JPn Neuroscience

The Journal of Physiology

J Physiol 591.4 (2013) pp 753–764

SYMPOSIUM REVIEW

Ion channels in genetic and acquired forms of epilepsy

Holger Lerche[1], Mala Shah[2], Heinz Beck[3], Jeff Noebels[4], Dan Johnston[5] and Angela Vincent[6]

[1] *Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Hoppe-Seyler-Str. 3, D-72076 Tübingen, Germany*

[2] *UCL School of Pharmacy, University College, London, WC1N 1AX, UK*

[3] *Life and Brain Center, Experimental Epileptology and Cognition Research, University of Bonn Medical Center, Sigmund-Freud Str. 25, 53105 Bonn, Germany*

[4] *Department of Neurology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA*

[5] *Institute for Neuroscience, College of Natural Sciences, University of Texas at Austin, TX 78712, USA*

[6] *Nuffield Department of Clinical Neurosciences, University of Oxford, Level 6, West Wing, John Radcliffe Hospital, Oxford OX3 9DU, UK*

Abstract Genetic mutations causing dysfunction of both voltage- and ligand-gated ion channels make a major contribution to the cause of many different types of familial epilepsy. Key mechanisms comprise defective Na^+ channels of inhibitory neurons, or $GABA_A$ receptors affecting pre- or postsynaptic GABAergic inhibition, or a dysfunction of different types of channels at axon initial segments. Many of these ion channel mutations have been modelled in mice, which has largely contributed to the understanding of where and how the ion channel defects lead to neuronal hyperexcitability. Animal models of febrile seizures or mesial temporal epilepsy have shown that dendritic K^+ channels, hyperpolarization-activated cation channels and T-type Ca^{2+} channels play important roles in the generation of seizures. For the latter, it has been shown that suppression of their function by pharmacological mechanisms or in knock-out mice can antagonize epileptogenesis. Defects of ion channel function are also associated with forms of acquired epilepsy. Autoantibodies directed against ion channels or associated proteins, such as K^+ channels, LGI1 or NMDA receptors, have been identified in epileptic disorders that can largely be included under the term limbic encephalitis which includes limbic seizures, status epilepticus and psychiatric symptoms. We conclude that ion channels and associated proteins are important players in different types of genetic and acquired epilepsies. Nevertheless, the molecular bases for

Holger Lerche (left) is Clinical Director and Head of the Department of Neurology and Epileptology at the Hertie Institute of Clinical Brain Research at the University of Tübingen, Germany. His main research interest is to unravel the genetics and pathophysiology of inherited epilepsies and related paroxysmal disorders using a combination of genetic and neurophysiological tools. He is also interested in molecular ion channel function, their specific roles in the brain and their pharmacology. After graduating from the University of Munich (LMU), he worked as a postdoc in neurophysiology and as a resident and consultant in neurology and epileptology at the Institute of Applied Physiology and the Department of Neurology of the University of Ulm. He undertook clinical and research fellowships in Bonn/Germany, London/UK and Melbourne/Australia. **Mala Shah** (right) did her PhD at University College London (UCL, UK) under the supervision of Dr Dennis Haylett. She then obtained a Wellcome Prize Travel Research Fellowship to work in the laboratories of Professors Daniel Johnston at Baylor College of Medicine (Houston, USA) and David Brown at UCL (UK). She subsequently received a lectureship at UCL School of Pharmacy (UK) where she is currently a Reader in Neuroscience. Her research interests include understanding how voltage-gated ion channels activated at sub-threshold membrane potentials affect hippocampal and cortical cell excitability under physiological as well as epileptogenic conditions.

The report was presented at the symposium *Why do some brains seize? Molecular, cellular and network mechanisms*, which took place at the Epilepsy Research UK Expert International Workshop, Oxford, UK on 15–16 March 2012.

DOI: 10.1113/jphysiol.2012.240606

most common forms of epilepsy are not yet clear, and evidence to be discussed indicates just how much more we need to understand about the complex mechanisms that underlie epileptogenesis.

(Received 8 July 2012; accepted after revision 21 October 2012; first published online 22 October 2012)
Corresponding author A. Vincent: Nuffield Department of Clinical Neurosciences, University of Oxford, Level 6, West Wing, John Radcliffe Hospital, Oxford OX3 9DU, UK. Email: angela.vincent@ndcn.ox.ac.uk

Abbreviations IGE, idiopathic generalized epilepsy; SE, status epilepticus; TLE, temporal lobe epilepsy.

Introduction

The epilepsies are disorders of neuronal network excitability. They can be divided into two major groups. In the first group, which is called 'symptomatic', an acquired or inborn structural or metabolic defect of the brain can be identified as the underlying cause of the disease. These forms of epilepsies have a mainly focal origin meaning that the seizures start from a point around the structural lesion. The clinical presentation of the resulting epileptic seizures depends on the respective brain region in which the seizures start and spread, and can vary from light symptoms such as a strange feeling in the stomach or paresthesia in a certain body area, to loss of consciousness and severe convulsions. Typical examples for epileptogenic lesions are tumours, stroke or hippocampal sclerosis, the latter causing mesial temporal lobe epilepsy, one of the most frequent and often pharmacoresistant forms of focal epilepsy. An example of increasing clinical importance is given by epilepsies with antibodies directed against proteins involved in membrane excitability such as ion channels. The second group, termed 'idiopathic', is genetically determined and characterized by the lack of structural or other predisposing causes. Both focal and generalized forms of epilepsy can be caused by genetic defects and the resulting epileptic phenotypes can range from mild seizures occurring only in neonates or infants, to severe epileptic encephalopathies with mental retardation, pharmacoresistant epilepsy and other neurological symptoms. The most common disease entity is 'idiopathic generalized epilepsy' (IGE) comprising the well-known absence, myoclonic and primary generalized tonic–clonic seizures. The detection of mutations causing idiopathic forms of epilepsy has dramatically advanced our understanding about the pathophysiology in the last 15 years, which is one major topic covered in this review.

There are three main ways in which ion channels are known to be involved in epilepsy. Firstly, there are specific mutations in familial idiopathic epilepsies; secondly, there are specific antibodies in acquired seizure-related disorders; and thirdly, there are changes of ion channel expression and function associated with modification of seizure activity which may contribute to all forms of epilepsy. Here we review these rapidly developing areas using tables to provide more details. For the sake of brevity we will focus on disorders with the main symptom of epilepsy. Recent other developments, for example, increasing research into epileptic seizures in Alzheimer's disease or connections to autism, are not covered here.

