Trends in Neurosciences

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REVIEWS

Review

Ion Channel Functions in Early Brain Development

Richard S. Smith[1,*] and Christopher A. Walsh[1,*]

During prenatal brain development, ion channels are ubiquitous across several cell types, including progenitor cells and migrating neurons but their function has not been clear. In the past, ion channel dysfunction has been primarily studied in the context of postnatal, differentiated neurons that fire action potentials – notably ion channels mutated in the epilepsies – yet data now support a surprising role in prenatal human brain disorders as well. Modern gene discovery approaches have identified defective ion channels in individuals with cerebral cortex malformations, which reflect abnormalities in early-to-middle stages of embryonic development (prior to ubiquitous action potentials). These human genetics studies and recent *in utero* animal modeling work suggest that precise control of ionic flux (calcium, sodium, and potassium) contributes to *in utero* developmental processes such as neural proliferation, migration, and differentiation.

Highlights

Developmental channelopathy is an ion channel disease with a pathophysiological origin during the gestational period.

Human genetics studies reveal that individuals with dysfunctional sodium and glutamate channels can have improperly folded cerebral cortices.

The gestational brain is comprised of diverse and transient cell types, including dividing and newly differentiated cells. Some of the mechanisms involved in developmental channelopathies during gestation are different from those acting postnatally during ion channel diseases.

Ion channel mutations that result in greater excitability (gain-of-function) are often associated with human cortical malformations.

Electrical Activity in the Developing Brain

In the instant following fertilization, human development begins with a Ca^{2+}-mediated depolarization, highlighting the fact that electrical activity guides the earliest blueprint of the human body [1]. During the initial stages of lamination in the developing mammalian cerebral cortex, long before stable synapse formation and abundant action potentials, slow depolarizing Ca^{2+} transients are observed ubiquitously in newborn neurons and progenitors [2,3]. Yet, little is known regarding how these prenatal neurophysiological properties contribute to cortical developmental processes across species, including human-related neurodevelopmental diseases. While cellular excitability has been extensively studied for its guiding role in shaping postnatal sensory neural circuits and synapses [4,5], data now also support an early instructional role for precise ion channel function in prenatal mouse, ferret, and human cerebral cortex development.

Recent studies have identified ion channel dysfunction associated with defects of brain development in humans, including voltage-gated sodium channel (Na_V) and glutamate receptor families that, when mutated, alter cellular excitability [6–8]. Connecting these ion channel disease pathologies to developmental excitability, recent animals studies have implicated aberrant membrane potentials in regulating early cortical processes such as cellular proliferation and neural cell fate determination in mice [9,10], as well as cell migration in the gyrencephalic ferret brain [6]. Within prenatal populations of transiently dividing cells and differentiating neurons, these aforementioned cellular process are critical to gyri and sulci formation in the human brain (<24 weeks' gestation), using several ion channel types and electrical coupling to generate slower cellular depolarization kinetics, without abundant action potentials [6,11–13]. Alternatively, in the postnatal cortex, the action potential dominates neurophysiology, and aberrant action potential pathophysiology is associated with multiple channelopathies, including epilepsy and pain [14]. Therefore, unlike adult channelopathy pathophysiology, **developmental channelopathies** (see Glossary) likely hijack the fetal-specific slower biophysical kinetics and cellular properties to generate distinct disease features.

[1]Division of Genetics and Genomics, Manton Center for Orphan Disease Research, and Howard Hughes Medical Institute, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA

*Correspondence:
Richard.Smith@childrens.harvard.edu (R.S. Smith) and
Christopher.walsh@childrens.harvard.edu (C.A. Walsh).

When classifying an ion channel dysfunction to a channelopathy, pathogenic channels are often first described functionally as **gain-of-function (GOF)** or **loss-of-function (LOF)**, yet the presence of highly heterogeneous phenotypes resulting from identical GOF mutations across individuals suggests the influence of multiple factors in disease severity. Therefore, in addition to the degree of ion channel dysfunction (e.g., GOF), other significant variables likely contributing to heterogeneity include temporal/spatial gene expression, cell-type susceptibility, environment, and cell/circuit compensatory mechanisms. However, these variables are not mutually exclusive, as developmental channelopathies likely represent a continuum of blended ion channel dysfunction between *in utero* and postnatal roles, shifting key biological processes timelines. Moreover, in humans, several cortical channelopathy diseases develop within the first years after birth, including infantile epilepsies and **encephalopathies** [14,15], as well as noncortical diseases such as infantile onset spinocerebellar ataxia [16]. In this review, we highlight ion channel genes associated with human prenatal cortical developmental channelopathies and the permissive immature properties distinct from postnatal channelopathies (e.g., epilepsy) that permit *in utero* diseases. Last, we discuss emerging *in utero* animal model studies evaluating bioelectric features (i.e., membrane potential) in developing cortex circuit processes, including ion channel disease modeling in ferrets, which due to their gyrencephalic brain serve as an important model for folding-associated cortical malformations.

