular, a finding that has not been reported to date in humans with CDFE. The findings in this animal model suggest that further examination of these interneuron populations in postmortem brains is worthwhile.

Deletion of *SHANK3*, which like *CNTNAP2* has also been implicated in LKS, and CSWS [54], in mice results in ASD-associated behaviours without seizures [74] and a PV neuronal deficit [27]. By contrast, a mouse model of *SHANK3* duplication syndrome displays both endophenotypes, and at the cellular level reduced $GABA_A$-mediated miniature post-synaptic inhibitory current (mIPSC) frequency are evident [57]. Mice mutant for *Mecp2* reproduce many features of the Rett mutant phenotype [75]. GABAergic neurons express MeCP2 at significantly higher levels than in non-GABAergic neurons [76]. Strikingly, selective deletion of *Mecp2* in GABAergic neurons recapitulated the phenotype of Rett syndrome, including epileptiform EEG changes [76]. Furthermore, MECP2 is an epigenetic regulator of many neuronal genes including *GABRB3*, and reduced expression of this MECP2 target in Rett syndrome could contribute to the lowered seizure threshold [77]. Consistent with deficits in human ASD subjects, both *Mecp2* and *Shank3* mutants also display abnormal multisensory integration in the insular cortex for auditory and tactile stimuli, which has been shown to depend on GABA inhibition [27].

Haploinsufficiency of the murine orthologue of the Dravet's syndrome gene, *Scn1a* results in spontaneous seizures and reproduces many of the neuropsychiatric features including stereotyped behaviours and social interaction deficits [78; 79]. The sodium channel $Na_v1.1$ is expressed in PV neurons, which become less excitable when the α subunit is mutated leading to the loss of inhibitory drive [78; 80]. Rescue of the neuropsychiatric deficits by low doses of a benzodiazepine reinforces the view that

the PV neuronal network has a pivotal role in disorders associated with ASD and epilepsy [79]. Differential effects on other subtypes of cortical interneurons were observed when Tsc1 was selectively knocked out just in mouse cortical interneurons [81]. The resulting increase in mTOR signaling in this cell type led to reduced numbers of NPY and calretinin expressing cortical interneurons, increased interneuron size and migration defects.

The 'fitful' mouse mutant exhibits recurrent limbic and generalized tonic-clonic seizures upon handling, by three months of age that is caused by a heterozygous mutation in the GTPase, Dnm1 [82]. Homozygotes have a more severe neurological phenotype including lethal seizures. By contrast, *Dnm1* heterozygotes harboring a targeted null mutation do not suffer from epilepsy [83]. *Dnm1* is alternatively spliced resulting in the generation of *Dnm1a* and *Dnm1b* isoforms. The fitful mutation results in the predominant generation of the *Dnm1b* transcript, which has been proposed to impair neurotransmitter release by interfering with endocytosis in a dominant negative manner. The mutation particularly affects the function of GABA-ergic interneurons because normal inhibitory function is dependent on a high level of tonic activation of these cells, which is not sustainable when the rate of vesicle recycling is slowed [84].

A preferential deficit of GABA-ergic interneurons has also been observed in experimental models of epilepsy [30]. Moreover, GABA was shown to prevent seizures, and drugs that interfere with GABA-ergic function are epileptogenic. In humans with temporal lobe epilepsy (TLE) and in experimental models of TLE, GABA-ergic neuronal cell loss is present amongst other neuronal deficits [30]. These findings have led to the proposal that epilepsy is a consequence of deficient GABA signaling. The mechanism by which deficient GABA signaling promotes seizures has been explored in rodent hippocampal slices bathed in Mg^{2+}-free medium containing the potassium channel blocker, 4-aminopyridine (4AP) [30]. The initial synchronous activation of interneurons that typically precedes ictal discharges in focal epilepsy results in a depolarization block, associated with silencing of their activity and the generation of sustained excitatory glutamatergic cell discharges. In contrast to these findings, other experimental models have emphasized the active involvement of GABA in seizure pathogenesis. In support of this view, abolition of GABA activity fails to result in ictal discharges, and instead induces activity resembling inter-ictal spikes and

afterdischarges. However, reduction or enhancement of GABAergic transmission can produce seizures, which have been proposed to occur through a $GABA_A$ mediated prolonged depolarization event in the post-synaptic membrane [30]. Furthermore, an enhancement of cortical GABA-ergic transmission was found in a mouse genetic model of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), in which a mutation was introduced into the alpha-4 subunit of the nicotinic acetylcholine receptor [85]. These studies on cortical interneuron dysfunction in epileptogenesis suggest possible mechanisms through which pervasive reduction in inhibitory tone in ASD could trigger epilepsy.