Genetic defects in voltage-gated or ligand-gated ion channels

Table 1 lists gene mutations found in different epilepsies and Figure 1 shows the localization of some of the relevant channel proteins in different neuronal subtypes and compartments. Classical linkage methodology, on large pedigrees with rare monogenic syndromes, identified mutations in familial idiopathic focal epilepsies: for instance, the genes encoding nicotinic acetylcholine receptors in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) or potassium channels (K_V7) in benign familial neonatal seizures (BFNS). In 'sporadic' cases, in which only one member of a family is affected, candidate gene approaches have been successful in identifying *de novo* pathogenic mutations, the most prominent being *SCN1A* nonsense mutations in Dravet syndrome A few mutations have also been identified in families with the most common IGEs, childhood and juvenile absence or myoclonic epilepsies, with different $GABA_A$ receptor subunit defects being of particular importance (Table 1). However, the substantial complexity of ion channel gene variant profiles among individuals with epilepsy as well as unaffected controls precludes a simple monogenic channelopathy model in the majority of cases, and particularly IGE is considered to be a prototype of a complex genetic disorder in which many – both rare and common – genetic variations play a pathogenic role. Pathophysiological studies demonstrated that two key defects are: (i) a neuronal dysinhibition that can be caused both by loss-of-function defects in different subunits of the postsynaptic $GABA_A$ receptor and presynaptic loss-of-function defects of the sodium channel $Na_V1.1$ expressed specifically in inhibitory interneurons, or (ii) dysfunction of axon initial segments, the neuronal structure in which action potentials are generated and in which many of the described channels (for example, $Na_V1.2$ sodium and K_V7 potassium channel subunits) are mainly localized. In addition, these clinically originated

Table 1. Genes and proteins mutated in idiopathic epilepsies and epileptic encephalopathies

	Abbreviation	Gene	Protein	References
Idiopathic focal epilepsies				
Benign familial neonatal seizures	BFNS1/EBN1	*KCNQ2*	$K_V7.2$ (K^+ channel)	Biervert *et al.* 1998; Singh *et al.* 1998
	BFNS2/EBN2	*KCNQ3*	$K_V7.3$ (K^+ channel)	Charlier *et al.* 1998
Benign familial neonatal–infantile seizures	BFNIS	*SCN2A*	$Na_V1.2$ (Na^+ channel)	Heron *et al.* 2002; Berkovic *et al.* 2004
Autosomal dominant nocturnal frontal lobe epilepsy	ADNFLE	*CHRNA4*	α_4 subunit (nACh) receptor	Steinlein *et al.* 1995
		CHRNB2	β_2 subunit (nACh) receptor	De Fusco *et al.* 2000
		CHRNA2	α_2 subunit (nACh) receptor	Aridon *et al.* 2006
Idiopathic generalized epilepsies and associated syndromes				
Childhood absence epilepsy with febrile seizures	CAE+FS	*GABRG2*	γ_2 subunit ($GABA_A$ receptor)	Wallace *et al.* 2001
Absence epilepsy and episodic ataxia	CAE+EA2	*CACNA1A*	$Ca_V2.1$ (Ca^{2+} channel)	Jouvenceau *et al.* 2001; Imbrici *et al.* 2004
Juvenile myoclonic epilepsy	JME	*GABRA1*	α_1 subunit ($GABA_{A)}$ receptor	Cossette *et al.* 2002
		EFHC1	EF hand motif protein	Suzuki *et al.* 2004
Genetic (generalized epilepsy with febrile seizures plus (GEFS+)	GEFS+	*SCN1A*	$Na_V1.1$ (Na^+ channel)	Escayg *et al.* 2000
		SCN1B	β_1 subunit (nACh receptor)	Wallace *et al.* 1998
		GABRG2	γ_2 subunit ($GABA_A$) receptor	Baulac *et al.* 2001
Generalized epilepsy and paroxysmal dyskinaesia	GEPD	*KCNMA1*	$K_{Ca}1.1$ (K^+ channel)	Du *et al.* 2005
Epileptic encephalopathies				
Dravet syndrome (severe myoclonic epilepsy of infancy)	SMEI	*SCN1A*	$Na_V1.1$ (Na^+ channel)	Claes *et al.* 2001
Other syndromes				
Focal epilepsy and episodic ataxia	EA1+FE	*KCNA1*	$K_V1.1$ (K^+ channel)	Zuberi *et al.* 1999

The table lists part of the affected ion channel genes in humans illustrating how different clinical syndromes arise from inherited disorders of ion channels. Only unequivocally proven genetic defects that have been described more than once in the literature are included. Full references for Table 1 can be found in Supplementary information. The neuronal localization of some of the channel proteins is shown in Figure 1.

studies identified novel genes, defined their neuronal functions and sometimes established new physiological principles (such as $Na_V1.1$ as the major sodium channel in GABAergic interneurons). Moreover, K_V7 channels have proven to be a novel therapeutic target (reviewed by Weber & Lerche, 2008; Reid *et al.* 2009).

Novel genetic technologies allowing sequencing of the whole coding genomic DNA (whole exome) or even the whole genome now allow identification of new mutations and involvement of loci that could not be efficiently explored before by classical methods. As an example for another ion channel alteration in epilepsy, a *de novo* mutation was recently identified in the sodium channel gene *SCN8A* in a patient with a severe epileptic encephalopathy. This mutation leads to a dramatic gain-of-function with increased sodium inward current and membrane hyperexcitability (Veeramah *et al.* 2012).

Ion channel defects modelled in mice

Transgenic and spontaneously mutant mice have proven crucial for identifying novel molecular pathways for epilepsy, validating the causality of candidate genes isolated from human pedigrees, and for unravelling their pathogenic mechanisms. Beginning with the first spontaneous single gene murine model of epilepsy, the mutant mouse *tottering*, mutations in at least 17 distinct genes have been reported that produce spontaneous electrographic seizures accompanied by behavioural manifestations (Table 2). Along with these spontaneous models, genetic engineering has allowed the creation of an expanding list of targeted null alleles, transgenic over-expression using selected or endogenous promoters, and knock-in of human mutant point mutations.