Human Genetic Studies of Individuals with Brain Malformations, and Gene Expression Studies

The advent of next-generation sequencing approaches to study individuals with **malformations of cortical development (MCDs)**, along with the introduction of intensive surveys of the developing human brain transcriptome, epigenome, and somatic mutations, including those from the Brain Somatic Mosaicism Network [17] and the PsychENCODE consortium [18], have facilitated the identification of novel mechanisms involved in developing neural circuit processes, including genes linked to cell-cycle proliferation, migration, and differentiation [19] (Figure 1, Key Figure). To this end, the gold standard for identifying novel biological pathways continues to be human genetics, including the recent work identifying a cortical malformation-causing pathway rooted in dysfunctional ion conducting proteins (sodium channels and glutamate receptors).

Disease Follows Gene Expression

For a mutated gene to have a pathogenic effect in a given neural tissue, its expression must be present at sufficient levels either at the time of generation of the neural circuit (e.g., MCDs), or at the time of the dysfunction (e.g., epilepsy; Figure 1). Human developmental and postnatal channelopathies of sodium and glutamate channels follow this susceptibility model, with embryonically enriched channel subtypes/subunits being more susceptible to MCDs (*GRIN2B* and *SCN3A*) and postnatally enriched subunits (*GRIN2A* and *SCN1A*) being associated with epilepsies [6,20,21]. While this pre/postnatal differential regulation of sodium and glutamate channels also persists in rodents [22,23], transgenic mouse models targeting these disease genes lack robust structural cortical phenotypes [24], suggesting a rodent-specific molecular and physiological profile when comparing with humans. However, a conserved functional role for sodium channels within the developing brain may exist as far back as the fly, with a sodium channel gene (*para*) promoting proliferation [25]. New single-cell RNA technologies enable fine-resolution mapping of gene spatial structure in the human fetal cortex by placing sodium channels (*SCN3A*) within nonexcitable cells, including progenitor cells enriched in humans, as well as newborn neurons [6]. Moreover, the expansion of single-cell RNA surveys across human developmental time points [18,26], including cell types critical to the expansion of human cortical **gyrification**, provides a tractable platform for screening novel disease candidate genes, as well as expanding mechanistic hypotheses.

Glossary

Bioelectricity: mode of electrical signaling across individual cells or fields of cells that is slower than neural spiking, particularly during development.
Developmental channelopathy: disease with a pathophysiological origin during gestational periods, caused by dysfunction of ion conducting proteins.
Encephalopathy: general term describing diseases of the brain that lead to dysfunctional properties (e.g., loss of cognitive function in epileptic encephalopathies).
Gain-of-function (GOF): mutation resulting in new or enhanced activity of a protein. In the context of ion channels, GOF typically results in increased ion flux into cells.
Gyrification: developmental process of forming the stereotyped folds (gyri and sulci) of the cerebral cortex in certain species.
Incomplete penetrance: when only a subset of individuals who carry a dominant disease-causing mutation display the characteristic disease phenotype (this is commonly observed in inherited channelopathies).
Loss-of-function (LOF): mutation resulting in lost or decreased activity of a protein. In the context of ion channels, LOF typically results in decreased ion flux into cells.
Malformations of cortical development (MCD): structural abnormalities of the cerebral cortex arising due to aberrant developmental processes (e.g., cell division and migration).
N-methyl-D-aspartate receptors (NMDARs): ligand-gated ion channels consisting of two GluN1 subunits (*GRIN1*) and two subunits of GluN2 or GluN3 (*GRIN2A-D* and *GRIN3A-B*, respectively).
Polymicrogyria (PMG): a condition characterized by an overfolded cerebral cortex (from Greek: poly – many, micro – small, gyri – folds).

Glutamate Receptors and Brain Malformations

Glutamate is the most abundant neurotransmitter in the brain, activating three ionotropic receptors [AMPA, kainate and **N-methyl-D-aspartate receptors (NMDARs)**] to execute critical functions such as cellular excitability and plasticity. NMDARs are heterotetrameric ligand- and voltage-gated ion channels expressed throughout the brain, consisting of two GluN1 subunits (*GRIN1*) and two subunits that are either GluN2 or GluN3 (*GRIN2A-D* and *GRIN3A-B*, respectively). Upon the binding of glutamate and glycine to NMDARs subunits, the ionotropic NMDARs conduct Ca^{2+}, and to a lesser extent Na^{+} and K^{+} ions, resulting in increased cellular excitability [27]. The ionic influx, primarily Ca^{2+}, activates abundant intracellular signaling cascades and cellular targets, akin to metabotropic activation [27]. While glutamate receptor dysfunction has been studied in the human and rodent postnatal brain [28], new data in humans suggest that glutamate receptor dysfunction during fetal development can cause cerebral cortex malformations [7,8] (Figure 2A), as well other neurological conditions [29,30]. The most common malformation of cortical development resulting from *GRIN1* and *GRIN2B* mutations is **polymicrogyria (PMG)**, which is characterized by an overfolded cerebral cortex (Figure 2B) [31]. Several gene families, including canonically described microtubules tied to migration disorders, are known to cause PMG when mutated, yet the mechanisms of cortical disruption and the connecting thread to developmental channelopathy pathology remain poorly understood [32].

