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Molecular mechanisms of epilepsy

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Decades of experimental work have established an imbalance of excitation and inhibition as the leading mechanism of the transition from normal brain function to seizure. In epilepsy, these transitions are rare and abrupt. Transition processes incorporating positive feedback, such as activity-dependent disinhibition, could provide these uncommon timing features. A rapidly expanding array of genetic etiologies will help delineate the molecular mechanism(s). This delineation will entail quite a bit of cell biology. The genes discovered so far are more remarkable for their diversity than their similarities.

Epileptic activity can be induced acutely by blocking synaptic and voltage-gated inhibitory conductances[1,2] or by activating synaptic and voltage-gated excitatory conductances[3]. Seizures are blocked by the opposite manipulations: increasing inhibition[4] or decreasing excitation[5]. Several decades of these types of pharmacological experiments have established the idea that an imbalance between inhibitory and excitatory conductances leads to seizures (i.e., is ictogenic) in otherwise normal brain tissue[6]. This imbalance is most clearly embodied clinically in toxic exposures such as domoic acid, which activates excitatory GluK1 glutamate receptors, or by overdoses of theophylline, which blocks the inhibitory adenosine A_1 receptor[7,8]. In these cases, immediate, repeated and medically intractable seizure activity is induced in otherwise normal subjects. An imbalance between excitation and inhibition is thus a validated ictogenic mechanism. Difficulties arise in extending this mechanism to epileptogenesis—that is, as a mechanism that creates a persistent increase in the probability of spontaneous seizures.

Chronic epilepsy rather than toxic exposure is responsible for the vast majority of seizures in patients, and this condition requires an expansion of the theory of an imbalance between inhibitory and excitatory conductances. This expansion is needed for two reasons. First, unlike the acute exposures, the timing of seizures in chronic epilepsy is unpredictable and seizures are relatively rare, representing much less than 1% of the total brain activity except in the most severe epileptic encephalopathies[9]. Thus, in chronic epilepsy, not only is an ictogenic mechanism required but also it or an additional mechanism must explain the timing of episodic transitions from normal activity to seizures.

The second area of difficulty in applying a theory of imbalanced inhibition and excitation is that the etiology of epilepsy does not usually suggest such an imbalance. In epilepsies arising from a genetic cause, analyses of the genetic etiology have occasionally found causal loss-of-function mutations in inhibitory conductances[10]; however, loss-of-function mutations are also found in several excitatory conductances[11,12], and the majority of causal mutations involve loss of function in genes that do not directly alter the balance of inhibition and excitation[13]. Commercially available diagnostic genetic sequencing services now feature panels of several hundred genes that have been associated with epilepsy. Most of these genes do not encode membrane conductances. This is in line with the intermittent nature of seizures described above; genetic abnormalities that compromise important inhibitory conductances would be expected to continuously alter brain function. Thus, such mutations are most frequently associated with severe epileptic encephalopathies, in which there are no normal epochs of brain activity, and frequent seizures[14–16].

In acquired epilepsies, spontaneous seizures begin after injury to a normal brain as a consequence of trauma, stroke, infection or status epilepticus. Steady-state imbalances in excitation versus inhibition are difficult to demonstrate in established animal models of acquired epilepsy. Damage to inhibitory neurons is compensated for by increases in GABAergic synaptogenesis before the onset of seizures in the pilocarpine model[17]. Compensatory glutamatergic synaptogenesis also occurs[18], but steady-state network imbalances in excitation versus inhibition are not apparent in experimental and human epilepsy[19].

Thus, researchers need to expand the experimentally derived idea of time-invariant ictogenic imbalances between inhibition and excitation to encompass both the timing of seizures and the wide variety of etiologies of human epilepsy.

