Emerging insights into the genesis of epilepsy

James O. McNamara

Epilepsies are a diverse collection of brain disorders that affect 1–2% of the population. Current therapies are unsatisfactory as they provide only symptomatic relief, are effective in only a subset of affected individuals, and are often accompanied by persistent toxic effects. It is hoped that insight into the cellular and molecular mechanisms of epileptogenesis will lead to new therapies, prevention, or even a cure. Emerging insights point to alterations of synaptic function and intrinsic properties of neurons as common mechanisms underlying the hyperexcitability in diverse forms of epilepsy.

Epilepsy, a brain disorder that is characterized by recurrent seizures, refers to a collection of disorders that affect 1–2% of the population worldwide. A seizure is a brief change in behaviour caused by the disordered, synchronous and rhythmic firing of populations of neurons in the central nervous system (CNS). Seizures are thought to arise from discharging lesions of the cerebral cortex[1]. The behavioural manifestations of a seizure are determined by the normal functions of the region of cortex in which neurons fire abnormally. For example, seizure-induced firing of neurons in the thumb area of the motor cortex in the right precentral gyrus evokes rhythmic jerking movements of the left thumb. Seizure disorders or epilepsy syndromes have been classified into more than 40 distinct types[2] based upon characteristic symptoms and signs, seizure types, cause, age of onset and electroencephalographic patterns.

The enormous diversity of epilepsy syndromes stands in sharp contrast to Huntington's disease, for example, the cause of which has been linked to mutations of a single gene. The single feature that is common to each of the syndromes is a persistent increase of neuronal excitability that is occasionally and unpredictably expressed as a seizure. Recent advances indicate that some cellular or molecular mechanisms might be common to distinct forms of epilepsy. Progress in molecular genetics has advanced understanding of the molecular aetiology of genetic diseases. Discoveries have pinpointed mutations of genes encoding voltage- and ligand-gated ion channels of neurons as the aetiology of some forms of human epilepsy, thereby implicating alterations of intrinsic properties and/or synaptic function as the principal causal factors. An interesting convergence is emerging as investigations of mechanisms of epilepsies that are caused by cortical damage, with no overt genetic cause, suggest that synapse function may lie at the heart of the increased neuronal excitability. This review considers epilepsies caused both by genetic factors and by cortical damage, and is especially apposite as insights into the underlying mechanisms of epilepsies are emerging at a rapid pace and appeal to a multidisciplinary readership.

Genetics of human epilepsy

Genetic causes contribute to a diversity of human epilepsies. They are responsible for some rare forms inherited in a mendelian pattern (for example, autosomal dominant or autosomal recessive; see later), and are solely or mainly responsible for some common forms such as juvenile myoclonic epilepsy (JME) or childhood absence epilepsy (CAE). In contrast to the rare forms caused by a single mutant gene, JME and CAE are almost certainly due to inheritance of two or more susceptibility genes[3]. Genetic determinants may also contribute some degree of risk to epilepsies caused by damage to the cerebral cortex[3], but the magnitude of this risk is much less than, for example, with JME.

Considerable advances in understanding the genetics of mammalian epilepsy have been made during the past five years. Before 1994, there was a single mouse with an identified gene defect that was linked to a phenotype of cortical epilepsy; since that time, 33 single-gene mutations have been linked to an epileptic phenotype[4]. Comparable progress has likewise been made in the genetics of human epilepsy. Whereas not a single gene causing a form of human epilepsy had been identified a decade ago, mutations of more than a dozen such genes have now been identified. In most of these disorders, epilepsy is symptomatic of a profound disturbance of brain function which can include cognitive impairment, cerebellar dysfunction and other impairments. In some instances these impairments are maximal at birth, whereas in others they arise in adolescence or adulthood and are progressive.

In many of the symptomatic epilepsies, the epilepsy itself seems to be a consequence of some profound neurodegenerative disease. For example, epilepsy is one manifestation of a disorder characterized by the onset in adolescence of progressive cognitive impairment and cerebellar degeneration which is known as Unverricht–Lundborg disease[5]; by contrast, most patients with epilepsy are neurologically normal. But the mechanisms underlying the hyperexcitability in this neurologically devastating disease may differ substantially from the mechanisms that operate in epilepsies in which the patient is otherwise normal (idiopathic epilepsies). One form of symptomatic epilepsy that may shed light on mechanisms of epilepsy in neurologically normal individuals may be some disorders of cerebral cortical development in which the mutant genes have been identified, such as periventricular heterotopia[6]. Because epilepsy can commonly occur as the sole manifestation of a small, localized defect of cortical development, study of the genetics and underlying neurobiology of disorders such as periventricular heterotopia may shed light on mechanisms of epilepsy in neurologically normal individuals.

In contrast to forms of monogenic epilepsy that are symptomatic of some underlying disturbance of brain structure or function, other monogenic forms exist in which epilepsy is the sole manifestation in most or all affected individuals. These forms are termed 'idiopathic' epilepsies owing to the absence of other abnormalities of brain structure or function; the mutant genes causing three idiopathic epilepsies have been identified. Each of the mutant genes encodes an ion channel gated by voltage or a neurotransmitter. An intriguing theme has emerged from this subset, namely that these epilepsies are simply one of many identified paroxysmal disorders. The hallmark of a paroxysmal disorder is that the affected individual is normal most of the time, yet experiences a brief dysfunction of a given organ, often precipitated by stress. This repertoire of paroxysmal disorders includes cardiac arrhythmias, periodic paralyses of skeletal muscle, episodic ataxia and familial hemiplegic migraine. Remarkably, in each instance the mutant gene encodes a component of a voltage-gated ion channel[7].

Generalized epilepsy with febrile seizures plus (GEFS+) is one of

the three idiopathic epilepsies of humans for which a mutant gene has been identified, namely a point mutation in the β-subunit of a voltage-gated Na^+ channel (SCN1B)[8]. GEFS+ manifests typically as febrile seizures before three months or after three years of age; in addition, multiple forms of seizures occur in the absence of fever. The mutant gene was identified by linkage analysis using an autosomal dominant model of a large pedigree, which established a locus on the long arm (q) of chromosome 19 at band 13.1 (19q13.1). A point mutation, a C⟶G transversion at nucleotide 387, was identified that changed a highly conserved cysteine to a tryptophan; this disrupted a putative disulphide bridge that was thought to be critical for maintaining an immunoglobulin-like fold in the extracellular domain. Although recognized as a distinct entity only recently, GEFS+ seems to be fairly common, although the pedigree reported is the only one in which the mutation of *SCN1B* has been identified.