Highlighting the specialized role of NMDARs in the prenatal cerebral cortex, functional NMDARs are present long before stable synapses form [33], with functional GluN2B and GluN1 subunits expressed in cortical progenitor cells and neurons [20,21,34]. During mid-fetal human cortex development, the composition of NMDAR subunits flips (*GRIN2B→GRIN2A*) [21]. This flip in the ratio of GluN2B:GluN2A containing subunits is thought to be a key switch for learning behaviors

Key Figure

Timeline of Ion Channel Disease and Neurodevelopmental Processes

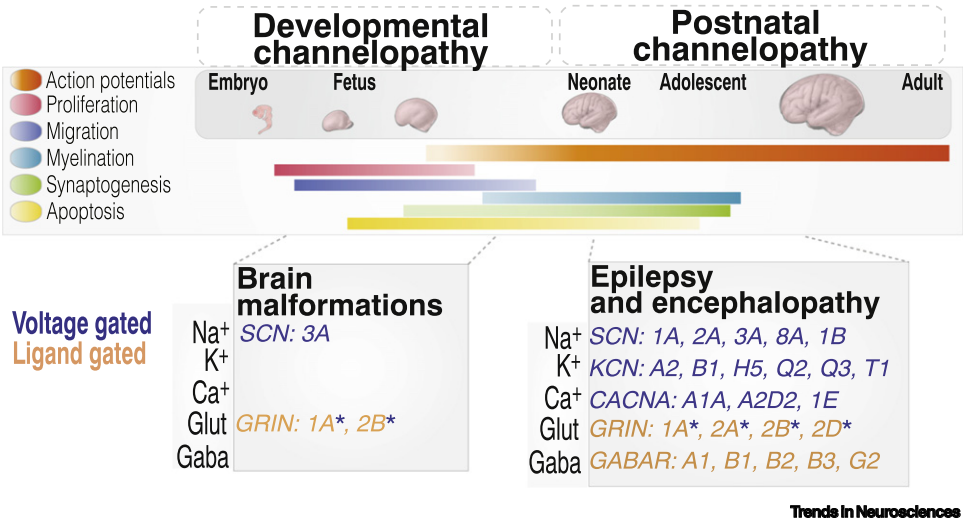

Figure 1. Top, timeline of human gestation from early cortical formation, including key developmental cellular processes and milestones [109], highlighting the development of abundant action potentials in the cerebral cortex [12]. Bottom, ion channel genes (*SCN3A*, *GRIN1*, and *GRIN2B)* implicated in cortical malformations all maintain high expression in the developing human cortex [6,20,21]. The specified genes are representative and are not a comprehensive list.

in mice [23]. Unlike individuals with *GRIN1* and *GRIN2B* mutations causing MCDs, pathogenic *GRIN2A* (GluN2A) mutations have not been described to cause MCDs [35]. Despite the lack of *GRIN2A* causing structural cortical malformations (i.e., PMG), *GRIN2A* postnatal encephalopathies are generally more severe than the PMG-causing *GRIN2B* encephalopathies [29], likely rooted in *GRIN2A*'s postnatal enhanced enrichment pattern. However, unlike the *GRIN2B→GRIN2A* transition, *GRIN1* is ubiquitously expressed throughout life and individuals with pathogenic *GRIN1* mutations have severe and wide-ranging phenotypes in addition to MCDs, including intractable epilepsy and impaired cognitive function [8].

To date, MCD-associated *GRIN1* and *GRIN2B* mutations are primarily functionally described as GOF pathophysiology, enhancing NMDAR activation and excitability [7,8,36]. Lending further support to the NMDAR GOF MCD hypothesis, MCDs can result in human metabolic conditions of elevated glycine [37]. Since glycine and glutamate cobinding is required for NMDAR activation, NMDAR mutations that decrease glycine affinity (i.e., making it more potent) can overactivate NMDARs [27,38]. Mechanisms of NMDAR GOF are varied, including early removal of Mg^{2+} block, increased permeation of Ca^{2+}, and glutamate affinity for binding sites. As NMDARs are heterotetrameric, GOF effects can be described as dominant positive, meaning that one mutated GluN subunit can affect the other subunits to enhance NMDAR activation. In mice, this overactivation of glutamate receptors causes excess Ca^{2+} influx, triggering intracellular signaling cascades that can result in excitotoxicity and cell death [39,40]. In human neural progenitor cell cultures, glutamate can increase progenitor proliferation [41]. Modulating Ca^{2+} influx and excitability as progenitor cells generate neurons can affect several developmental processes, including bidirectional control of neurogenesis [42], NMDAR-mediated shift in neuronal differentiation [43], supporting neuronal survival [44] and guiding radial migration [45], calcium mobilization defects in Zellweger syndrome [46], and inactivation of DNA synthesis [47] (reviewed in [48,49]). One link could include the of role of Ca^{2+} in cytoskeletal remodeling, such as microtubule polymerization and assembly, which represent key functions of MCD-related genes, *LIS1*, *DCX*, and *Tubulinopathies* [19,32].