Timing of seizures

One explanation for the timing of seizures in chronic epilepsy is an episodic shift in the balance of inhibition and excitation, which raises questions about the mechanisms of these shifts. Other possibilities include seizure mechanisms with low probability, for example, circular or reentrant activity that can occur only in network states that exist very rarely. The probability of entry into a seizure under such conditions may not be a direct consequence of the molecular mechanism of ictogenesis. For example, in autosomal dominant nocturnal frontal lobe epilepsy (ADFNLE) arising from gain-of-function mutations in a nicotinic cholinergic receptor, seizures only occur in non–rapid eye movement sleep[20]. Thus, network (brain) states may be an important determinant of seizure timing. Network states that are less probable than non–rapid eye movement sleep may be responsible for proportionately lower frequencies of seizures: for example, in catamenial epilepsy, seizures occur predominantly at specific stages of the menstrual cycle[21]. Although global brain states may help explain changes in seizure probability, they do not directly explain seizure timing.

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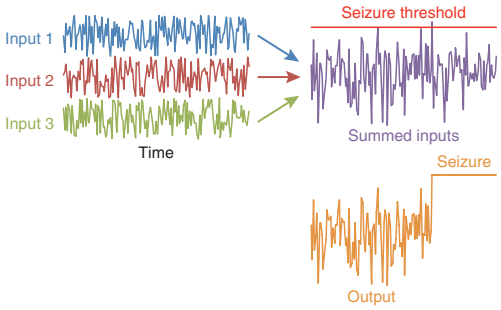

Figure 1 Seizure timing. Episodic surges in network activity may rarely cross a 'seizure threshold' of activity levels at which positive feedback mechanisms such as activity-dependent disinhibition dominate network dynamics. The variance of the summed inputs to the epileptic network is proportional to the sum of the input variances, leading to rare surges of input intensity. In the case of activity-dependent disinhibition, levels of input above this seizure threshold rapidly degrade inhibition in the epileptic network, leading to further increases in network activity. This process culminates in a seizure.

For both ADFNLE and catamenial epilepsy, most of the at-risk periods are characterized by the absence of seizure activity. Thus, in the high-risk periods, additional mechanisms must induce further ictogenic alterations of the balance of inhibition and excitation.

Unpredictable and low-frequency events can be readily generated by low-probability–state transitions in nonlinear systems[22,23]. Although this body of analysis does not lend itself to elucidating molecular mechanisms, it does emphasize that the improbable state itself must be at least temporarily stable. If the ictal state were not stable, there would be continual exits at random times from the seizure state, which generally does not occur: both experimental and human seizure durations are stereotyped, ranging from seconds for absence epilepsy to 1 or 2 min for primary and secondarily generalized tonic-clonic seizures.

One way to explain both low-probability ictal transitions and ictal stability is to incorporate positive feedback into the ictogenic mechanism (**Fig. 1**). For example, if transient imbalances in inhibition and excitation engendered further imbalance, that process could occasionally build to the point of engendering a seizure. Activity-dependent reductions in the efficacy of neuronal inhibition, or activity-dependent disinhibition, comprises one such mechanism or set of mechanisms[24–26]. If disinhibition occurred only at extremes of local network activity, then the probability of ictal transition would depend on the probability of that exceptional level of local network activity. Presumably these levels of activity could occur over the same range of probabilities as those characterizing spontaneous seizures. Once a seizure was induced by an activity-dependent reduction in the balance of inhibition to excitation, the seizure itself could continue to produce activity levels that were sufficient to suppress inhibition or enhance excitation, providing the necessary positive feedback to sustain the seizure.

Molecular mechanisms of activity-dependent shifts

One way to delimit candidate ictogenic mechanisms is to consider the duration of the epochs of elevated network activity that lead to seizures. Some candidate mechanisms can be excluded if one assumes that the ictogenic mechanism must operate on the time scale relevant to the epoch of increased activity. Unfortunately, researchers do not really know how long these high-activity epochs might be. Clues have been provided by inducing seizures in both experimental animals undergoing kindling[27] and patients undergoing electroshock treatment[28]; for such subjects, stimuli of a few seconds are sufficient to engender seizures. The duration of these stimuli represents the lower bound of epoch duration because the applied stimuli synchronously activate many afferents, and such synchrony is unlikely to be engendered by physiological activity.