In epilepsy associated with focal cortical dysplasia (FCD) and TLE altered expression of the potassium channel, KCC2 and the ion channel cotransporter, NKCC1 have been proposed to switch GABA from inducing post-synaptic hyperpolarization to triggering depolarization instead [86; 87]. A failure to convert GABA action from excitatory to inhibitory during embryonic development has also been proposed to be a key event in ASD that can be induced by genetic and environmental factors. As in epilepsy, the ratio of NKCC1 to KCC2 expression promotes augmentation of glutamatergic activity by GABA in ASD animal models [88]. The inhibitory action of GABA can be restored in these models by the diuretic agent, bumetanide which is a highly specific blocker of NKCC1. In human ASD subjects, bumetanide improves fMRI-based activation of brain regions that typically show a deficient response in behavioural paradigms in ASD. Moreover, clinical improvements in ASD behaviours result from treatment with bumetanide [88]. These findings, and the observation that dysplastic patches of cortical tissue reminiscent of focal cortical dysplasia in epileptic brain tissue are highly prevalent in ASD [89], suggest convergent neuroanatomical deficits and pathophysiology in ASD and epilepsy.

The contribution of PV interneurons in particular to cortical network oscillations known to be disrupted in ASD suggests that the observed deficits in PV interneuronal function in multiple animal models of ASD and epilepsy syndromes are clinically relevant. These models provide evidence for both haploinsufficiency and dominant negative modes of action. Importantly, the extent to which the observed deficits in PV and other interneuron subtypes account for autistic behaviours and seizures has been rigorously investigated in only certain diseases, in particular Rett syndrome.

Furthermore, animal models of epilepsy and ASD raise the possibility of more complex modes of interneuron dysfunction in seizure-associated forms of ASD, which it will be important to address in future studies. Nevertheless, the encouraging response in animal models to enhancement of GABA signaling implies broader relevance of an inhibitory deficit to the symptomatic manifestations of neurodevelopmental diseases. Furthermore, the reversibility of ASD-like behaviours by GABA agonists in these models implies that the cognitive and behavioural deficits are not contingent on recurrent seizure induced excitotoxicity. Precisely how these genetic mutations alter connectivity within brain circuit motifs and the knock on effects on computational functions, and ultimately behavior, remains unclear [36]. However, the increasing sophistication of optogenetic technology makes the investigation of these key questions experimentally tractable in ASD animal models. The relative contributions of other cell types should also become clearer as new animal models target expression of mutant alleles to specific cell populations.

Conclusions and Future Directions

The pathophysiological basis for the association between ASD and epilepsy is beginning to emerge by combining detailed clinical evaluation with imaging, neurophysiological and genetic technologies. The surprising outcome of genotyping studies is the great diversity of genetic loci which appear to produce similar disease phenotypes. Conversely, resequencing approaches are revealing the phenotypic diversity associated with individual gene loci. Despite the lack of a 'one-to-one' relationship between genotype and phenotype, genes with diverse functions appear to cluster in networks that subserve common cellular processes. Interneuron function represents one important point of convergence of genetic and other neurological investigations. These biological insights raise new questions that will be important to address if therapeutic advances are to be made. Is involvement of the immune system, microglia and astrocytes in ASD, also suggested by genetic studies, mediated by interneurons [64; 90]? How can the variability of the endophenotypes associated with specific genetic defects be explained in neurobiological terms, in particular what is the contribution of cortical interneuron dysfunction to phenotypic variability? What differences in interneuron function and cortical circuit assembly account for situations where ASD and epilepsy are comorbid compared to when only behavior is perturbed? The link with greater IQ deficit in the former context and the known association

between lower IQ and the severity of the de novo mutation [91] suggests that when epilepsy and ASD are co-morbid, cortical circuit disruption is more profound. Furthermore, does treatment of epilepsy or epileptiform EEGs per se in ASD improve behavior and cognition? Much larger studies are needed to address these questions.

From a treatment perspective, the convergence of diverse causes onto a restricted set of neuronal functions makes the identification of therapeutic targets more tractable. Animal models offer hope that damage to neural circuits caused by prolonged behavior changes and seizures is not irreversible [79; 92]. However, the complex spectrum of behaviours and physical symptoms encapsulated by ASD and its comorbidities requires the development of new tools and biomarkers that can be used to dissect neuronal function in humans with these disorders. Further characterization of cortical physiology in genotyped human cases should clarify whether therapies designed to restore interneuron function have the potential to reverse or alleviate autistic behavior and seizure related morbidity.

Key Points

- Epilepsy is observed in up to 30% of ASD subjects and epileptic encephalopathies are associated with ASD.
- ASD and epileptic encephalopathies are genetically highly heterogeneous and have shared genetic causes.
- In ASD and epilepsy the balance of excitation to inhibition (E/I) in the cortex is tipped in favour of excitation.
- Diverse genetic disorders are mediated by cortical interneuron dysfunction resulting in an increased E/I ratio in ASD associated with epilepsy.