Sodium Channels and Brain Malformations

Na_Vs are highly conserved proteins across species, and several knockout models of Na_V subtypes are lethal (including in fly and mice). Similarly, in humans, complete Na_V knockouts (*SCN1A*, *SCN2A*, and *SCN3A*; resulting in complete LOF of both copies of the gene) are typically not observed in the population [The Genome Aggregation Database (gnomAD)], although individuals with point mutations and copy number changes within Na_V genes are present and these mutations result in a range of developmental and adult disorders. The spectrum of channelopathy diseases resulting from pathogenic Na_V mutations depends primarily on the Na_V subtype, the mutation's functional consequence (e.g., GOF), and the gene's temporal–spatial expression pattern. Na_V genes expressed abundantly in postnatal brains, such as *SCN1A* and *SCN2A,* are associated with generalized epilepsy with febrile seizures and other seizure types ($GEFS^+$); Dravet syndrome reflects *SCN1A* mutations [14], and benign familial neonatal–infantile seizures and Ohtahara syndrome reflect *SCN2A* mutations [14,48,49]. Of these Na_V diseases, the most extensively studied is Dravet syndrome (*SCN1A*), and most pathogenic mutations in this gene result in Na_V LOF pathophysiology and are accompanied by intractable epilepsy, but without a detectable MCD (Figure 3) [50]. However, a few examples of *SCN1A* variants have been reported to be associated with small cortical lesions, including focal cortical dysplasia and periventricular nodular heterotopia [51].

Na_V's vital role in action potential genesis has been a centerpiece of neurophysiological research for more than 60 years. However, less has been known about the function of Na_Vs in the prenatal cerebral cortex, where action potentials are sparse [6,12]. New data suggest that an embryonically enriched Na_V subtype, *SCN3A* (encoding $Na_V1.3$), can disrupt fetal cortical development

in humans when mutated, causing MCDs [6]. MCD-causing *SCN3A* pathogenic mutations are distributed across key functional Na_V transmembrane domains, including segments critical for voltage-dependent inactivation (DIV-S6) and voltage-sensing (S4), resulting in GOF effects [6] (Figure 4). Within inherited GOF *SCN3A* mutations, a wide phenotypic variability in brain diseases exists, as affected brain areas range from unilateral perisylvian to a broader frontal–parietal distribution, and resulting cortical malformations are mostly accompanied by oral motor deficits and not accompanied by seizures [6]. Other *SCN3A* alleles that are functionally described as LOF have been associated with cases of neonatal onset seizures and epileptic encephalopathy (EE) without detectable MCDs [52–54] (Figure 4). Additionally, some epilepsy-associated *SCN3A* variants generate a GOF increase in persistent current (I-Na_p), but without MCD [54–56], further highlighting the need for continued genotype–phenotype functional studies. Similarly, some epilepsy-associated *SCN1A* mutations generate increased I-Na_p [57].

In both humans and rodents, $Na_V1.3$ is differentially enriched during gestational periods in the cortex, in contrast to *SCN1A* which is enriched postnatally [6,22]. Consequently, $Na_V1.3$'s spatiotemporal structure is primed for developmental pathology, as it is enriched in prenatal cor-