Unexpected pathogenic mechanisms are emerging from comparative analysis of current defects in excitatory and inhibitory networks. For instance, as already mentioned above, different types of sodium channels expressed in both glutamatergic and GABAergic cell types play unequal roles in excitability, providing an explanation for the network disinhibition arising from *Scna1* deletion (Yu *et al.* 2006; Ogiwara *et al.* 2007). Another example is how mutations of calcium *Cacna1a* and *Cacnb4* channel subunit genes that decrease P/Q-type currents may lead to the 'acquired' downstream enhancement of low-voltage T-type calcium currents sufficient to produce thalamocortical spike-wave epilepsy (Zhang *et al.* 2002, Ernst *et al.* 2009).

Genotype–phenotype correlations of inherited ion channel disorders are far from being well understood. For example, gain- and loss-of-function mutations in the same gene, or in related members of the same gene family, can give rise to alternative seizure phenotypes. In addition, even when the electrographic seizures themselves appear similar, epilepsies arising from different ion channel genes may be accompanied by remarkably different behaviours and co-morbidities, such as the absence of hippocampal remodelling (Singh *et al.* 2008), or sudden unexpected death (Goldman *et al.* 2009; Glasscock *et al.* 2010) in the K^+ channel family.

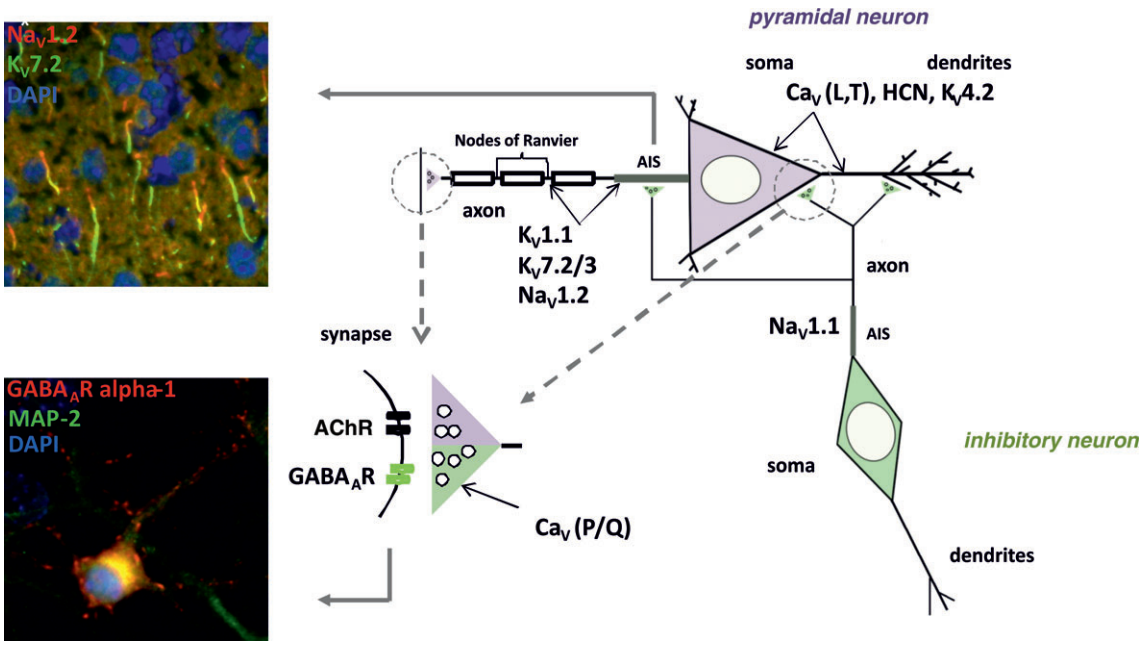

Figure 1. Neuronal localization of some relevant voltage- and ligand-gated ion channels
Shown is a schematic view of an excitatory pyramidal (purple) and an inhibitory (green) neuron and their synaptic connections. Distinctive intracellular compartments are targeted by different populations of ion channels, examples of which as mentioned in this review are shown here: in the somatodendritic compartment, Ca_V (L- and T-type), HCN and some K_V channels; at axon initial segments (AIS) and nodes of Ranvier in pyramidal neurons, $K_V1.1$, $Na_V1.2$, $K_V7.2$, $K_V7.3$; at AIS of inhibitory neurons, $Na_V1.1$; in the presynaptic terminals, Ca_V P/Q type; in the postsynaptic compartment, $GABA_A$ and acetylcholine receptors. Upper insert: Colocalization of $K_V7.2$ and $Na_V1.2$ channels at AIS of cortical neurons in an adult mouse brain, as revealed by immunofluorescent staining using an anti-$K_V7.2$ (green) and an anti-$Na_V1.2$ (red) antibody of sections obtained from an unfixed brain; DAPI (blue) was used to mark the nuclei. Lower insert: Distribution of $GABA_A$ receptors in a primary cultured hippocampal neuron shown by immunofluorescent staining using an anti-GABAAR alpha-1 subunit antibody (red). An anti-MAP2 antibody (green) was used as a somatodendritic and DAPI (blue) as a nuclear marker (Figure kindly provided by Dr. Snezana Maljevic, modified after Maljevic and Lerche, 2011).

Table 2. Genes for voltage-gated ion channel subunits with spontaneous epilepsy phenotypes in mice