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Conflicts of interest

None declared.

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Table 1. Summary of DSM-V classification of Autism Spectrum Disorder

Domain	Criterion 1	Criterion 2	Criterion 3	Criterion 4	Additional criteria	Number of criteria required for diagnosis
Social Interaction and Social Communication	Deficits in social-emotional reciprocity	Deficits in non-verbal communicative behaviour used for social interaction	Deficits in developing and maintaining relationships to a developmentally appropriate level	-	-	All three
Restricted Interests and Repetitive Behaviours	Stereotyped or repetitive speech, motor movements or use of objects	Excessive adherence to routines, ritualized patterns of verbal or non-verbal behavior, or excessive resistance to change	Highly restricted fixated interests that are abnormal in intensity or focus	Hyperreactivity or hyporeactivity to sensory input or unusual interest in sensory aspects of environment	-	At least two
					Symptoms must be present in early childhood	Required
					Symptoms are disabling	Required

Table 2 – The epileptic encephalopathies.
Epileptic encephalopathies (EE) are a group of disorders characterized by infantile or childhood onset of refractory seizures, cognitive involvement and ASD. Developmental regression is well-recognised. EEs often display an electroencephalographic (EEG) signature and most types have a poor prognosis. The association with ASD is well-recognised, but high quality quantitative data on the association of ASD with EE is sparse.
Abbreviations: CSWS, continuous spike-waves during slow wave sleep

Disease	Age at Presentation	Semiology	EEG	ASD/ASD behavior	Prognosis
Ohtahara Syndrome/ Early Infantile Epileptic Encephalopathy	3 months	Tonic spasms Focal seizures Myoclonic seizures	Burst suppression		Death often in infancy
West Syndrome	3 – 7 months	Infantile spasms which can evolve to Lennox-Gastaut Syndrome	Hypsarrhythmia	9 – 35% [19]	Severe disability or death
Lennox-Gastaut Syndrome	3 – 5 years	Multiple seizure types	Paroxysmal fast activity and generalized slow spike-and-wave discharges	Present	Severe disability or death
Dravet's Syndrome/Severe Myoclonic Epilepsy in Infancy	< 12 months	Prolonged febrile convulsions; myoclonus; atypical absences; complex focal seizures	Generalised/ multifocal polyspike and slow wave	24% (most cases have some ASD features) [93]	Severe disability or death
Early myoclonic encephalopathy	neonatal	Myoclonus with or without focal seizures	Burst suppression	Common [94]	Severe disability or death
Malignant Migrating Partial Seizures of Infancy	< 6 months	Focal seizures	Multifocal epileptiform discharge		Severe disability or death
Epilepsy with Continuous Spike and Waves during Slow Wave Sleep	1 – 10 years	Unilateral clonic or tonic-clonic; absence seizures	CSWS	Present	Seizure remission, improvement of cognitive and behavioural deficits
Landau-Kleffner Syndrome	3 – 7 years	Unilateral clonic or tonc-clonic; absence; fluctuating verbal auditory agnosia;	Posterior temporal sharp and slow waves, facilitated by slow sleep; CSWS	Common	Seizure remission and language improvement by mid-teens in majority

Table 3
Genetic factors in syndromic forms of epilepsy and ASD and in epilepti
encephalopathy.
Syndromic associations are recognised for multiple genes, but reseque
studies have revealed that genes classically associated with a single
syndrome are also associated with other neurodevelopmental phenoty
(see text for details). Genes marked by an asterisk (*) are associated v
epileptic encephalopathy. Only the most commonly associated genes a
listed. 'X' indicates interneuron involvement and/or involvement of othe
cortical cell types. For *MBD5*, *CHD2* and *ALG13* the 'Interneuron involv
and 'Other cell type/process' boxes are unfilled due to a lack of data fr
animal models on the cells/circuits affected.

Abbreviations:
EE, Epileptic Encephalopathy
E/I ratio, Excitation-to-Inhibition ratio
BECTS, Benign Epilepsy with Centro-Temporal Spikes
BFNIS, Benign Familial Neonatal Infantile Seizures
CAE, Childhood Absence Epilepsy
CDFE, Cortical Dysplasia Focal Epilepsy syndrome
FXS, Fragile X Syndrome
GEFS+, Generalised Epilepsy with Febrile Seizures plus
JME, Juvenile Myoclonic Epilepsy
LGS, Lennox-Gastaut syndrome
MAE, Myoclonic Atonic Epilepsy
MPSI, Malignant Migrating Partial Seizures of Infancy
OS, Ohtahara Syndrome (EIEE, Early Infantile Epileptic Encephalopatl
Suppression-Burst); OS and EIEE are synonymous.
PEFS+, Partial Epilepsy and Febrile Seizures plus
TS, Tuberous Sclerosis
WS, West Syndrome