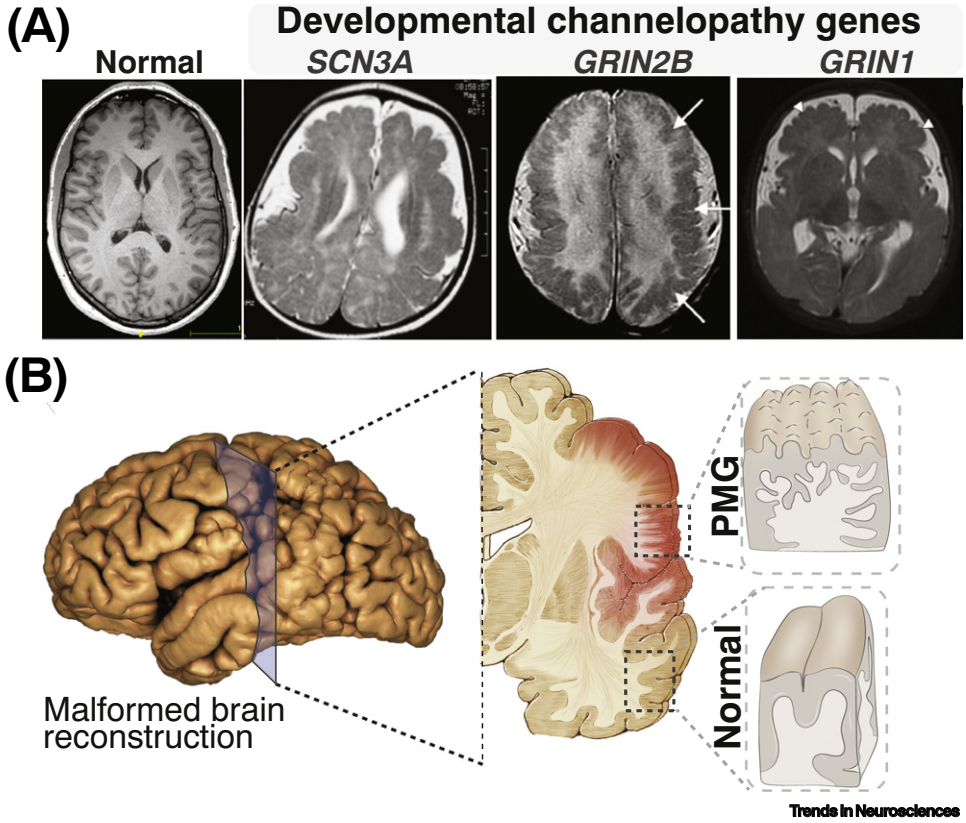

Figure 2. Cortical Malformations Associated with Sodium and Glutamate Receptor Dysfunction. In humans, the most common cortical malformation disease is polymicrogyria (PMG); a developmental brain malformation characterized by the presence of multiple, abnormally small gyri in the cerebral cortex [31]. (A) Magnetic resonance imaging (MRI) of individuals with mutations within ion channel genes, sodium channel α subunit 3, *SCN3A* (voltage-gated sodium channel; $Na_V1.3$), N-methyl-D-aspartate receptor (NMDAR) subunits *GRIN1* (GluN1) and *GRIN2B* (GluN2B), when mutated are capable of causing PMG, with various degrees of severity. Reproduced, with permission, from [6–8]. (B) Left, MRI reconstruction of an individual with SCN3A mutation and resulting perisylvian PMG. Coronal section highlights the affected area. Right, Illustration of neocortical architecture of a normal (bottom) and diseased (top, developmental malformation, PMG) hemispheres, including gray and white matter. Red regions indicate affected PMG area, depicting gyral fusing to give a 'sawtooth'-like appearance. Reproduced, with permission, from [6].

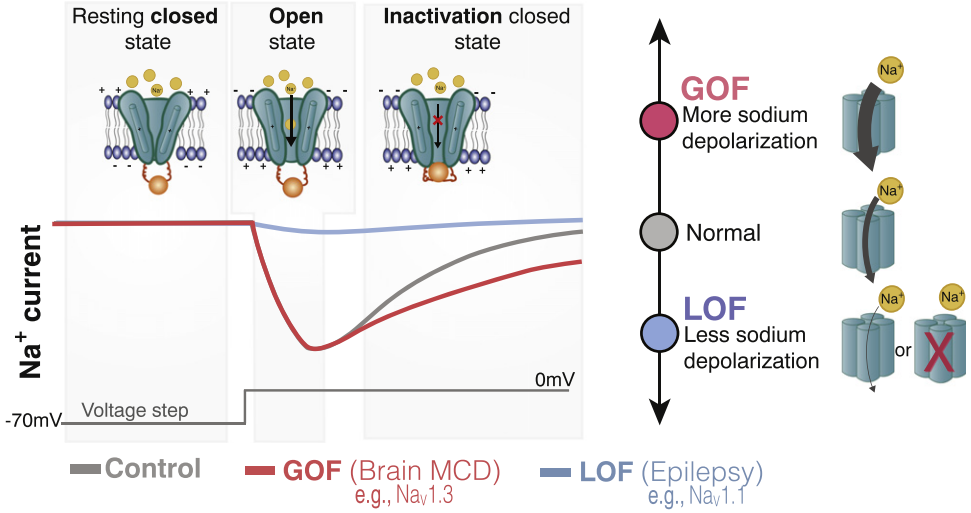

Figure 3. Biophysical Properties of Sodium Channel Mutations That Result in Diverse Channelopathies. Top left, schematic of sodium channel gating and correlation with representative whole-cell recordings. Two of the voltage-clamp recordings are of pathogenic voltage-gated sodium channels (Na_Vs), illustrating a gain of function (GOF) mutation [red trace, *SCN3A* malformation of cortical development (MCD) F1759Y, increased noninactivating persistent sodium current] [6] and a loss of function (LOF) mutation (blue trace, Dravet associated *SCN1A* G1674R impairs $Na_V1.1$ trafficking) [105]. Gray trace represents control experiment. Bottom, square trace represents a depolarizing step (-70 to 0 mV), and voltage-clamp Na_V traces are normalized for visualization. Right, Na_V illustration depicting Na^+ ion flux in the context of GOF and LOF mutations. LOF Na_V mutations are represented as defective Na_V biophysical properties, or by decreased cell surface expression properties which result in decreased Na^+ influx [53].