On a time scale of seconds, short-term synaptic plasticity could be a relevant mechanism of ictogenesis. Potentially ictogenic mechanisms of short-term synaptic plasticity include depression at inhibitory GABAergic synapses onto principal neurons[24] and depression of glutamatergic synapses onto inhibitory neurons[29], as well as facilitation of glutamatergic synapses between principal cells[30] and of GABAergic inhibitory synapses onto inhibitory interneurons[31]. In short, either depression or facilitation, with loci at either GABAergic or glutamatergic synapses, could be proconvulsant depending on the type of postsynaptic neuron. Neurotransmitter vesicle fusion with the presynaptic membrane requires the coordinate activity of a set of proteins subserving docking, priming and fusion of the vesicles as well as calcium entry, sensing and export[32]. Sustained fusion of neurotransmitter vesicles with the presynaptic membrane requires efficient neurotransmitter and vesicle recycling. Mutations in many of these presynaptic proteins have been shown to be associated with epilepsy[33,34], whereas the anticonvulsants pregabalin and levetiracetam interact with presynaptic calcium channels and docking proteins[35]. Although these findings support the idea that abnormal short-term synaptic plasticity could underlie ictogenesis, it has not yet been demonstrated that abnormal short-term synaptic plasticity is necessary or sufficient to engender seizures or epilepsy[36].

Postsynaptic mechanisms of synaptic plasticity acting on slightly longer time scales that are still likely to be relevant to epochs of increased network activity include neurotransmitter reuptake, receptor desensitization, receptor membrane trafficking, and post-translational modifications of receptor subunits affecting transmitter affinity and channel gating. Mutations in proteins subserving some of these functions have been associated with epilepsy. For example, epilepsy is engendered by mutations in the gene encoding the stargazin member of the transmembrane AMPA receptor regulatory protein family that modulates many aspects of AMPA-type glutamate receptor subunit membrane trafficking[37,38], as well as by mutations in genes encoding the LGI1 and ADAM22 proteins that link stargazin to the postsynaptic density[39]. Some of these mechanisms, such as membrane trafficking of NMDA and $GABA_A$ receptors, have been implicated in the pathological prolongation of seizure activity initiated in normal animals by chemical or electrical stimulation[40,41]. Intracellular signaling networks may participate in these activity-dependent shifts in the balance of excitation and inhibition[42,43].

Collectively the epileptogenic mutations in pre- and postsynaptic proteins support the idea that functional shifts in the balance of inhibition versus excitation arising from abnormal synaptic plasticity could transform high but normal levels of network activity into ictal activity. Another prominent mechanism of activity-dependent disinhibition involves dysregulation of ionic concentrations in the intra- and extracellular spaces. Ions that have been implicated in activity-dependent ictogenesis include potassium, calcium, protons and chloride[44–47]. The relevant proteins involved in ionic homeostasis are broadly distributed in the brain, and they include transmembrane channels and transporters in the membranes of the neuronal cytoplasm and subcellular organelles. Mutations in many of the genes encoding these proteins have been associated with epilepsy[15,33,48]. A great deal of experimental work has gone into testing the idea that activity-dependent shifts in ion concentrations contribute to ictogenesis. For example, tetanic stimulation of afferents as well as chemically induced seizure activity result in increases in K^+_o as potassium efflux

overwhelms the ability of NaKATPase to maintain the appropriate cation gradients[46]. The increase in K^+_o shifts the potassium reversal potential to depolarized values so that the resting membrane potential of neurons and co-transport of other ions is compromised. Similarly, large influxes of Ca^{2+} through voltage- and ligand-gated conductances overwhelms the capacity for restorative calcium transport, resulting in reductions in extracellular calcium[49,50], altering both neurotransmitter release probability and the magnitude of calcium-dependent potassium conductances.