Some clinical features of GEFS+ are of particular interest[9]. The same mutation causes distinct types of seizure, including tonic-clonic, absence, myoclonic and atonic seizures, in different members of the same family. The distinct types of seizure probably result from inheritance of the mutant gene in the context of other susceptibility genes, but the contribution of environmental factors remains a possibility. Some members of the *SCN1B* pedigree who did not inherit the mutant Na^+ channel nonetheless exhibited febrile seizures, providing evidence for the genetic heterogeneity of febrile seizures. The initial characterization of the mutant channel in *Xenopus* oocytes also generated results that may help explain the mechanism by which the genotype produces the phenotype (Box 1).

Benign familial neonatal convulsions constitute another idiopathic epilepsy syndrome for which mutant genes have been identified, namely mutations of two distinct but related, novel K^+-channel genes[10–12]. Inherited in an autosomal dominant pattern, seizures begin typically around day 3 of life and remit weeks to months later. The seizures generally consist of sustained muscle contractions focally or throughout the body and often progress to rhythmic muscle contractions. Neurological development is usually normal. Seizures recur later in life in ~15% of patients, a likelihood that exceeds the risk of the general population by at least sevenfold. Linkage analysis of large pedigrees established a locus initially on chromosome 20q13.3 (ref. 13); subsequently, another locus was established on chromosome 8q24 in distinct pedigrees[14]. Screening of libraries of fetal brain complementary DNA with genomic DNA from the locus led to the discovery of *KCNQ2* as the mutant gene underlying the 20q13.3 form of the disease[10,11]. Identification of expressed sequence tags with a sequence that was homologous to *KCNQ2* led to genomic localization of these tags to chromosome 8q24 and the subsequent identification of *KCNQ3* (ref. 12).

The K^+-channel genes underlying benign familial neonatal convulsions show sequence and functional homology to a previously identified K^+ channel, KCNQ1; mutations of *KCNQ1* cause the cardiac arrhythmia known as the long-QT syndrome and also a cardioauditory syndrome[15,16]. Because potassium channels are tetramers assembled from identical or homologous α-subunits, it seemed possible that KCNQ2 and KCNQ3 co-assemble to form heteromeric channels. Consistent with this concept, northern-blot analyses showed that both of these channels are expressed in the brain, and *in situ* hybridization revealed pronounced overlap in their patterns of expression[17]. The two proteins seem to be associated because immunoprecipitation of either subunit resulted in immunoprecipitation of the other when expressed in *Xenopus* oocytes. Characterization of the functional properties when expressed in *Xenopus* oocytes showed that co-expression of KCNQ2 and KCNQ3 produces currents more than tenfold larger than the homomeric channels alone, implying the formation of KCNQ2/KCNQ3 heteromers. By contrast, no evidence of heteromeric channels of KCNQ2 or KCNQ3 with KCNQ1 was found. Each of the mutations exhibited behaviour consistent with loss of function, the same pattern observed with *SCN1B* in GEFS+. For example, co-expression of mutant KCNQ2 subunit with wild-type KCNQ2 and KCNQ3 at a 1:1:2 ratio led to reduction of currents in the range of 20–40%[17]. Because a single copy of the mutant gene is sufficient to cause benign familial neonatal convulsions, this indicates that a 25% loss of heteromeric KCNQ2/3 channel function may be sufficient to cause the neuronal hyperexcitability in this epilepsy[17].

Autosomal dominant nocturnal frontal-lobe epilepsy is the third form of idiopathic epilepsy for which a mutant gene has been identified. In contrast to the voltage-gated ion-channel genes responsible for GEFS+ and benign familial neonatal convulsions, mutations causing this frontal-lobe epilepsy encode a ligand-gated ion channel, namely the α4 subunit of the neuronal nicotinic acetylcholine receptor (CHRNA4)[18]. Autosomal dominant frontal-lobe epilepsy is a rare form of epilepsy in which the seizures ordinarily begin in childhood, occur at night, are frequent, brief and show predominantly motor manifestations that are occasionally violent. Linkage analysis of a large family from South Australia established a locus on chromosome 20q13.2 (ref. 19). The presence of the CHRNA4 in the same region of chromosome 20q13.2 led to the identification of a point mutation (*S248F*) in affected members of the pedigree[18]. Subsequent analysis of a pedigree from Norway with the same form of epilepsy revealed a distinct mutation in the same gene, namely an insertion of three nucleotides at position 776 (*776ins3*), which encodes a leucine[20]. The mutations in both of these pedigrees affected the same region of the α4 subunit, the putative second transmembrane domain. A second locus on chromosome 15q24 has now been reported[21], which is a region known to contain multiple additional subunits of nicotinic cholinergic receptors.

Characterization of the physiological and pharmacological properties of CHRNA4 has been done using oocytes in which the mutant subunit was co-expressed with the β2 subunit of the neuronal acetylcholine receptor (nAChR); the β2 subunit was selected because α4β2 nAChRs are the most common nAChRs in brain. Characterization of both the *776ins3* and the *S248F* α4 mutants revealed many altered physiological and pharmacological properties compared with wild-type receptors[20,22–24], but a reduction of Ca^{2+} flux through the receptor was common to both mutant receptors. Because at least some nAChRs are thought to be located presynaptically and can promote the release of transmitters such as GABA (γ-aminobutyric acid) from synaptosomes[25,26], a reduction of ACh-mediated Ca^{2+} flux through the mutant receptor may reduce the amount of GABA released from presynaptic terminals and trigger a seizure by synaptic disinhibition[23].