tical radial glial progenitor cells (RGCs), newborn neurons, and Cajal–Retzius cells [6,58]. During lamination in the developing cortex, several cell types and transition states exist (e.g., cell cycle), including dividing RGCs and differentiating neurons (which possess sodium currents), but with vastly different neurophysiology compared with adult spiking neurophysiology [13]. Within spiking and nonspiking cells, sodium can activate several downstream effector targets, including other ion channels (Ca^{2+}, K^+), and can trigger a cascade of pathways, in some cases cell death [39,59]. *SCN3A* GOF mutations demonstrate increased, noninactivating, persistent Na^+ currents (Figure 3), yet how cell types in the developing cortex (progenitor, migrating, or mature neurons) handle excess Na^+ flux is only beginning to be understood (e.g., receptor expression, cell state, and Vm). Moreover, modeling of *SCN3A* GOF mutations in the gyrencephalic ferret brain revealed migratory deficits, as well as surprising non-cell autonomous effects [6], which suggest a pathological basis in altered neurotransmitter/neuropeptide release or electrical coupling onto neighboring cells [39,60]. Furthermore, mice with hypomorphic *Scn3a* (LOF) have normal developmental features, but with evoked seizures [53]. Future work to elaborate identified *SCN3A* mutations resulting in MCDs versus infantile epilepsy will elucidate the delicate balance of sodium flux in the developing cortex and how modifications can differentially contribute to pre- and postnatal channelopathy diseases.

Ion Channel Dysfunction Alters Postnatal Brain Development

The end of the gestational period does not mark the end of maturation of cortical circuits, as several developmental processes continue postnatally, including cell death, myelination, and synaptic pruning (Figure 1). Uncontrolled seizures, for example, can lead to poor cognitive outcomes and intellectual disability in children [61,62]. Two classes of ion channel diseases have been described that affect neurodevelopment in the postnatal period, EEs and developmental encephalopathies (DEs). EEs are broadly defined diseases relating epileptic activity to risk for

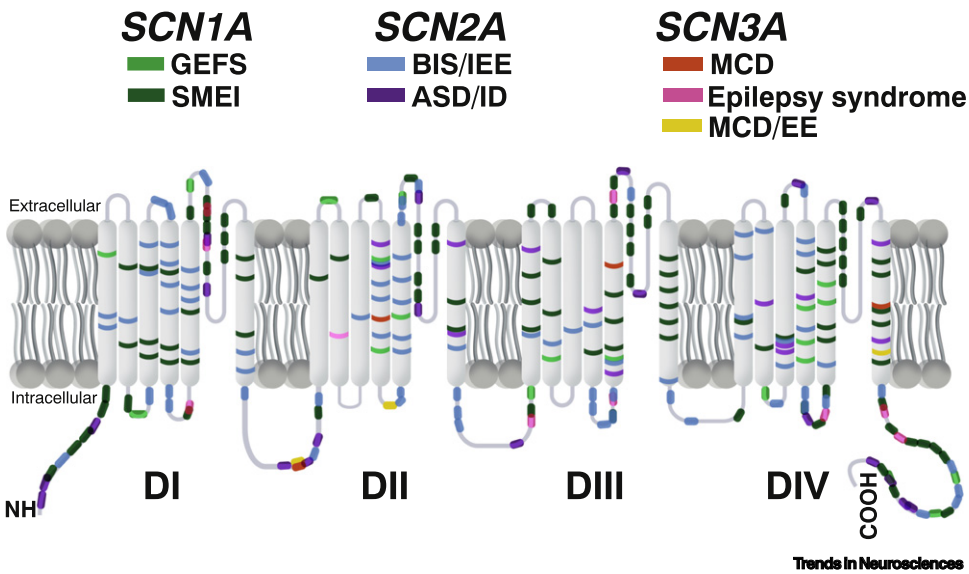

Figure 4. Locations of Mutations within Sodium Channel Subtypes Resulting in Developmental and Postnatal Channelopathies in Humans. Transmembrane topology of voltage-gated sodium channel (Na_V) channel depicting location of missense variants within the central nervous system enriched sodium channel subtypes, *SCN1A*, *SCN2A*, and *SCN3*A ($Na_V1.1$, $Na_V1.2$, and Na_V 1.3, respectively). *SCN1A* related conditions: generalized epilepsy with febrile seizures and other seizure types ($GEFS^+$, light green), severe myoclonic epilepsy of infancy (SMEI, dark green); *SCN2A* related disorders: infantile epileptic encephalopathy (IEE, blue), benign infantile seizures (BIS, Blue), autism spectrum disorder/intellectual disability (ASD/ID, purple); *SCN3A* related disorders: malformations in cortical development (MCDs, orange), and epilepsy syndrome (pink; includes focal, tonic, partial, IEE), or blended MCD/EE phenotype (orange/pink). *SCN1A*, *SCN2A*, and *SCN3A* are paralogous genes with conserved functional domains, with positional data for amino acid location overlaid and approximated from [6,14,53–55,82,106–108]. Na_V's consist of four transmembrane domains (DI, DII, DIII, and DIV), with each domain consisting of six segments that contribute biophysical properties to channel function.

decreased cerebral function, whereas DEs describe disorders in which developmental delay emerges before the onset of epileptic activity or in the presence of mild epileptic activity [63]. In this regard, MCDs resulting from *SCN3A*, *GRIN1A*, and *GRIN2B* mutations are not easily classified within these postnatal encephalopathies, since the structural damage is occurring *in utero* and it remains to be precisely defined what is a pathogenic epileptogenic signal in the gestational brain. While early postnatal seizures are largely cortical, they shift to other brain regions later in life (such as the hippocampus) [64], thus resulting in a differential susceptibility to damage as life progresses [65]. Moreover, Dravet syndrome is generally considered an EE [66], whereby epileptic activity underlies progressive brain dysfunction, yet cognitive delay symptoms may develop independent of seizures, suggesting an additional sodium-mediated developmental pathophysiology separate from classic EEs [67]. Future studies of this developmental shift in seizure origin, as well as defining an *in utero* epileptogenic network, represent an important expansion of the etiology of these developmental diseases.