Gene	Current/protein	Seizure type	Mutation Ko, knock-out Ki, knock-in	Reference
Sodium				
Scna1a	Nav1.1 α subunit	convulsive	Ko	Yu *et al.* 2006; Ogiwara *et al.* 2007
Scna1a			Targeted R1648H human Ki	Martin *et al.* 2010
Scna1b			Ko	Chen *et al.* 2004
Scn2a	Nav1.2 α subunit	convulsive	transgenic GAL879-881QQQ	Kearney *et al.* 2001
Calcium				
Cacna1a	Cav2.1 P/Q-type α subunit	absence	Spontaneous alleles (tottering, leaner, rocker roller, tg4J, tg5J)	Fletcher *et al.* 1996; Zwingman *et al.* 2001; Mori *et al.* 2000; Miki *et al.* 2008
Cacna1a	Cav2.1 P/Q-type	absence	Cerebellar selective PCP2-Cre promoter Ko	Mark *et al.* 2011
Cacna1a	Cav2.1 P/Q-type	absence	Ko	Llinas *et al.* 2007
Cacnb4	β4 regulatory subunit	absence	Spontaneous (*lethargic)*	Burgess *et al.* 1997
Cacna1g	Cav3.1 T-type α subunit	absence	Bac transgene overexpression	Ernst *et al.* 2009
Cacna2d2	$\alpha 2\delta$ regulatory subunit	absence	Spontaneous several alleles	Barclay *et al.* 200; Brill *et al.* 2004
Potassium				
Kcna1	Kv1.1	convulsive	Ko	Smart *et al.* 1998
Kcna1	Kv1.1	absence	Overexpression	Sutherland *et al.* 1999
Kcna2	Kv1.2	convulsive	Ko	Brew *et al.* 2007
Kcnh3	Kv12.2	non-convulsive	Ko	Shang *et al.* 2010
Kcnmb4	β4 regulatory subunit calcium-activated	non-convulsive	Ko	Brenner *et al.* 2005
Kcnc2	Kv3.2	convulsive	Ko	Lau *et al.* 2000
KcnJ6	Girk2 ATP sensitive	convulsive	Ko	Signorini *et al.* 1997
Kcnq1	Kv7.1	convulsive	Human Ki T311I A340E	Goldman *et al.* 2009
Kcnq2	Kv7.2	convulsive	Human Ki A306T	Singh *et al.* 2008
Kcnq3	Kv7.3	convulsive	Human Ki G311V	Singh *et al.* 2008
Cyclic nucleotide gated				
Hcn2	Ih hyperpolarization-activated cyclic nucleotide-gated	absence	Ko spontaneous *apathetic*	Ludwig *et al.* 2003; Chung *et al.* 2009
Chloride				
Clcn3	Ichloride	convulsive	Ko	Dickerson *et al.* 2002

Full references can be found in Supplementary information. The neuronal localization of some of the channel proteins is shown in Figure 1.

Various specific digenic interactions have been explored in mouse models, illustrating the prominent additive (Kearney *et al.* 2006; Hawkins *et al.* 2011), and suppressant (Glasscock *et al.* 2007), effects of ion channel mutations on epilepsy phenotypes. These pairwise combinations are highly informative, but still too simple to explain the complex inheritance of human epilepsy.

Recent analysis of ion channel mutation profiles in idiopathic epilepsy reveals a marked complexity, with no two individuals showing sharing gene variant pattern, but similar channel variant pattern diversity, including 'deleterious' variants in known 'monogenic' epilepsy genes, in unaffected individuals (Klassen *et al.* 2011). In mouse models, it is well known, but often not emphasized,

that epilepsy phenotypes, as well as induced seizure thresholds (Frankel *et al.* 2001), are strongly dependent on the genetic background of inbred mouse strains. Thus, the contribution of specific ion channel defects to brain excitability disorders is complex even when they are associated with a clear mendelian disease phenotype in a particular mouse strain or human pedigree. This complexity poses a major challenge to the clinical genetic assessment of the individual's epilepsy risk.

Ion channel plasticity in acquired epilepsy

Temporal lobe epilepsy (TLE), which is the most common form of acquired epilepsy, can be initiated by an insult such as traumatic head injury, febrile seizures or status epilepticus (Engel, 1996), but inflammatory or autoimmune diseases may also be a cause (Bien *et al.* 2007). Following these insults, there is often a 'latent period' before the onset of chronic TLE. The insult preceding TLE can be mimicked in animals by the administration of chemoconvulsants such as kainic acid or pilocarpine to induce status epilepticus (SE), or kindling in which focal seizures are repeatedly induced using electrical stimulation and febrile seizures. Studies both on the mouse tissue and human tissue from TLE patients have provided valuable information on some of the potential mechanisms underlying TLE. In excitatory glutamatergic neurons, the cell surface expression and biophysical properties of numerous voltage-gated ion channels are altered (Table 3), leading to fundamentally altered integrative properties of these neurons.

The expression and function of one particular ion channel, the hyperpolarization-activated cyclic nucleotide gated (HCN) channel, is implicated in these changes. HCN channel activity is reduced in the cortex and hippocampus within a few hours of SE induction in animal models (Shah *et al.* 2004; Jung *et al.* 2011), and the reduction persists for weeks in the models (Shah *et al.* 2012). Furthermore, a decrease in HCN mRNA and current function has also been found in cortical and hippocampal tissue obtained from TLE patients (Bender *et al.* 2003; Wierschke *et al.* 2010), suggesting that decreased HCN channel current is playing a role in epileptogenesis.

HCN channels are cation channels that are activated at potentials more hyperpolarized to −40 mV (Biel *et al.* 2009). They are predominantly expressed in cortical and hippocampal pyramidal cell dendrites where, intriguingly, they reduce pyramidal cell excitability by restricting synaptic integration and excitability (Shah *et al.* 2010). HCN channels, however, are also expressed in certain interneurons (Aponte *et al.* 2006; Dugladze *et al.* 2007; Matt *et al.* 2011) as well as pre-synaptically (Bender *et al.* 2003; Aponte *et al.* 2006; Huang *et al.* 2011).

Thus, does a reduction in HCN channel function *per se* facilitate the onset of chronic TLE? Four HCN subunits (HCN1–4) have been cloned to date. HCN1 subunits are predominantly expressed in the cortex and hippocampus (Biel *et al.* 2009) and HCN1 null mice are more susceptible to seizures induced by chemoconvulsants or kindling, suggesting that a loss of HCN channel function is likely to enhance epileptogenesis (Huang *et al.* 2009; Santoro *et al.* 2010). Further, transiently restoring HCN channel expression by disrupting the interaction between the neuron-restrictive silence factor (NRSF) and HCN1, delays the onset of spontaneous seizure activity (McClelland *et al.* 2011), and several anti-convulsants used for TLE, such as lamotrigine, augment HCN channel function (Poolos *et al.* 2002).