Unanswered questions and future directions. Identification of each of the mutant genes underlying these rare forms of human epilepsy raises similar questions: how does the genotype produce the phenotype? The answers to this question will begin to emerge from characterization of the molecular phenotype in reduced preparations such as *Xenopus* oocytes, as described above. Critical in each instance will be generation of mutant mice expressing the phenotype, and study techniques will need to span all of the neurobiology disciplines and be applied to a spectrum of experimental preparations, including behaving animals *in vivo* as well as reduced preparations isolated from the mutant animals (for example, cultured neurons and cortical or hippocampal slices *ex vivo*). For each of these mutants, addressing these questions is a huge undertaking. If the goal of a research programme is to develop new therapies for large numbers of patients with epilepsy, the sceptic might argue that study of these rare epilepsies is not justified because of the small number of patients afflicted. But insights from the rare forms of human epilepsy may provide new therapies that will benefit large numbers of patients with epilepsy with little or no genetic determinants (Box 1).

Together, these three epilepsy syndromes account for a tiny fraction of all patients with epilepsy, certainly far less that 1%. Many additional, rare mendelian forms of idiopathic epilepsy exist and the search for the genes is likely to succeed. A more challenging

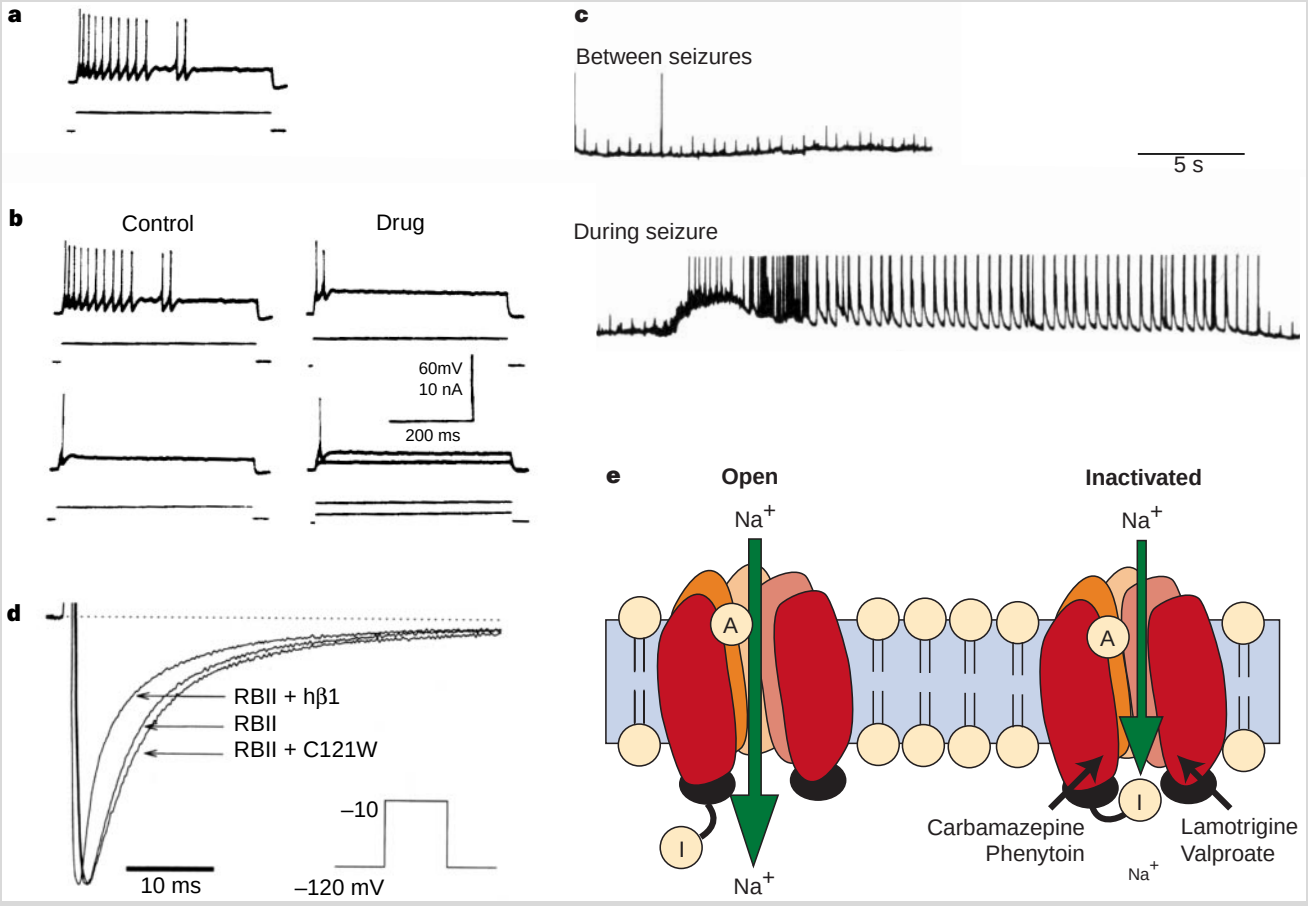
Box 1 *SCN1B*: a lesson for antiseizure drug development?

The discovery of *SCN1B* as the mutant gene underlying GEFS+ is particularly interesting in the historical context of antiseizure drug development. Phenytoin is one of the oldest antiseizure drugs in clinical use. Its antiseizure properties were discovered more than 50 years ago by Merritt and Putnam[81,82], investigators who tested a variety of structural derivatives of the leading antiseizure drug of the time, phenobarbital. They discovered that phenytoin exerted antiseizure effects in an animal model yet lacked the sedative effects of phenobarbital. Approximately 40 years later, phenytoin's cellular and molecular mechanism was determined in studies of cultured spinal-cord neurons[83]. Recordings from these neurons (Box 1 Figure, panels **a**, **b**; reproduced with permission from ref. 83) show that phenytoin selectively inhibits high-frequency trains of action potentials produced by large, but not smaller depolarizations of the neuron. Phenytoin and several other commonly used antiseizure drugs are thought to inhibit seizures by stabilizing the voltage-dependent sodium channel in its inactivated state, thereby limiting sustained repetitive firing of a neuron[83]. Because repetitive firing is the hallmark of a neuron's behaviour during a seizure[84], as observed in intracellular recordings from CA1 pyramidal neurons (Box 1 Figure, panel **c**; reproduced with permission from ref. 85), this action preferentially affects a neuron during a seizure rather than between seizures.