Prolonged activation of inhibitory GABAergic conductances can also degrade inhibition as a consequence of excitatory shifts in the $GABA_A$ reversal potential. The GABA receptor is permeable to chloride and bicarbonate, although under most circumstances the higher concentration and permeability of chloride make it the dominant charge carrier[51]. When high levels of GABAergic synaptic activity are sustained for periods on the order of 1 s, the chloride flux through the $GABA_A$ receptor channel overwhelms the transport capacity of the cation-chloride co-transporters that maintain the steady-state transmembrane chloride gradients[52,53]. This is due to the finite maximum velocity of the transporters as well as the finite velocity of NaKATPase, which maintains the appropriate driving forces for the cations whose diffusion provides the energy for chloride co-transport[54]. Under these circumstances, chloride becomes relatively passively distributed so that the driving force for net ion movement through the $GABA_A$ channel is largely due to bicarbonate. The cycle of intracellular hydration of CO_2 to create HCO_3^-, permeation of the HCO_3^- via $GABA_AR$ to the extracellular space and then dehydration to CO_2 supports the continued efflux of HCO_3^- via $GABA_AR$ with no change in HCO_3^- gradient. Free CO_2 diffusion keeps the bicarbonate approximately symmetrically distributed on both sides of the membrane so that bicarbonate currents reverse near 0 mV. Thus, the shift to net bicarbonate flux causes the GABA conductance to become very strongly depolarizing so that instead of inhibition, GABAergic circuits subserve excitation as long as the GABA conductance is in excess of co-transport capacity[52,53,55].

The activity-dependent synaptic and ionic mechanisms of disinhibition described above can result in ictal activity in normal tissue subject to sufficiently prolonged synchronous input *in vivo* and *in vitro*[56,57] (**Fig. 1**). Presumably, healthy individuals do not experience seizures because the activity-dependent changes in synapses and ion concentrations do not cross the threshold needed to generate a self-reinforcing, positive feedback cycle of increased activity, disinhibition and consequent further increases in activity. However, after injury a number of changes may make such cycles more probable. In the undercut model of post-traumatic epilepsy, NaKATPase levels and co-transport capacity are diminished, rendering ion concentrations less stable[58]. Loss-of-function mutations in NaKATPase are associated with epilepsy[59], and pharmacological reductions in NaKATPase activity engender seizure activity in normal tissue[60], supporting the idea that degradations in ionic homeostasis during periods of increased network activity are a key mechanism of ictogenesis.

Setting the stage for activity-dependent shifts

If rare, high levels of network activity create positive feedback cycles that reduce the balance of inhibition and excitation, why isn't everyone epileptic? Has the preceding discussion simply described the processes underlying the generalization of seizures rather than ictogenesis? These questions suggest two possibilities: epilepsy may arise from uncommonly high levels of network activity or from distinct vulnerabilities to activity-dependent shifts in the balance of inhibition to excitation. In the preceding section I focused on vulnerabilities to high but normal levels of synaptic activity. In this section, I consider mechanisms that may lead to uncommonly high levels of network activity.

High, ictogenic levels of network activity could occur more readily in circuits with abnormal feedback caused by cortical dysgenesis or the compensatory rewiring that occurs after brain injury. These circuit alterations are discussed in detail elsewhere in this issue. Here I consider the molecular mechanisms that might drive the formation of such an imbalanced circuit. A number of mutations in the PI3K, IGF and mTOR pathway have been reported in association with brain malformations that are strongly associated with epilepsy, including focal cortical dysplasias, the gliotic tubers of tuberous sclerosis and hemimegalencephaly[61,62]. The mTOR cascade is an important anabolic cellular signaling pathway that is activated by growth factors such as IGF as well as an abundance of metabolic substrates. Activation of this pathway may be a necessary step in the outgrowth of afferent and efferent neural processes[63,64], and overactivation of the mTOR pathway may lead to unnecessary neuronal connectivity and epilepsy. Supporting this hypothesis is the more robust repression of seizure activity by mTOR inhibitors early in development[63,65] when process outgrowth may be more active and potentially rate limited by substrate availability, making synaptogenesis sensitive to anabolic cues, versus more modest effects observed in the mature brain in which process outgrowth is much more limited[66].