By contrast, in some forms of acquired epilepsy such as that following febrile seizures, HCN channel expression and function is increased in hippocampal pyramidal neurons (Chen *et al.* 2001; Brewster *et al.* 2002; Dyhrfjeld-Johnsen *et al.* 2008). Moreover, dendritic HCN current, I_h, is enhanced in hippocampal pyramidal neurons in mice with targeted deletions in the fragile X FMR1 gene (Brager *et al.* 2012), although FMR1 knock-out mice do not exhibit spontaneous seizures but are more susceptible to audiogenic seizures (Musumeci *et al.* 2000). Similarly, only about a third of the rodents subjected to febrile seizures develop chronic epilepsy (Walker & Kullmann, 1999; Dube *et al.* 2009). Hence, whether I_h upregulation under these conditions is a homeostatic change or epileptogenic remains to be further investigated.

In addition to HCN channels, the mRNA levels and activity of the low-threshold T-type Ca^{2+}, $Ca_V3.2$, channel are transiently elevated in the hippocampus following SE induction (Su *et al.* 2002; Becker *et al.* 2008). Inhibition of these channels is also likely to benefit TLE treatment as seizure frequency and incidence is significantly lowered in $Ca_V3.2$ null mice (Becker *et al.* 2008). Intriguingly, hippocampal mossy fibre sprouting, a hallmark of TLE, is absent following SE induction in $Ca_V3.2$ null mice (Becker *et al.* 2008), suggesting that Ca^{2+} entry through $Ca_V3.2$ channels may have effects additional to alterations in neuronal excitability.

Although there is considerable evidence that HCN and $Ca_V3.2$ channels undergo plasticity very early on in the epileptic process, other channels are likely to be involved in seizure induction. Certainly during chronic TLE, the expression and biophysical properties of a substantial number of K^+ and Na^+ channels are altered, examples of which are shown in Table 3. A better understanding of how ion channel expression and function contributes to epileptogenesis, is important and may lead to more targeted treatments for TLE and other epilepsies.

Ion channel plasticity contributing to epileptic phenotypes may also occur following inherited lesions in non-ion channel gene mutations. A striking example is the aberrant excitability and electrographic seizures identified in models of amyloid precursor protein beta

Table 3. Voltage-gated ion channels that undergo plasticity during acquired epilepsies

Channel/current	Form of epilepsy	Nature of change	Impact on cell excitability	Reference
HCN channel/ HCN current I_h	Temporal lobe epilepsy (TLE)	Sustained reduction in current density following status epilepticus (SE) induction	Enhanced pyramidal and interneuron excitability	Dugladze *et al.* 2007; Jung *et al.* 2007; Marcelin *et al.* 2009; Shah *et al.* 2004; Shah *et al.* 2012; Shin *et al.* 2008; Wierschke *et al.* 2010
HCN channels/ HCN current I_h	Febrile seizure-induced epilepsy	Enhanced HCN channel expression and current	Enhanced rebound activity following inhibitory post-synaptic potentials (IPSPs)	Bender *et al.* 2003; Chen *et al.* 2001; Dyhrfjeld-Johnsen *et al.* 2008
HCN current I_h	Fragile X syndrome	Enhanced HCN channel current	Impaired long-term potentiation (LTP in pyramidal neurons)	Brager *et al.* 2012
$Ca_V3.2$ channels/ T-type Ca^{2+} current	TLE	Transient elevation of expression and current from SE to chronic epilepsy	Enhanced pyramidal cell bursting	Becker *et al.* 2008; Su *et al.* 2002
$K_V4.2$ channels/ A-type K^+ current	TLE	Reduction 1 week after SE and persisting during chronic TLE	Enhanced pyramidal cell dendritic excitability	Bernard *et al.* 2004; Monaghan *et al.* 2008
BK channels	TLE	Reduction in expression during chronic TLE	??	Pacheco Otalora *et al.* 2008
$K_{ir}2$ channels/ inward rectifier current	Chronic TLE	Enhanced expression	Reduced dentate gyrus granule cell excitability	Young *et al.* 2009
KCNN1 (SK1) KCNN2 (SK2) and KCNN3 (SK3) channels	TLE	Transient reduction during chronic TLE	Increased number of hippocampal population spikes	Oliveira *et al.* 2010
Persistent sodium current	TLE	Sustained increase following SE	Enhanced neuronal excitability	Agrawal *et al.* 2003; Chen *et al.* 2011; Epsztein *et al.* 2010; Hargus *et al.* 2011; Vreugdenhil *et al.* 2004

Full references can be found in Supplementary information. The neuronal localization of some of the channel proteins is shown in Figure 1.

(Abeta) overexpression (Palop *et al.* 2007). The seizure activity emanates from the hippocampal and neocortical circuitry and has been described in a wide variety of human and mouse models of Alzheimer's disease (Noebels, 2011). Recent analysis of tissue from Alzheimer's disease patients and a mouse model identified decreased levels of sodium $Na_V1.1$ currents and *SCN1A* subunit expression in parvalbumin-containing interneurons (Verret *et al.* 2012). When these currents were restored in the mouse model by transgenic expression, inhibitory synaptic activity and gamma oscillations were restored, hypersynchrony was reduced and memory deficits were ameliorated, indicating that ion channel-mediated disinhibition makes a strong contribution to epileptogenesis in this model.

Antibodies to voltage-gated or ligand-gated ion channels

Antibodies to AMPAR3 were reported in a few patients with Rasmussen's encephalitis, a devastating

Table 4. Seizure-related syndromes associated with antibodies to ion channels or receptors

Target channel	Antibodies to:	Clinical syndrome	Clinical features	Associated features	Key references
Kv1 complex includes LGI1, CASPR2	LGI1, CASPR2	Limbic encephalitis	Amnesia, change in personality or psychosis, temporal lobe and other seizure types, mainly partial complex seizures	Serum hyponatraemia	Vincent *et al.* 2004; Irani *et al.* 2010
Kv1	LGI1	Faciobrachial dystonic seizures (FBDS)	Brief frequent dystonic seizures usually unilateral often involving the arm and ipsilateral face	Can precede limbic encephalitis Often resistant to AEDs Immunotherapy-responsive	Irani *et al.* 2011
AMPAR1/2	AMPAR1/AMPAR2 mainly	Limbic encephalitis	As above but with more evident psychosis		Lai *et al.* 2009
AMPAR3	AMPAR3 in a few reports, otherwise none defined	Rasmussen's encephalitis	Intractable unilateral seizures with hemiplegia	Patients develop epilepsia partialis continua (EPC)	Bien *et al.* 2004
GABAbR	GABAbR	Limbic encephalitis	As above but often dominated by TLE		Lancaster *et al.* 2010
NMDAR	NR1	Psychiatric features and seizures	Seizures are part of the presentation but are not well defined.	Most patients progress over days or weeks to a complex encephalopathy	Dalmau *et al.* 2011; Niehusmann *et al.* 2009

Full references can be found in Supplementary information. The neuronal localization of some of the channel proteins is shown in Figure 1.

unihemispheric condition in childhood (Rogers *et al.* 1994), but they are not found frequently among cases of this rare epilepsy syndrome (Watson *et al.* 2004). More recently, antibodies to other neuronal surface proteins have been found in patients with seizures presenting as part of a more widespread inflammatory brain disorder (see Vincent *et al.* 2011*a*). The ion channel and related proteins that are the targets for these specific antibodies are listed in Table 4.