SCN1B encodes a subunit of the Na^+ channel, the molecular target of these antiseizure drugs. Because the antiseizure drugs stabilize the Na^+ channel in its inactivated state, one might predict that a Na^+-channel mutation that caused epilepsy would cause defective inactivation, and the initial characterization of the phenotype of the mutant *SCN1B* supports this prediction[8]. Neuronal Na^+ channels consist of a large α-subunit and two smaller β-subunits; the α-subunit expressed alone exhibits functional properties that are modified by co-expression with the β-subunits. Whereas co-expression of wild-type β1 subunit with the α-subunit in oocytes resulted in Na^+ channels that inactivated more rapidly than when the α-subunit was expressed alone, co-expression of the mutant β1 subunit with the α-subunit resulted in Na^+ channels that inactivated slightly slower than when the α-subunit was expressed alone (Box 1 Figure, panel **d**; reproduced with permission from ref. 8). Because both mutant and wild-type β1 subunits were efficiently translated in an *in vitro* system, the phenotype in the oocyte is unlikely to be due to inadequate expression of the mutant subunit. If the same phenotype occurs in oocytes and neurons, the defective inactivation could fail to limit the sustained repetitive firing of a depolarized neuron and be expressed as a seizure.

If, hypothetically, antiseizure drugs promoting Na^+-channel inactivation had not been discovered, identification of *SCN1B* as the mutant gene underlying GEFS+ would have nevertheless pointed to Na^+ channels as a valuable molecular target for antiseizure drug development. Moreover, drugs in current clinical use are thought to restrict seizures through a limited number of mechanisms, including Na^+-channel inactivation, limiting activation of the T-type of Ca^{2+} channel and enhancing presumed synaptic activation of $GABA_A$ receptors (different drugs are thought to act at pre- or postsynaptic sites)[86]. Thus, discovery of drugs that combat seizures through new mechanisms may enable clinicians to treat the large numbers of patients who do not respond to available drugs. Indeed, it is now clear that antiseizure drugs acting on Na^+ channels are effective in many different seizures, even those with little or no overt genetic cause (Box 1 Figure, panel **e**; reproduced with permission from ref. 86).

Stated differently, the mutant genes underlying rare forms of human epilepsy may suggest molecular targets that could prove useful for many patients with common forms of epilepsy. For example, drugs acting on K^+ channels like KCNQ2 and KCNQ3 could generate a new class of antiseizure drugs. Because a relatively minor reduction of K^+ current may produce epilepsy[17], perhaps a drug that only modestly enhanced the K^+ current could effectively inhibit seizures. Likewise, determining the cellular and molecular mechanisms by which the mutant CHRA4 produces seizures may lead to new pharmacological approaches to combat seizures.



task—for the epilepsies as for diabetes, hypertension and other common diseases—is to identify the susceptibility genes underlying the common forms of epilepsy, such as JME, in which multiple genes must be inherited to produce the phenotype. The success in demonstrating that the ϵ4 isoform of apolipoprotein E increases risk of earlier onset of Alzheimer's disease (see review by Selkoe, this supplement) provides hope that there will be similar success with the epilepsies. Continued cooperation among clinicians, genetic epidemiologists and molecular geneticists, together with progress in sequencing the human genome, identification of single-nucleotide polymorphisms and technical breakthroughs in genotyping and sequencing, will strengthen the likelihood of success.

Mechanisms of partial epileptogenesis

The emergence of epilepsy after injury of the cerebral cortex by traumatic, vascular, infectious and other insults has been recognized by clinicians for centuries, yet scientists lack a clear understanding of the cellular and molecular events initiated by injury that culminate in increased cortical excitability. Typically, a latent interval ranging from weeks to several years elapses between the occurrence of the injury and the emergence of the epilepsy. A diversity of potential mechanisms exist by which a small region or 'focus' of cortex could become hyperexcitable or 'epileptic' after injury, and it seems likely that different mechanisms may operate in different patients or even at different stages of the disease in the same patient. Insight into two such mechanisms has recently emerged.

Autoimmune attack on neuronal antigen

In 1942, Kopeloff *et al.*[27] demonstrated that an immune attack on a foreign antigen injected into monkey cortex could trigger epileptic seizures and hypothesized that an autoimmune mechanism might be operative in epilepsy arising after brain infection or injury. Despite such insightful predictions, more than 50 years passed without convincing evidence for an autoimmune mechanism in any of the epilepsy syndromes confined to the nervous system. But in the past five years, evidence has emerged implicating an autoimmune mechanism in a rare form of human epilepsy, Rasmussen's encephalitis (RE).

RE is a rare, progressive, neurodegenerative illness of unknown cause that typically affects otherwise normal children in the first decade of life[28]. The anatomic distribution of the insult is unusual in that the disease destroys the cortex of a single cerebral hemisphere. The disease is manifest as severe seizures that are refractory to antiseizure medication, loss of motor, sensory, visual and language functions normally subserved by the involved hemisphere, and dementia. The diagnosis is established by these clinical features combined with hemispheric atrophy evident in neuroimaging studies together with a characteristic inflammatory histopathology. Surgical removal or functional inactivation of the involved hemisphere is the standard therapy, leaving the child free of seizures but with a severe neurological deficit.

Glutamatergic synapses are the predominant excitatory synapses in the mammalian CNS. The subset of glutamate receptors (GluR) that subserves most of the fast synaptic transmission is named after their most selective agonist, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid). These receptors are composed of subunits GluR1–4. In the course of raising antibodies against proteins containing part of the extracellular domain of recombinant glutamate receptors fused to a bacterial protein (trpE), behaviours typical of epileptic seizures were observed in two of three rabbits immunized with GluR3 protein[29]. The brains of these two rabbits exhibited the histopathology characteristic of RE, namely perivascular lymphocytic infiltrates confined to the cortex, microglial nodules concentrated in the outer cortex and lymphocytic infiltrate in meninges[29]. By contrast, more than 50 rabbits immunized with fusion proteins containing portions of other GluRs and the β-subunit of nAChR remained clinically normal yet produced appropriate antibody responses.