Gene	Location	Electroclinical syndrome	Known syndromic association	Molecular function	Interneuron involvement	Other cell type/process	Effect on E/I ratio	Reference for animal model
FMR1 Fragile X Mental Retardation Protein 1	Xq27.3		FXS	Translational repression; GABRB3 expression; mGluR	X	X	⇧	Neurosci Lett, 412(3), 227-232, 2007; Cold Spring Harb Perspect Biol. 4(3),a009886
MECP2 Methyl-CpG-Binding Protein 2	Xq28		Rett	Histone modification	X		⇧	Nature, 468(7321), 263-269, 2010
TSC1/2* Tuberous Sclerosis 1 (hamartin) and 2 (tuberin)	9q34.13 (*TSC1*) 16p13.3 (*TSC2*)	WS	TS	mTOR Complex 1 hyperactivity	X	X	⇧	Cereb Cortex 22, 2111-2119, 2012 Neuron, 78(3), 510-522, 2013
SHANK3	22q13.3	LKS, CSWS	Phelan-McDermid syndrome	Synaptic scaffold protein	X	X		Neuron, 83(4), 894-905, 2014; Nature, 503(7474), 72-77, 2013 Neuron, 78(1), 8-27, 2013
SCN1A* Sodium Channel Neuronal Type 1 Alpha Subunit	2q24.3	Dravet's, MPSI, Other EE, GEFS+, PEFS+		Sodium channelopathy	X		⇧	Nat Neurosci, 9(9), 1142-1149, 2006; Nature, 489, 385-390, 2012
KCNQ2/3*	20q13.33	BFNIS		Potassium	X			J Neurosci

Subfamily Member 2/3								
GABRB3* Gamma-Aminobutyric Acid Receptor Beta-3	15q12	CAE		GABA receptor	X		⇧	J Neurosci 18(20), 8505-8514, 1998
CNTNAP2 Contactin-Associated Protein-Like 2 (Caspr2)	7q35-q36		CDFE	Clustering of K^+ channels	X		⇧	Cell, 147, 235-246, 2011
SYNGAP1* Synaptic Ras-GTPase-Activating Protein 1	6p21.32			Synaptic function	X	X	⇧	Eur J Neurosci **31**(3), 529-543, 2010 ; Neuron 82, 1317–1333, 2014
CHRNA7 Cholinergic Receptor Neuronal Nicotinic Alpha Polypeptide 7	15q13.3	CAE, JME, BECTS	15q13.3 microdeletion syndrome	Acetylcholine receptor family member	X		⇧	Mol Cell Neurosci 61, 163-175, 2014; Neuroscience 207, 274-282, 2012
DNM1* Dynamin 1	9q34	WS, LGS		Synaptic function	X		⇧	PLOS Genetics, 6(8), 1-14, 2010
CDKL5* Cyclin-Dependent Kinase-Like 5	Xp22.13		Rett-like syndrome	Interaction with MECP2; Dendrite growth		X		PLOS One, 9(5), e91613, 2014
SCN2A* Sodium Channel Voltage-Gated	2q24.3	BFNIS, GEFS+		Sodium channelopathy		X	⇧	Epilepsia, 53(11), 1849–1859, 2012

Type 2 Alpha Subunit							
SCN8A	12q13.13	WS, LGS		Sodium channelopathy	X	⇧	Front Genet 4, Article 213, 2013; Hum Mol Gen 24(2), 506-515, 2014
STXBP1* Syntaxin-Binding Protein 1	9q34.11	OS (EIEE) Dravet's		Neurotransmitter release	X		Science 287(5454), 864-869, 2000
TRIO* Triple Functional Domain	5p15.2			Synaptic function; Axon guidance	X		Proc Natl Acad Sci U S A, **97**(22), 12074-8, 2000; The Neuroscientist 19(3), 255–273, 2013
ADNP Activity-dependent Homeobox	20q13.13			Transcription factor; chromatin modifier	X		Journal Pharmacol Exp Therap, 323(2), 438-449, 2007
MBD5* Methyl-CpG-Binding Domain Protein 5	2q23.1		2q23.1 microdeletion syndrome	Chromatin remodelling			EMBO Molecular Medicine 6, 1003-1015, 2014
CHD2* Chromodomain Helicase DNA-Binding Protein 2	15q26.1	MAE, LGS		Chromatin remodeling			J Cell Physiol, 209, 162-171, 2006; Am J Hum Genet, 93(5), 967-975, 2013

ALG13* ***Asparagine-Linked Glycosylation 13***	Xq23	WS		Glycosylation pathway	-