Infantile encephalopathies (IEs) are a type of EE, and can be caused by improperly functioning receptors and ion channels (e.g., glycine, K^+, and Na^+, etc.), resulting in a spectrum of conditions that are generally not associated with MCDs [62,67]. For example, a sodium-activated potassium channel (*KCNT1*) causes a heterogeneous condition of IE with onset in the first 6 months, acquired microcephaly (small brain), and developmental delay, suggesting that damage in the early neonatal period can alter postnatal brain development [68,69]. Early infantile EE (EEIE13) resulting from *SCN8A* (NaV1.6) mutations is also disruptive to neonatal brain development, resulting in developmental regression and intellectual disability [70]. In contrast,

epilepsy syndromes that remit on a shorter timeline (<6 weeks), such as those caused by mutations in potassium channel subunits $K_V7.2$ and 7.3 (*KCNQ2* and *KCNQ3*, respectively), are generally associated with normal cognitive development [71]. Moreover, mixing of phenotypes does exist, as *KCNQ2* can cause epileptic and cognitive deficits in both GOF and LOF models at the exact same codon (albeit with different amino acid substitutions), suggesting a bidirectional cellular effect of altered excitation as the pathological basis [72]. *GRIN2B* mutations can result in early life epilepsies, including infantile spasms, and are largely described as GOF [30,73]. In some individuals with *KCNQ2*, *SCN3A*, or *STXBP1* encephalopathy, epilepsy resolves relatively early in adolescence, but the developmental consequences persist long thereafter [15,52]. Similar to the above-described heterogeneous EE phenotypes, ion channel mutations associated with MCDs also generate diverse disease features, including a role for *GRIN2A* [35] and *SCN3A* in speech development [6]. Moreover, individuals with *SCN2A* mutations display a complex phenotype that can include a wide range of epilepsy and encephalopathy syndromes, as well as autism [14,48,49] (Figure 4). Future work expanding the genotype–phenotype relationships for single genes (e.g., *GRIN2B*) will be critical to understanding convergent and divergent mechanisms of pathophysiology.

Ca^{2+} channel subunits are also implicated in wide-ranging postnatal channelopathies, including *CACNA1A* and *CACNA1E* in DE and EE [74,75], *CACNA4A* in familial hemiplegic migraine and episodic ataxia [76], and *CACNA1C* in Timothy syndrome, which includes autism phenotypes [77]. Last, proteins that interact with pore-forming ion channel subunits can also result in encephalopathies, such as Na_V auxiliary β subunits (β-Na_V), which support the α subunit Na_V ion conduction properties [78]. In rodent models, β-Na_V knockdown can alter cortical neurite development, including noncanonical roles of Fyn kinases and secreted cell adhesion molecules, as well as cell-type-specific pathology rooted in maturation of GABAergic neurons [79–81]. Cell-type susceptibility represents a key avenue for future research on EE pathophysiology, as well as on developmental psychiatric diseases classified as interneuronopathies, such as *SCN2A* in autism and epilepsy syndromes [82].

Single Gene Ion Channel Disorders Are Heterogeneous and Unique to Each Individual

Penetrance is a feature of dominantly inherited genetic diseases measured by the proportion of carriers showing the characteristic phenotypes. Plainly, three individuals within a multigenerational family can possess the same pathogenic channel mutation, but have three different disease phenotypes; one individual might have the severe disease, another might have a less severe version, and the last no apparent disease phenotype. This genetic feature of **incomplete penetrance** and variable phenotypes is common across channelopathies, with an excess of 60% of carriers not displaying a complete disease phenotype [83]. Of the brain channelopathies, decreased penetrance is typically observed with GOF mutations in a dominant disease inheritance model [6,57]. The large phenotypic variability of channelopathies is best illustrated in monozygotic twin studies (identical ion channel gene mutations), demonstrating both concordant and discordant features depending on the mutation classification (e.g., GOF) and ion channel type being affected [84,85]. Between individuals, the basis for decreased penetrance and variable phenotypes is likely multifactorial, including individual variations in gene expressivity [86], modifier genes [87,88], epigenetic factors [89], and environmental interactions [90]. For example, *PLOG* variants can act as genetic modifiers of Dravet syndrome and cause brain damage [91], and channel mutations that would otherwise be benign under normal conditions can result in pathogenicity following exposure to noxious stimuli [89]. Future work exploring the selective susceptibility of specific mutations and penetrance-modifying mechanisms represents critical knowledge towards the design of precision therapies.