These data indicated that an immune response directed against GluR3 is sufficient to cause an RE-like disease in experimental animals, and so suggested that RE itself was an autoimmune disease directed against the GluR3 protein. This led to the discovery of a correlation between the presence of RE and serum immunoglobulin-γ (IgG) antibodies to GluR3 detected by immunoblot and immunoreactivity to transfected cells expressing GluR3. Repeated plasma exchanges in a seriously ill child transiently reduced serum titres of GluR3 antibodies, decreased seizure frequency and improved neurological function. Effectiveness of plasma exchanges as treatment of RE was demonstrated in additional patients[30], although the magnitude of the improvement varied substantially among individual patients. These results were extended by subjecting the plasma of an RE patient to Protein A affinity chromatography[31]; instead of removal of all plasma proteins and replacement with albumin, as done previously[29,30], this chromatography method allowed selective removal of IgG and IgM from the plasma. The results implied that the mechanism by which plasma exchange produces improvement involves removal of circulating IgG and/or IgM, an idea supported by the correlation between clinical benefit[29] and reduced titres of GluR3 antibodies[31].

Whether or how the anti-GluR3 itself contributed to the disease manifestations remained to be established. Anti-GluR3 isolated from ill GluR3-immunized rabbits and patients with RE was found to activate the AMPA subtype of glutamate receptor in mouse cortical neurons[32]. Because excessive activation of glutamate receptors can destroy cortical neurons through an excitotoxic mechanism, it seemed plausible that circulating anti-GluR3 might promote excessive activation and actual excitotoxic injury. Previous results[29] were entended by inducing epileptic seizures in a subset of rabbits immunized with a GluR3 protein fused to the distinct bacterial protein, glutathione *S*-transferase (GST)[33]. In contrast to earlier findings[32], no excitatory properties of the sera were detected in whole-cell patch-clamp recordings of cortical neurons, raising the possibility of distinct specificities of the anti-GluR3 in the different rabbits in the two studies. Despite the absence of detectable excitatory effects of the anti-GluR3, robust cytotoxic effects of the anti-GluR3 were found. The mechanism of the cytotoxic effects was not excitotoxic; instead, anti-GluR3 promoted death of cultured cortical cells by a complement-dependent mechanism. Importantly, anti-GluR3 with complement-dependent cytotoxic properties was found in both ill and healthy GluR3-immunized rabbits, demonstrating that cytotoxic anti-GluR3 is not sufficient to produce the illness.

Access of circulating anti-GluR3 to epitopes normally confined to the brain and thus hidden behind the blood–brain barrier must occur if the anti-GluR3 is to contribute to the disease. To test this hypothesis, IgG immunocytochemistry was performed and IgG immunoreactivity was demonstrated on neurons and their processes in the neocortex and hippocampus in ill but not in healthy rabbits[33]. To assess the relevance of the animal model to the human disease, immunocytochemical studies were done on brain tissue removed from RE patients and other epilepsies. Immunoreactivity for IgG, classical complement-pathway protein C4, as well as C8 and the terminal membrane-attack complex (MAC) was detected on neurons and their processes in the cortex of a subset of patients with RE[34]. Together, these results suggest that access of IgG to neuronal epitopes in the CNS triggers complement-mediated neuronal damage and contributes to the pathogenesis of both the animal model and RE.

In addition to this evidence of humoral autoimmunity, evidence for involvement of cell-mediated immunity in RE has also emerged[35]. Using the DNA sequence of the chain of the T-cell receptor as a means of phenotyping T cells, the population of T lymphocytes in RE brain was found to be distinct from that in the systemic circulation. That is, subpopulations of T lymphocytes were overrepresented in the RE brain tissue in comparison to the T

lymphocytes in the blood. This subset may be part of a selective immune attack aimed at one or more antigens. Identification of the antigens targeted by these T cells would be a valuable clue to the immunopathogenesis of the disease.

Unanswered questions and future directions. The results from the study of RE raise many questions. How does the disease start? Why does it progress? Why does the disease remain confined typically to a single hemisphere? The structural homology between the extracellular domain of GluRs and proteins expressed in bacteria[36] raises the possibility that molecular mimicry between a protein expressed in an invading pathogen 'tricks' the immune system into recognizing self as foreign. Evidence implicating a pathogenic role of circulating antibodies implies that a defect of the blood–brain barrier must be present to permit access of these large proteins to neuronal epitopes in the CNS. Perhaps a localized defect of the blood–brain barrier, mediated by focal inflammation or seizures, could promote access of the antibodies and thereby contribute to both lateralization of the disease to a single hemisphere and its progressive destruction.

Can activation of the complement cascade produce the phenotype of epileptic seizures and, if so, how? How might immune attack on a synaptically localized epitope modify the efficacy of synaptic transmission and how might this contribute to the neurological manifestations of the disease? Might immune-mediated damage to the cerebral cortex trigger axonal sprouting and formation of new circuits with increased excitability so that the mechanism of the neuronal hyperexcitability late in the disease is similar to that proposed in the following section? Can passive transfer of the clinical phenotype be accomplished with antibodies isolated from the animal model and from humans with RE? Can the animal model of the rabbit or one established in a mouse or rat be exploited to develop specific immunotherapy for RE? While awaiting development of specific immunotherapy, can optimal use of available immunotherapies obviate the need for hemispherectomy?

Just as insights from rare mendelian epilepsies may prove useful for common epilepsies, insights gained from the study of RE may shed light on the pathogenesis of more common forms of epilepsy and also other diseases of the CNS. For example, might an autoimmune mechanism underlie the pathogenesis of other forms of epilepsy, such as that in the 5% of patients undergoing temporal lobectomy for refractory epilepsy who show localized inflammatory histopathology?[37] Might an autoimmune mechanism underlie the pathogenesis of a subset of other CNS diseases of unknown and potentially diverse aetiologies, such as dementias, psychoses and post-traumatic encephalopathy? Development of a simple, sensitive and specific assay to detect anti-GluR3, which can be used to survey sera quickly and reliably from many individuals, will permit these ideas to be tested.

Hyperexcitability due to axonal sprouting after injury

Ramón y Cajal[38] was the first to show intracortical axonal sprouting of injured neocortical neurons and proposed that this sprouting might increase activity within cortical circuits. Although many investigators have shown sprouting and formation of new synapses after lesions at multiple sites in the CNS, the idea that sprouting promotes epileptogenesis has received increased attention in the past decade.