Most of the patients have a form of limbic encephalitis – a syndrome that includes amnesia, seizures and psychiatric or behavioural changes, and MRI or cerebrospinal fluid (CSF) evidence of inflammation (Vincent *et al.* 2004). Antibodies that immunoprecipitate dendrotoxin-labelled K_V1 channels extracted from rodent brain tissue, and bind to LGI1, a secreted protein tightly associated with these channels *in situ* (see also Table 1), are also found in a recently described seizure type termed faciobrachial dystonic seizures. These involve brief (a few seconds), very frequent (up to 200 per day), usually unilateral dystonic epileptic events. These seizures may precede the onset of limbic encephalitis, and are frequently anti-epileptic drug (AED) resistant, but seizure frequency is markedly reduced following immunotherapies (Irani *et al.* 2011). Antibodies that immunoprecipitate the K_V1 complexes are also found in a proportion (around 10–15%) of patients with idiopathic forms of epilepsy (McKnight *et al.* 2005; Vincent *et al.* 2011b; Quek *et al.* 2012).

Most antibodies reduce the surface expression of their target by binding divalently to adjacent cell-surface proteins and causing their internalisation – a mechanism that has been shown to apply to NMDAR antibodies binding to hippocampal neurons in culture (Hughes *et al.* 2010). Direct functional effects on ion channel function have not yet been demonstrated, but purified IgG from one patient with K_V1/LGI1 antibodies increased the release probability at the mossy fibre–CA3 synapse, similar to that of dendrotoxin, and suggesting that the antibodies modify K_V1 channel function (Lalic *et al.* 2011). It is still early days and these disorders present many challenges

including how and where the antibodies access the brain parenchyma, whether they induce permanent damage with possible compensatory changes, or changes that are fully reversible, and the relative roles of serum and CSF antibodies.

Challenges and questions for the future

Although a large amount of the data on ion channel defects shed light on the aetiology of seizures in both genetic and acquired epilepsies, there is still a lack of understanding of the precise mechanisms underlying epileptogenesis leading to a chronically epileptic brain. In addition to adding to the cellular and molecular changes that occur in epilepsy, it will be necessary to ask new qualitatively different questions.

How complex are the genetic epilepsies and what is the role of genetic background in defining the disease phenotype? Is it justified to call the familial forms of epilepsy with known gene mutations monogenic? There is no doubt that many single genes are responsible for the respective epilepsy syndromes, as summarised in Table 1, but all these syndromes also show considerable phenotypic variability. For example, mutations with complete loss of function of *SCN1A* are found more frequently in Dravet syndrome than in generalized epilepsy with febrile seizures plus (GEFS+), which is more often caused by missense mutations; however, the opposite relationship has also been described (http://www.molgen.vib-ua.be/SCN1AMutations/). The inconsistant relationship between the phenotype and a single monogenic mutation may be explained in part by other co-expressed ion channel variants in the genomic background, as seen in mouse models. Large sequencing efforts in groups with different phenotypes might be able to detect such variability in humans, but proof-of-concept studies are so far lacking.

Despite the existence of monogenic or major gene effects in some patients (Table 1), in the majority such defects have not been found (Heinzen *et al.* 2012). Search for individual patterns of variability among ion channels and related proteins has not yet provided clear differences between patients and controls (Klassen *et al.* 2011). However, more detailed bioinformatic analyses may demonstrate pathways that are affected more commonly in cases than in controls. In addition, the genetic variation in ion channel genes is only one piece of the puzzle. Variations in copy numbers of several chromosomal regions are significantly more frequent in IGE patients than in controls (Sisodiya & Mefford, 2011), suggesting non-ion channel genetic influences. Time will tell whether whole exome or genome sequencing efforts will be able to clarify a significant part of the genetic origin of complex genetic epilepsies.

Are there neuronal compartments that have been insufficiently examined with respect to epilepsy-related changes? While a large number of beautiful studies have addressed changed properties of somatodendritic compartments, we currently know very little about the local integrative properties of small-calibre dendrites, even though these processes receive the majority of excitatory inputs in most excitatory neurons. It will be necessary to apply techniques suitable for the analysis of small-calibre dendrites such as multiphoton glutamate uncaging and imaging, along with novel techniques for obtaining patch-clamp recording from these processes, to study local integration at these sites. Similar approaches will be required to interrogate other critical excitability compartments, including the axon initial segment and presynaptic terminals. The properties of aberrantly sprouted axons and their collateral terminals involved in seizure-induced neosynaptogenesis also require exploration.

How are changes integrated at the mesoscale (i.e. the level of micronetworks and assemblies of these hundreds of neurons)? The manifestations of all neurological disorders, such as seizures in the case of epilepsy, rely on a disturbed interplay of different neuron types in the CNS. However, in both genetic and acquired epilepsies, we know surprisingly little about the precise changes in excitability and synaptic integration in different types of principal neurons and interneurons. In the case of some genetic epilepsy models, changes in interneurons appear to be crucial in mediating hyperexcitability, but these studies have so far not dissected the role of specific interneuron types (Yu *et al.* 2006; Ogiwara *et al.* 2007). It will undoubtedly be important to understand disease-related modifications in different, well-defined cell types in the CNS. This applies to genetic epilepsy models, the effects of putative disease-related autoantibodies, as well as to acquired epilepsies. Furthermore, it will be necessary to apply and further develop the techniques to record and manipulate the activity of large-scale networks of principal cells and interneurons in normal and diseased brain in order to understand how the different neuron types interact in the epileptic brain to cause aberrant synchronization (Feldt *et al.* 2011).