Concluding Remarks

Historically, channelopathies were commonly viewed as diseases of aberrant cellular excitation in postnatal nervous tissue, with roots in altered action potential firing features. Here, we describe developmental channelopathies as diseases of prenatal neural circuits involving nonexcitable cortical progenitor cells and newly born neurons. Moreover, the absence of abundant action potential electrogenesis in the fetal brain [12], in tandem with the several immature cellular states, likely contributes a unique neurophysiological signature that differs between pre- and postnatal neural circuits. Within the prenatal rodent cortex, altering resting membrane potential is sufficient to disrupt progenitor proliferative properties and cell fate [9,10], and in ferrets increased sodium led to both cell- and non-cell autonomous effects leading to gyri misfolding [6], suggesting small excitability changes are sufficient to disrupt cortical development across several trajectories. However, open questions remain, including how ion channel mutations differentially influence intrinsic cellular properties across transient cell types (immature vs mature). For example, consider the simple scenario of modeling a single mutation within two anatomically and functionally similar neuron types (sensory vs sympathetic ganglion neurons), which results in different outputs (hyper- and hypoexcitability, respectively) [92]. One can similarly envision a more pronounced differential disease pathology affecting the very heterogeneous cortical cell types in their developmental properties. Complicating gene-to-mutation classifications further, even within a monogenic ion channel gene disorder (i.e., *GRIN1*), pathogenic mutations within in the same functional domains can result in highly heterogeneous manifestations, including non-PMG- and PMG-causing mutations [8]. Similar heterogeneous disease features are observed in sodium channels (Figure 4). Future studies elaborating each allele individually and across cell types, rather than relying on functional domain predictions and modeling, will be critical to accurate therapeutic development.

Human malformations of cortical development due to NMDAR point mutations have not been mechanistically described in animal models (to our knowledge). While human MCD-associated NMDAR genes have been studied in rodent knockdown models, including a *GRIN2B* knockdown associated with a mild neuronal migration deficit [93], and a *GRIN1* knockout generating mild layer IV defect [24], these lesions are subtle compared with human MCDs and represent LOF pathophysiology (human MCDs are generally GOF). While some rodent models of MCDs have proven effective for studying some human cortical defects (e.g., *EML1*) [94], their use in modeling gyrification defect diseases (i.e., PMG) is limited [95]. Important molecular, temporal, and genetic differences during gestation that result in the rodent lissencephalic cortex generally do not recapitulate human MCDs well. One of these molecular differences is that rodents have fewer progenitor cell types than that observed in gyrencephalic mammals, including basal radial glial cells (bRGCs), which suggests rodents lack the progenitor diversity required for cortical expansion [96]. Using primate models in this context is a possibility to consider, but of note, small primates such as marmosets are lissencephalic, and therefore alternative non-primate animal models to study gyrencephalic brains seems critical. One example is the domestic ferret (*Mustela putorius furo*), which possess a folded neocortex and increased progenitor cell diversity [97]. The developmentally tractable ferret brain therefore offers a promising platform for mechanistic testing of cortical neurodevelopmental diseases associated with gyrification deficits [97], such as developmental channelopathies [6,98,99]. Last, while rodent studies fail to demonstrate robust structural cortex malformations, they likely represent a viable model for studies of nonmalformation causing neurodevelopmental diseases, especially those hypothesized to affect interneuron maturation (e.g., *SCN2A* and ASD [82]).

Bioelectricity studies evaluating electrical signals across singles cells or groups of cells in developing organisms is a rapidly growing field and hypothesizes roles for excitability in a wide variety of developmental processes and tissue types [100]. For example, K^+ channels have been

Outstanding Questions

How do mutations distributed within an ion channel gene (e.g., *GRIN1*) generate a spectrum of resulting diseases (malformation vs epilepsy)?

How is an epileptogenic network defined in *in utero* versus postnatal brains?

How does a single pathogenic ion channel mutation affect cell types differently within the same individual (dividing cells, excitatory ones, inhibitory, and sensory)?

What are the noncanonical targets of normal ion flux in the fetal cortex?

What is the spatial–temporal expression structure of EE genes during early postnatal life?

How does ion channel dysfunction resulting in aberrant electrical activity in the prenatal brain compare with well characterized malformation pathways?

demonstrated to promote limb development in zebra fish [101], and are also associated with dysmorphic facial features in humans [102,103]. Within the prenatal embryo, key cellular processes within nonexcitable cells are readily modified by bioelectric properties, including membrane potential changes linked to oscillations which are fundamental for cell-cycle state and proliferation [49]. In two recent cortical development bioelectric studies, *in utero* electroporation of K^+ channels (hyperpolarized) or chemogenetic receptors (depolarized) in cortical progenitors and neurons suggests that depolarization maintains neurogenic properties [10], while increased early activity in migrating neurons may also act as a stop signal for migration [9]. Importantly, bioelectric changes in progenitor cells are capable of having widespread effects since progenitor cells maintain electrical synapses, enabling coupling of electrical activity [11,104]. In the future, neurophysiological research of excitation in the gestational cortex, as well as the connection between dysfunctional bioelectric mechanisms and canonical MCD-causing pathways (cellular proliferation, migration, and neural cell fate determination), should be explored (see Outstanding Questions).

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