The condition of a sufficiently anabolic state is perhaps the most basic limitation on the development of synaptic connectivity, which is further influenced by a wide variety of cues that vary with location, cell type and developmental stage. Accordingly, epileptic circuitry can be engendered by a host of defects that affect neuronal migration[61], process outgrowth[67] and synaptic plasticity[68–70]. For example, the protein product of *ARX*, or aristaless related homeobox gene, is a transcription factor that is an important determinant of neuronal migration. *ARX* mutations have been associated with failure of interneuron migration to their proper cortical target[71]. If a population of interneurons do not successfully complete tangential migration to a cortical circuit owing to mutations in *ARX*, it is likely that the cortical circuit will be chronically mis-inhibited. This is because the populations of interneurons that do successfully innervate this area may not be able to assume all of the duties of the interneurons that did not reach their target. The successfully migrating neurons may not have the appropriate firing properties, calcium buffers or synaptic connectivity to successfully inhibit the network under all circumstances[72]. Such a network would be prone to disinhibition during peaks of network activity, engendering seizures in either the mis-inhibited circuit itself or the downstream synaptic targets of the mis-inhibited circuit. A host of signals regulate interneuron differentiation and migration[73], and mutations in many of these signals are associated with severe epileptic encephalopathies[14].

Interneurons may migrate to the right place in time to form the proper connections but still be unable to provide the levels of negative feedback necessary to prevent ictogenic network activity. An example of this may be the autosomal-dominant SCN1A sodium channel mutation that underlies Dravet syndrome, a serious epileptic encephalopathy that begins in childhood. A leading mechanistic hypothesis is that haploinsufficiency of *SCN1A* results in lower densities of the Nav1.1 sodium channel and that the concomitant reduction in sodium currents and action potential firing causes the most substantial functional compromise in fast-firing GABAergic basket cells[11]. In this scheme, basket cell function is sufficient to regulate network activity under all but the higher range of normal network activity.

Neuronal homeostasis

There are many epilepsies associated with neuronal loss and attributable to either brain injury or degenerative disorders. The strategies that neurons use to rewire circuits are even less well understood than the strategies used to wire the original circuits during development. Neuronal homeostasis refers to the process by which neurons regulate their excitability. Thus, in conditions that engender excess activity, neurons downregulate excitatory conductances and upregulate inhibitory conductances; the reverse is true under conditions of chronically reduced excitation[74]. After injury to a brain circuit, these mechanisms would be an important means to prevent the development of overactive neural networks. Under most circumstances, this is successful: even after severe brain injury, the majority of patients do not develop epilepsy[75]. One reason that some patients develop epilepsy may be that the mechanisms underlying neuronal homeostasis fail to prevent the development of a seizure-prone network[58,76].

Although there are substantial empirical data supporting the idea of neuronal homeostasis, the molecular mechanisms are not well understood[74]. Presumably the wiring and rewiring of neuronal circuits are governed by neuronal homeostasis. Just as neurons may change their complement of voltage-gated membrane proteins to maintain firing after deafferentation, the formation and weighting of new synapses after injury to inputs may be driven by similar mechanisms. Because these mechanisms of neuronal homeostasis are unknown, researchers do not know how, or whether, seizures affect neuronal homeostasis. Although ictal activity levels are high, the rarity and brevity of seizures may reduce their impact on the feedback mechanisms governing synaptic homeostasis. For example, a daily seizure lasting 1 min may be a catastrophe for the patient but implies an ictal duty cycle of less than 0.1%. The duration of epochs of activity necessary to influence neuronal homeostasis are not known. If the epileptic circuit is substantially deafferented as a consequence of injury or dysgenesis, such that inter-ictal activity is too low, it may be that the overall activity of the epileptic circuit (including seizures and epochs of postictal depression) averages to a level that is within the acceptable range of the homeostatic mechanisms. Or it may be that ictal hyperactivity and the characteristic postictal depression average to an acceptable level of activity, and the processes that regulate neuronal homeostasis average the two periods together. Epilepsy might also arise from defects in the homeostatic mechanisms, for example, an inability to assign an appropriately negative weight to synaptic solutions that result in seizures.

Because researchers do not have a good idea as to which intracellular signaling networks regulate neuronal activity levels, genetic epilepsies may provide our best clues as to what some of the necessary elements of this network might be. Many of the genes whose mutations are linked to epilepsy are involved in intracellular signaling and thus could be involved in neuronal homeostasis[13].