The form of axonal sprouting most extensively studied in the context of epileptogenesis is 'mossy-fibre sprouting' (MFS), which refers to the synaptic reorganization of the mossy-fibre axons of dentate granule cells into the inner molecular layer of the dentate gyrus of the hippocampus[39–41]. It is the most extensively studied because mossy-fibre axons are readily identified with a histochemical stain for divalent cations, a Timm stain, which detects mossy fibres because of their high content of the divalent cation, zinc. MFS has been documented in humans with a damaged or sclerotic hippocampus, termed Ammon's horn sclerosis[42–44] (Fig. 1a, b), as well as in numerous animal models of temporal lobe epilepsy[39,45,46], but is not seen in normal animals[39,45–48]. The sclerotic hippocampus is thought to be a common cause of drug-resistant epilepsy arising from the temporal lobes of humans for two reasons: a sclerotic hippocampus is found by neuroimaging in most patients and its surgical resection usually cures the epilepsy[49]. This implies the presence of hyperexcitability intrinsic to the hippocampus and raises the question as to potential mechanisms by which the sclerotic hippocampus is hyperexcitable and causes epilepsy.

The discovery of MFS in the sclerotic hippocampus led to its candidacy as a mechanism of the hyperexcitability, an idea that can be outlined as follows. A simplified version of hippocampal circuitry is that it consists of a trisynaptic feedforward excitatory pathway in which information from the entorhinal cortex is transmitted sequentially through a series of excitatory synapses; first to dentate granule cells, from there to CA3 pyramidal cells, and lastly from CA3 to CA1 pyramidal cells[50]. Among brain regions of a normal animal, the hippocampus is prone to exhibit seizures; within the hippocampus, the CA3 pyramidal cells are especially prone to epileptiform activity[51], in part because these cells normally form excitatory synapses with their neighbouring CA3 pyramidal cells. In contrast, seizure-like activity is difficult to induce in normal dentate granule cells (for example, by application of convulsants to slices of hippocampus *in vitro* in which many synaptic connections remain intact). This is partly owing to the lack of recurrent excitatory synapses with their neighbouring granule cells and also because of the presence of strong polysynaptic inhibitory synapses onto the granule cells. Experimental evidence shows that the dentate granule cells limit seizure propagation through the hippocampal network[52,53]. However, the formation of recurrent excitatory synapses between dentate granule cells, as is thought to occur after MFS, may transform the dentate granule cells into an epileptogenic population of neurons, much like CA3, and could promote seizure initiation and/or propagation through the hippocampal pathways.

One key question related to MFS is whether synapses formed by the sprouted mossy fibres actually excite other granule cells. Anatomic studies have documented synapses from sprouted mossy fibres onto granule cells[39–41,43,54], a result that is consistent with recurrent excitatory synapses; but synapses onto GABAergic neurons[47] have also been found, raising the possibility that MFS may promote inhibition of dentate granule cells through a disynaptic pathway. To determine whether sprouted mossy fibres form recurrent excitatory synapses, whole-cell patch-clamp recordings were taken of dentate granule cells in hippocampal slices isolated from kainate-treated animals with MFS or from controls[55]. Excitatory synapses were analysed in isolation from inhibitory synapses by inclusion of the $GABA_A$-receptor antagonist, bicuculline. Activation of the granule cells by locally applied glutamate microdrops triggered abrupt increases in the frequency of excitatory postsynaptic potentials (EPSPs) in 64% of cells from kainate-treated animals but in none of the controls, results that are consistent with the presence of recurrent excitatory synapses between granule cells formed by MFS (Fig. 1c). Furthermore, seizures were evoked by electrical activation of the granule cells in 81% of slices with MFS but in none of the control slices. Together, these results suggest that at least some of the MFS form functional recurrent excitatory synapses between dentate granule cells which, in the presence of reduced inhibition, could promote seizure occurrence. The fact that the animals from which hippocampal slices were isolated only occasionally exhibited spontaneous seizures indicates that intact synaptic inhibition typically holds these hyperexcitable networks in check.

The occurrence of MFS in a sclerotic hippocampus with extensive loss of neurons led to the hypothesis that the stimulus that initiates MFS is death of susceptible neurons, with axons of the surviving

neurons (that is, the dentate granule cells) filling in vacated synapses. Investigators have further hypothesized that seizures themselves can kill the susceptible neurons and thereby set into motion a vicious cycle whereby seizures cause neuronal death which results in MFS which in turn causes more seizures.

Can seizures kill neurons? Continuous seizure activity for tens to hundreds of minutes, a condition known as status epilepticus, has been shown to kill neurons, even in well oxygenated experimental animals[56]. The hierarchy of susceptibility to death among populations of hippocampal neurons is similar to the pattern observed in the sclerotic hippocampus in humans with severe temporal-lobe epilepsy[57], and death involves both necrotic and apoptotic processes[58–60] (see discussion of these processes in the review by Lee *et al.*, this supplement). Magnetic resonance imaging of infants shortly after prolonged seizures has identified an enlarged hippocampus, followed several weeks later by atrophy[61]; thus it seems almost certain that status epilepticus in humans is capable of producing hippocampal sclerosis. But although status epilepticus can clearly kill neurons, whether a brief seizure lasting tens of seconds—or even multiple brief seizures that occur in many patients with epilepsy—is sufficient to kill neurons and produce hippocampal sclerosis is less certain. Quantitative stereological methods were used to assess this issue in the kindling model in which varying numbers of isolated seizures were induced experimentally[62] and a positive correlation was established between decreased neuronal density in the hilus of the dentate gyrus of the hippocampus and increasing numbers of seizures. The reduction of neuronal density was at least partly replicated by other investigators but an increase of hilar volume[63,64]—perhaps due to proliferation of astroglia[64]—rather than a loss of neurons was claimed to be responsible for the decreased density. Whether hundreds of isolated, brief seizures over a period of years is sufficient to induce widespread loss of neurons in the hippocampus is uncertain.