What is the *in vivo* relevance for changes in ion channels observed *in vitro*? The manifold changes in ion channels, described in part above, predict powerful changes of synaptic integration and neuronal input–output behaviour. However, it would be highly desirable to identify how neuronal firing behaviour *in vivo*

changes in chronic epilepsy. Studies using juxtacellular multielectrode arrays, single-unit, or direct intracellular and patch-clamp recordings as well as *in vivo* imaging techniques will be required to probe the *in vivo* relevance of *in vitro* membrane excitability findings.

What is the role of homeostasis in defining the disease phenotype? Neurons, and most probably all neural cells, have an immense inherent capability for homeostasis, manifested as the ability to conserve functional properties in the face of continuous environmental perturbation and turnover of their proteolipid components. The arsenal of neuronal homeostatic mechanisms includes regulation of both synaptic and intrinsic properties to maintain neuronal functions. In view of this fact, it is perhaps surprising that in epilepsy the underlying ion channel defects are not homeostatically compensated. It will be important to understand fully which homeostatic mechanisms are active under normal excitability conditions in order to identify the conditions under which they fail.

References

Aponte Y, Lien CC, Reisinger E & Jonas P (2006). Hyperpolarization-activated cation channels in fast-spiking interneurons of rat hippocampus. *J Physiol* **574**, 229–243.

Becker AJ, Pitsch J, Sochivko D, Opitz T, Staniek M, Chen CC *et al.* (2008). Transcriptional upregulation of Cav3.2 mediates epileptogenesis in the pilocarpine model of epilepsy. *J Neurosci* **28**, 13341–13353.

Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW & Baram TZ (2003). Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci* **23**, 6826–6836.

Biel M, Wahl-Schott C, Michalakis S & Zong X (2009). Hyperpolarization-activated cation channels: from genes to function. *Physiol Rev* **89**, 847–885.

Bien CG, Urbach H, Schramm J, Soeder BM, Becker AJ, Voltz R *et al.* (2007). Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. *Neurology* **69**, 1236–1244.

Brager DH, Akhavan AR & Johnston D (2012). Impaired dendritic expression and plasticity of h-channels in the fmr1−/y mouse model of fragile X syndrome. *Cell Rep* **1**, 225–233.

Brewster A, Bender RA, Chen Y, Dube C, Eghbal-Ahmadi M & Baram TZ (2002). Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. *J Neurosci* **22**, 4591–4599.

Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ & Soltesz I (2001). Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. *Nat Med* **7**, 331–337.

Dube CM, Brewster AL & Baram TZ (2009). Febrile seizures: mechanisms and relationship to epilepsy. *Brain Dev* **31**, 366–371.

Dugladze T, Vida I, Tort AB, Gross A, Otahal J, Heinemann U *et al.* (2007). Impaired hippocampal rhythmogenesis in a mouse model of mesial temporal lobe epilepsy. *Proc Natl Acad Sci U S A* **104**, 17530–17535.

Dyhrfjeld-Johnsen J, Morgan RJ, Foldy C & Soltesz I (2008). Upregulated H-current in hyperexcitable CA1 dendrites after febrile seizures. *Front Cell Neurosci* **2**, 2.

Engel JJ (1996). Introduction to temporal lobe epilepsy. *Epilepsy Res* **26**, 141–150.

Ernst WL, Zhang Y, Yoo JW, Ernst SJ & Noebels JL (2009). Genetic enhancement of thalamocortical network activity by elevating α1g-mediated low-voltage-activated calcium current induces pure absence epilepsy. *J Neurosci* **29**, 1615–1625.

Feldt S, Bonifazi P & Cossart R (2011). Dissecting functional connectivity of neuronal microcircuits: experimental and theoretical insights. *Trends Neurosci* **34**, 225–236.

Frankel WN, Taylor L, Beyer B, Tempel BL & White HS (2001). Electroconvulsive thresholds of inbred mouse strains. *Genomics* **74**, 306–312.

Glasscock E, Qian J, Yoo JW & Noebels JL (2007). Masking epilepsy by combining two epilepsy genes. *Nat Neurosci* **10**, 1554–1558.

Glasscock E, Yoo JW, Chen TT, Klassen TL & Noebels JL (2010). Kv1.1 potassium channel deficiency reveals brain-driven cardiac dysfunction as a candidate mechanism for sudden unexplained death in epilepsy. *J Neurosci* **30**, 5167–5175.

Goldman AM, Glasscock E, Yoo J, Chen TT, Klassen TL & Noebels JL (2009). Arrhythmia in heart and brain: KCNQ1 mutations link epilepsy and sudden unexplained death. *Sci Transl Med* **1**, 2ra6.

Hawkins NA, Martin MS, Frankel WN, Kearney JA & Escayg A (2011). Neuronal voltage-gated ion channels are genetic modifiers of generalized epilepsy with febrile seizures plus. *Neurobiol Dis* **41**, 655–660.

Heinzen EL, Depondt C, Cavalleri GL, Ruzzo EK, Walley NM, Need AC *et al.* (2012). Exome sequencing followed by large-scale genotyping fails to identify single rare variants of large effect in idiopathic generalized epilepsy. *Am J Hum Genet* **91**, 293–302.

Huang Z, Lujan R, Kadurin I, Uebele VN, Renger JJ, Dolphin AC & Shah MM (2011). Presynaptic HCN1 channels regulate Cav3.2 activity and neurotransmission at select cortical synapses. *Nat Neurosci* **14**, 478–486.

Huang Z, Walker MC & Shah MM (2009). Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. *J Neurosci* **29**, 10979–10988.

Hughes EG, Peng X, Gleichman AJ, Lai M, Zhou L, Tsou R *et al.* (2010). Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *J Neurosci* **30**, 5866–5875.

Irani SR, Michell AW, Lang B, Pettingill P, Waters P, Johnson MR *et al.* (2011). Faciobrachial dystonic seizures precede Lgi1 antibody limbic encephalitis. *Ann Neurol* **69**, 892–900.

Jung S, Warner LN, Pitsch J, Becker AJ & Poolos NP (2011). Rapid loss of dendritic HCN channel expression in hippocampal pyramidal neurons following status epilepticus. *J Neurosci* **31**, 14291–14295.