Neuronal homeostasis gone awry

A second approach to deciphering molecular mechanisms of neuronal homeostasis is to consider genes for channels that must be exquisitely regulated to maintain normal neuronal excitability. Researchers first derived the concept of neuronal homeostasis by considering the consequences of transient pharmacological derangement of voltage-gated sodium channels and ligand-gated $GABA_A$ channels[77]. Are there genetic 'experiments of nature' that correspond to these ideas? I mentioned Dravet syndrome before as an early epileptic encephalopathy resulting from mutations in genes encoding ion channels, most commonly *SCN1A*, which encodes Nav1.1 sodium channels[11], although causal genes may also include presynaptic proteins and ligand-gated $GABA_A$ channels[78].

Loss-of-function mutations in sodium channel genes would be expected to underlie disorders of sensation, movement and cognition rather than epilepsy. However, a wide range of loss-of-function mutations in sodium channels result in epilepsy. As discussed earlier, a clever proposed pathogenesis is that fast-firing inhibitory interneurons are disproportionately affected by some of these mutations, and substantial experimental data support this idea[11]. However, why loss of a single allele is sufficient to cause disease is not known; at a molecular level, there is no evidence that the truncated protein exerts a dominant negative effect in experimental models[79]. This raises the possibility that the mutation alters the expression of sodium channels in the cytoplasmic membrane by interfering with the feedback mechanism neurons use to sense and adjust the sodium current density. There is some evidence for such a mechanism, in that sodium currents are elevated in induced pluripotent stem cell–derived neurons bearing human sodium channel mutations, although the developmental stage at which researchers measured currents was still very far from mature and the sodium current density was much lower than in the mature animal[80]. Nevertheless, this experiment raises the possibility that it is not haploinsufficiency that is the core problem but rather dysregulation of sodium current density that results in higher currents at some developmental stages and lower currents at others. This modulation of intrinsic excitability, along with the circuit alterations discussed earlier, are key elements of neuronal homeostasis[74]. It is possible that careful consideration of the molecular pathogenesis of epilepsies such as Dravet syndrome will provide important clues to the nature of the mechanisms of neuronal homeostasis, as well as the means by which they may be altered in some genetic forms of epilepsy.

The variety of etiologies of epilepsies

At the time of the last major review of the molecular mechanisms of epileptogenesis, researchers had only identified 13 genes known to cause epilepsy, and all of those genes coded for ion channels[68]. This was before the era of rapid, inexpensive gene sequencing, so the predominance of ion channels was in retrospect a function of what genes scientists were sequencing. Unbiased sequencing and copy number studies have uncovered over 400 genes closely associated with epilepsy[13]. The bulk of the mutations associated with epilepsy are not in inhibitory or excitatory ion channels, so the mechanisms by which mutations lead to seizures and epilepsy need to be broadened. It is possible that entirely new mechanisms of epilepsy will be discovered as a consequence of investigations into the cell biology of these newly discovered gene defects. However, it can also be argued that researchers will find instead a wider variety of ways to engender the core mechanism of episodic, self-reinforcing shifts in the balance of excitation and inhibition. In this scenario, new genes will be found to act, both singly and in concert with other factors, to influence network structure in a way that leads to episodic disinhibition and seizures. For example, there are many factors that determine the stability of network connectivity, including the survival of neurons, that may be linked to epilepsy. As an example, the intractable epilepsies associated with neurodegenerative diseases[81] such as neuronal ceroid lipofuscinoses[82] can be most readily explained as network instabilities engendered by loss of connectivity, including loss of neurons. Thus, the molecular mechanisms of the epilepsies may be considered as a diverse set of pathways leading to a final common pathophysiology of a self-reinforcing cycle of activity-dependent disinhibition.

These genetic experiments of nature have provided researchers with a wide variety of pathways to epilepsy. Where does that leave neuroscientists doing 'experiments of the laboratory'? The positive feedback mechanisms that underlie the distinctive timing features of seizures can now be approached by network studies made possible by new technologies in microscopy, activity-dependent biomarkers, optogenetics and computation. The timing of seizures is also ripe for study, with improvements in electrode technology, telemetry, signal analysis and seizure detection. Finally, every gene discovered has a complex cellular biology that will take some time to unravel. With luck, the large numbers of discovered genetic mutations can be exploited to develop categories of genetic etiologies. Those categories in turn may be very helpful in focusing researchers' pathophysiological investigations. I will not try to guess those categories at this stage of gene discovery, but I look forward to the point at which categorization becomes feasible.

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