An interesting twist on the 'death by seizure' story has emerged with respect to the hippocampal dentate granule cells in the past couple of years, as evidence seems to favour both 'death and birth by seizure'. In one study[65], 40 seizures induced in the hippocampus by focal stimulation were found to be sufficient to induce apoptosis of a tiny fraction of neurons in the dentate gyrus, presumably the dentate granule cells. A TdT-mediated dUTp nick-end-labelling (TUNEL) assay provided the evidence for apoptosis, as TUNEL-positive nuclei were identified and labelling was eliminated by pre-treatment

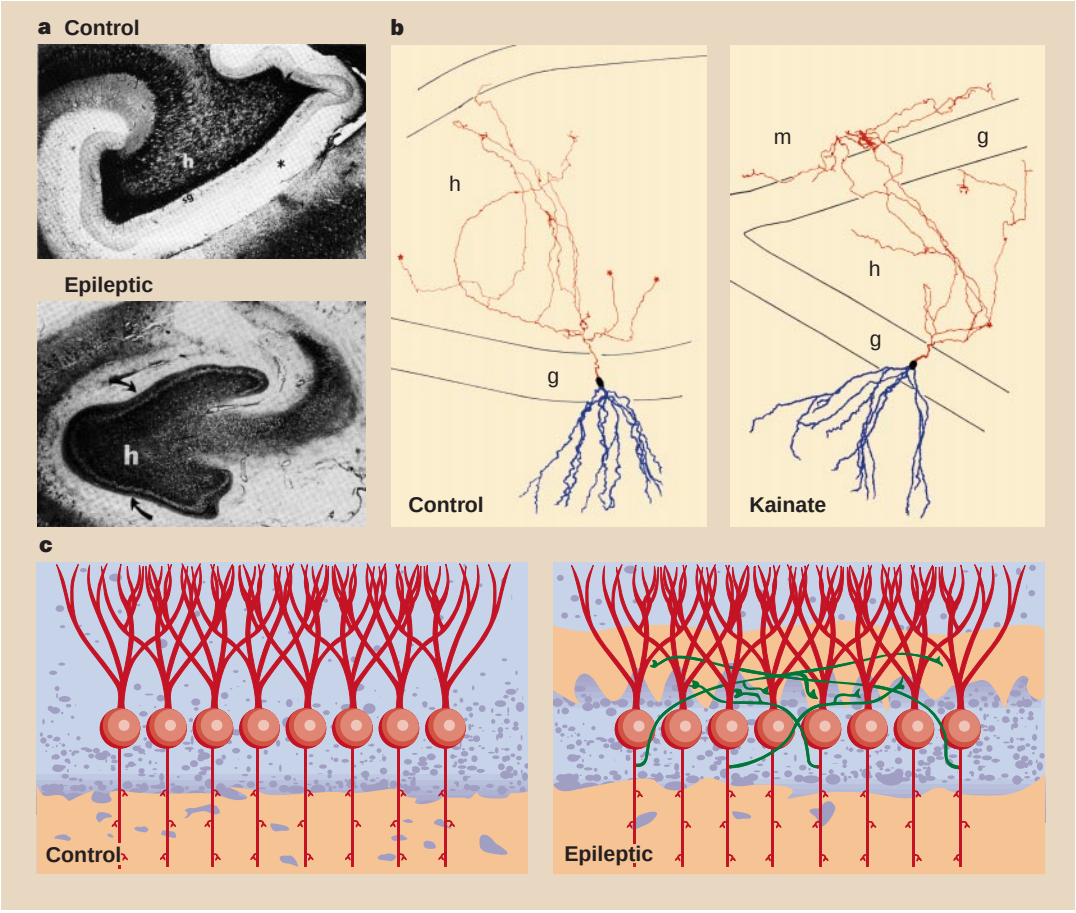


Figure 1 Synaptic reorganization of epileptic hippocampus. **a**, Mossy-fibre reorganization of the epileptic hippocampus. Timm histochemistry of a hippocampus that was surgically removed from a patient with severe epilepsy shows a dense band of Timm staining of presumed mossy-fibre axons of the granule cells in the inner molecular layer of the dentate gyrus (denoted by arrows in bottom panel). By contrast, no detectable Timm staining is observed in this region (denoted by asterisk in top panel) of the dentate gyrus in a control hippocampus from a nonepileptic rhesus monkey; h and sg refer to hilar region and dentate granule-cell layer, respectively. (Reproduced with permission from ref. 39.) **b**, Camera lucida reproductions of biocytin-filled dentate granule cell from normal (left) and kainate-treated (right) rat. Note the extensive ramification of axons in the region in the molecular layer (m) of the dentate gyrus in the kainate-treated, but not the normal rat; this is the predicted single-cell correlate of the Timm histochemistry. g and h refer to the granule-cell layer and hilus, respectively. (Reproduced with permission from ref. 80.) **c**, Recurrent excitatory networks may be formed by reorganized mossy fibres. Schematic representation of dentate granule cells superimposed on the dentate gyrus of a control (left) and kainate-treated (right) epileptic rat. Timm stain (denoted by light brown colouring) reveals the mossy fibres in the hilus of both animals but in the inner molecular layer of the dentate gyrus only in the epileptic rat. The granule-cell bodies and their dendritic trees are evident in both the control and the epileptic rat, but the sprouted mossy-fibre axons are only in the epileptic rat and overlay the Timm stain in the molecular layer; these schematized sprouted axons reflect the putative recurrent excitatory networks formed among the granule cells. (Reproduced with permission from ref. 55.)

with an inhibitor of protein synthesis; the actual death of some neurons was confirmed by silver impregnation studies. Importantly, TUNEL-positive neurons were not detected in brain regions outside of the dentate gyrus, implying that granule cells in particular are sensitive to seizure-induced apoptosis. The same study also showed that a single brief seizure could induce an increased number of TUNEL-positive nuclei, but no silver-stained neurons were detectable; whether the sensitivity of the silver stain was inadequate (and some neurons actually died) or whether the TUNEL-positivity reflects a nonlethal state of DNA repair is uncertain. Despite this uncertainty, the finding that 40 seizures could induce death of presumed granule cells was surprising because study of Nissl-stained sections after status epilepticus showed that the dentate granule cells are less susceptible to 'death by seizure' than their neighbours in the hippocampus such as the CA3 and CA1 pyramidal cells and mossy cells of the dentate hilus[57]. The explanation for this paradox probably involves another form of plasticity specific to the granule cells, namely 'birth by seizure'. In contrast to most neurons in the CNS, neurogenesis of the dentate granule cells persists even into adulthood. Interestingly, seizures induced by a diversity of stimuli increase the rate of granule-cell proliferation, evident in increased labelling of granule cells with bromdeoxyuridine[65–67]. Whether the net number of dentate granule cells remains constant, decreases or even increases in the epileptic brain is undoubtedly determined at least in part by these two competing processes of apoptosis and neurogenesis.