Kearney JA, Yang Y, Beyer B, Bergren SK, Claes L, Dejonghe P & Frankel WN (2006). Severe epilepsy resulting from genetic interaction between Scn2a and Kcnq2. *Hum Mol Genet* **15**, 1043–1048.

Klassen T, Davis C, Goldman A, Burgess D, Chen T, Wheeler D *et al.* (2011). Exome sequencing of ion channel genes reveals complex profiles confounding personal risk assessment in epilepsy. *Cell* **145**, 1036–1048.

Lalic T, Pettingill P, Vincent A & Capogna M (2011). Human limbic encephalitis serum enhances hippocampal mossy fiber-CA3 pyramidal cell synaptic transmission. *Epilepsia* **52**, 121–131.

Maljevic S & Lerche H. 2011. Epileptogenesis in idiopathic epilepsy. In: Shorvon S, Andermann F, Guerrini R (ed): The Causes of Epilepsy, Chapter 2, Cambridge University Press.

McClelland S, Flynn C, Dube C, Richichi C, Zha Q, Ghestem A *et al.* (2011). Neuron-restrictive silencer factor-mediated hyperpolarization-activated cyclic nucleotide gated channelopathy in experimental temporal lobe epilepsy. *Ann Neurol* **70**, 454–464.

McKnight K, Jiang Y, Hart Y, Cavey A, Wroe S, Blank M *et al.* (2005). Serum antibodies in epilepsy and seizure-associated disorders. *Neurology* **65**, 1730–1736.

Matt L, Michalakis S, Hofmann F, Hammelmann V, Ludwig A, Biel M & Kleppisch T (2011). HCN2 channels in local inhibitory interneurons constrain LTP in the hippocampal direct perforant path. *Cell Mol Life Sci* **68**, 125–137.

Musumeci SA, Bosco P, Calabrese G, Bakker C, De Sarro GB, Elia M, Ferri R & Oostra BA (2000). Audiogenic seizures susceptibility in transgenic mice with fragile X syndrome. *Epilepsia* **41**, 19–23.

Noebels J (2011). A perfect storm: Converging paths of epilepsy and Alzheimer's dementia intersect in the hippocampal formation. *Epilepsia* **52**, 39–46.

Ogiwara I, Miyamoto H, Morita N, Atapour N, Mazaki E, Inoue I *et al* (2007). Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J Neurosci* **27**, 5903–5914.

Palop JJ, Chin J, Roberson ED, Wang J, Thwin MT, Ly NB, Yoo JW, Ho1 KO, Yu1 GQ, Kreitzer A, Finkbeiner S, Noebels JL, Mucke L (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* **55**, 697–711.

Poolos NP, Migliore M & Johnston D (2002). Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. *Nat Neurosci* **5**, 767–774.

Quek AM, Britton JW, McKeon A, So E, Lennon VA, Shin C *et al.* (2012). Autoimmune epilepsy: clinical characteristics and response to immunotherapy. *Arch Neurol* (Epublish ahead of print).

Reid CA, Berkovic SF & Petrou S (2009) Mechanisms of human inherited epilepsies. *Prog Neurobiol* **87**, 41–57.

Rogers SW, Andrews PI, Gahring LC, Whisenand T, Cauley K, Crain B *et al* (1994). Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. *Science* **265**, 648–651.

Santoro B, Lee JY, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA *et al.* (2010). Increased seizure severity and seizure-related death in mice lacking HCN1 channels. *Epilepsia* **51**, 1624–1627.

Shah MM, Anderson AE, Leung V, Lin X & Johnston D (2004). Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. *Neuron* **44**, 495–508.

Shah MM, Hammond RS & Hoffman DA (2010). Dendritic ion channel trafficking and plasticity. *Trends Neurosci* **33**, 307–316.

Shah MM, Huang Z & Martinello K (2012). HCN and K_V7 (M-) channels as targets for epilepsy treatment. *Neuropharmacology* (Epublish ahead of print).

Singh NA, Otto JF, Dahle EJ, Pappas C, Leslie JD, Vilaythong A *et al.* (2008). Mouse models of human KCNQ2 and KCNQ3 mutations for benign familial neonatal convulsions show seizures and neuronal plasticity without synaptic reorganization. *J Physiol* **586**, 3405–3423.

Sisodiya SM & Mefford HC (2011). Genetic contribution to common epilepsies. *Curr Opin Neurol* **24**, 140–145.

Su H, Sochivko D, Becker A, Chen J, Jiang Y, Yaari Y & Beck H (2002). Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *J Neurosci* **22**, 3645–3655.

Veeramah KR, O'Brien JE, Meisler MH, Cheng X, Dib-Hajj SD, Waxman SG *et al.* (2012). De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. *Am J Hum Genet* **90**, 502–510.

Verret L, Mann EO, Hang GB, Barth AM, Cobos I, Ho K *et al.* (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* **149**, 708–721.

Vincent A, Bien CG, Irani SR & Waters P (2011*a*). Autoantibodies associated with diseases of the CNS: new developments and future challenges. *Lancet Neurol* **10**, 759–772.

Vincent A, Buckley C, Schott JM, Dewar BK, Detert N *et al.* (2004). Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. *Brain* **127**, 701–712.

Vincent A, Irani SR & Lang B (2011*b*). Potentially pathogenic autoantibodies associated with epilepsy and encephalitis in children and adults. *Epilepsia* **52**, 8–11.

Walker MC & Kullmann DM (1999). Febrile convulsions: a 'benign' condition? *Nat Med* **5**, 871–872.

Watson R, Jiang Y, Bermudez I, Houlihan L, Clover L & McKnight K *et al* (2004). Absence of antibodies to glutamate receptor type 3 (GluR3) in Rasmussen encephalitis. *Neurology* **63**, 43–50.

Weber YG & Lerche H (2008). Genetic mechanisms in idiopathic epilepsies. *Dev Med Child Neurol* **50**, 648–654.

Wierschke S, Lehmann TN, Dehnicke C, Horn P, Nitsch R & Deisz RA (2010). Hyperpolarization-activated cation currents in human epileptogenic neocortex. *Epilepsia* **51**, 404–414.

Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA *et al.* (2006). Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci* **9**, 1142–1149.

Zhang Y, Mori M, Burgess DL & Noebels JL (2002). Mutations in high-voltage-activated calcium channel genes stimulate low-voltage-activated currents in mouse thalamic relay neurons. *J Neurosci* **22**, 6362–6371.