Because seizures increase neurogenesis of dentate granule cells and also promote sprouting of mossy-fibre axons of the dentate granule cells, the question arose as to whether the newborn granule cells were solely or mainly responsible for seizure-induced MFS. Despite virtually complete elimination of granule-cell neurogenesis by whole-brain irradiation, pilocarpine-induced status epilepticus induced robust MFS, equivalent to control rats[68]. Thus MFS arises predominantly from mature rather than newborn dentate granule cells.

The precise factor that initiates sprouting of the mossy-fibre axons of the dentate granule cells into the supragranular region of the dentate gyrus is unknown. One hypothesis proposes that death of susceptible neurons such as the mossy cells of the dentate hilus leads to retraction of terminals on the dendrites in the proximal one-third of the dentate granule cells and that the mossy-fibre axons, and presumably others, 'fill in' the vacated synapses. An alternative hypothesis proposes that seizure activity induces a cascade of gene expression that produces axonal sprouting. In fact, seizures do trigger a cascade of gene expression beginning with immediate early genes that encode transcription factors such as c-*fos*[69,70], followed temporally by genes encoding neurotrophic factors[71,72] and axonal growth-associated proteins such as GAP-43 (ref. 73).

In favour of the first 'neuron death' hypothesis is the fact that the amount of MFS is much greater in both human tissue and experimental models in which extensive neuronal death has occurred. Moreover, combined lesions of distinct anatomic projections to the hippocampus in the absence of recognized seizures produce MFS[74]. In favour of the alternative 'activity' hypothesis is the fact that seizure-induced MFS is detectable in the absence of measurable neuronal loss[63]. Indeed, MFS can be induced by application of brief trains of stimuli that produce long-term potentiation (LTP) but are not sufficiently intense to produce seizures[75]; such non-seizure producing stimulations would not be expected to produce neuronal death.

To test the 'activity' hypothesis, seizure-induced MFS was quantified in mice carrying a null mutation in a gene whose expression is closely regulated by seizures, c-*fos* (ref. 63). The amount of MFS induced by repeated seizures was decreased in mice carrying the null mutation compared with wild-type littermates. No neuronal loss was detected after seizures in either the wild-type or c-*fos*-mutant mice compared with unstimulated control mice[63]. Together, these data support the conclusion that activity, either modest in the form of LTP-inducing stimuli or more intense in the form of seizures, can induce MFS in the absence of neuronal death, and it is likely that the mechanism involves activation of a cascade of gene expression. It seems plausible that both hypotheses may be correct and that the relative contribution of neuron death versus activity may differ depending on the clinical and experimental settings. It will be of particular interest to determine whether 'activity' contributes to the MFS arising after death of susceptible neurons and, if so, by what mechanism.

The ease of identifying mossy-fibre axons with the Timm stain led to the initial detection of axonal sprouting of the mossy fibres, but it seems likely that axonal sprouting with formation of hyperexcitable networks with increased numbers of recurrent excitatory synapses may occur in multiple populations of neurons in the hippocampus and elsewhere in the cortex. Work from several laboratories has established morphological and functional evidence of networks of increased recurrent excitatory synapses in multiple sites. For example, transection of the axons of excitatory CA3 pyramidal neurons in hippocampal slice cultures was shown to trigger formation of new axon collaterals in the CA3 region[76]. Dual intracellular recordings showed an increased probability of excitatory synaptic connections among CA3 pyramidal cells in the sprouted compared with the control condition. Like previous results with MFS[55], inclusion of low concentrations of the $GABA_A$ antagonist bicuculline generally resulted in epileptiform activity in lesioned but not control cultures, demonstrating the epileptiform propensity of the lesioned and sprouted network and also that powerful synaptic inhibition can limit occurrence of seizures for much of the time. Evidence of local networks of recurrent excitatory synapses in additional populations of hippocampal neurons, the excitatory CA1 pyramidal cells, has been identified in slices isolated from kainate-treated animals[77] and in hippocampal-slice cultures[78]. Injury also promotes formation of such local networks in the neocortex, as axonal sprouting of layer-5 pyramidal neurons was identified in slices of neocortex rendered epileptogenic by undercutting the cortex[79].

Unanswered questions and future directions. Although development of circuitry with recurrent excitatory synapses is emerging as a common theme in many experimental models, and potentially also in the sclerotic hippocampus of humans (at least with respect to MFS), the precise extent to which these connections contribute *per se* to the epileptogenesis is uncertain. That is, many potential mechanisms that could cause hyperexcitability may be occurring simultaneously in the models. The fact that mutations of voltage-gated ion channels cause some human epilepsies such as GEFS+ and benign familial neonatal convulsions (BFNC) warrants particular focus on these possibilities. It seems certain that the intrinsic properties of these neurons within the synaptically reorganized network will have a powerful influence on the excitability of the network.

Several questions arise if, as seems likely, the axonal sprouting in one or more sites in the nervous system contributes to the epileptic phenotype of the animal or human. Because the presence of synaptic inhibition seems to prevent the hyperexcitable network from exhibiting seizures continuously, might the periodic occurrence of seizures be caused by brief reduction of synaptic inhibition and, if so, what is the trigger? What are the molecular cues underlying formation of this hyperexcitable network and can these cues be regulated pharmacologically so as to prevent formation of the network? To what extent does the hyperexcitable network subserve recovery of function after brain injury? If the extent is substantial, then inhibiting formation of this network may have deleterious effects. □

James O. McNamara is at the Durham Veterans Affairs Medical Center and the Departments of Medicine (Neurology), Neurobiology, and Pharmacology and Cancer Biology, Duke University Medical Center, 401 Bryan Research Building, Research Drive, Box 3676, Durham, North Carolina 27710, USA.

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