

Targeting Epilepsy Genes

Minireview

Jeffrey L. Noebels

Developmental Neurogenetics Laboratory
Department of Neurology
Section of Neurophysiology
Baylor College of Medicine
Houston, Texas 77030

Had epilepsy never existed, it would have been rediscovered eight times over in the past year by those intent on knocking out mouse genes one at a time. Adding extra copies of genes worked too. Feared since ancient times for their unpredictable appearance in humans, seizures have suddenly found an unusual welcome as visible markers of cerebral dysfunction in transgenic mice. The high incidence of the phenotype was less surprising than the broad range of functional defects represented, since the gene errors for most human epilepsies are unknown and it is unclear whether they arise from a few common mutations or many rare ones. What can be learned from each of these newly created epilepsy models in mice? Do they pinpoint new excitability mechanisms and novel targets for human therapy, or do they lead the way to cures for diseases that don't exist?

First, a definition, since the epilepsies comprise a specific subset of cerebral dysrhythmias, rather than an inevitable result of any defect in membrane excitability or synaptic strength. Epilepsy is a disorder of recurrent, spontaneous episodes of aberrant synchronization in neural networks that can remain local, spread to other sites, or engage all cortical regions simultaneously. Neocortical or hippocampal circuits are always activated during an epileptic seizure; synaptically linked downstream regions are recruited in ways that serve to amplify or limit the discharge in time and space. Epilepsy is not myoclonus, a discharge usually arising in subcortical circuits that gives rise to a sudden unitary or rhythmic muscle contraction involving the body, limbs, or a limited muscle group. Nor is it the appearance of a seizure following a stimulus, since the capacity for afterdischarge is inherent in all higher nervous systems and is readily potentiated. The classification of inherited epilepsy syndromes has helped to differentiate between seizure phenotypes that arise at different ages, show characteristic electroencephalograph (EEG) or behavioral patterns, or exhibit specific anticonvulsant sensitivity. The pathophysiology of the bursting circuit in different syndromes is of particular interest, since a large number of defects can enhance excitability in neural networks, but fewer can also account for the prolonged periods of normal brain function characteristic of many seizure disorders. Two principal challenges are to explain the conditional behavior of the phenotype and to find the correct molecular targets for the large fraction of human lesions that are inexplicably resistant to current anticonvulsants.

Selecting the Candidates

Targeted mutations open the possibility of tracing the

steps taken by gene errors to destabilize neural networks; the first practical issue is which gene to select. In vitro studies have defined a pyramid of molecular candidates for reversible epileptiform bursting in neurons, beginning with ion channels and the receptors that gate them. Abnormal modulation of these primary currents by derangements in the extra- and intracellular ionic and second messenger environment provides the next layer of potential gene modifiers; the broad base of genes for developmental network assembly and plasticity expands the list of plausible targets for epilepsy candidate loci to many hundreds of alleles. While it could be argued that deleting all of the prime candidates for inhibition, (e.g., genes for repolarizing K^+ channels and GABA receptors) and screening for epileptic phenotypes would be superfluous, the developmental attributes of these null subunit syndromes cannot be predicted, and in some (see below) epilepsy does not occur. Conversely, structural point mutations in depolarizing ion channels might be anticipated to lead only to irreversible bursting rather than to a stable epileptic phenotype. However, paroxysmal phenotypes have arisen spontaneously in muscle channelopathies and could easily do so in brain. At this early stage of analysis, none of the large number of mapped spontaneous epilepsy mutants in mouse and man has been found to map to chromosomal positions of genes for the dominant modes of neuronal inhibition.

When a gene for epilepsy is identified, what more do we need to know? The recent elegant descriptions of Na^+ , K^+ , and Cl^- channel defects in striated and cardiac muscle excitability syndromes seem to imply that in some systems only the position of the mutated codon and the resultant channel biophysics are required to solve the disease phenotype. But there are other variables. How else can we account for the individual differences in onset, penetrance, and progression of the same inherited mutation when examined on different genetic backgrounds, a phenomenon seen both in human and in mouse mutants alike? Much of this phenotypic jitter must depend on compensatory gene activity; therein lies the major source of uncertainty in localizing the origin of epilepsy amidst the plasticity of the nervous system.

Whether or not the fortuitous mutant models actually correspond to naturally occurring genes for human epilepsies, they define key sites of vulnerability in the nervous system's formidable array of inhibitory control mechanisms. The fascination now rests in analyzing the categories of molecules they encode (Table 1) and in pursuing the essential question: how does the excitability defect lead to intermittent cortical dysrhythmia? Those mutations that perturb multiple steps are perhaps the most important to evaluate, since it is these intervening mechanisms, rather than the targets we already understand, that offer a cascade of new molecular entry points to reverse the lesion in the developing brain prior to the clinical onset of epilepsy.

Table 1. Single Gene Deletion/Overexpression Mutants with Epileptic Phenotypes

Gene	Protein	Age of Onset	Postnatal Lethality	Reference
Spontaneous seizures				
Deletions				
5-HT _{2c}	Serotonin receptor	<5 Weeks	+	Tecott et al., 1995
Synapsin I, II	Vesicle protein	>2 Months	-	Rosahl et al., 1995
CaMKII α	Ca ²⁺ /calmodulin protein kinase		-	Butler et al., 1995
TNAP	Nonspecific alkaline phosphatase	2 Weeks	+	Waymire et al., 1995
Centromere BP-B (ins)	Brain-specific DNA binding protein	3-4 Months	-	Toth et al., 1995
mKv1.1	K ⁺ channel	2 Weeks	+	Smart et al., 1995
Weaver (<i>spon</i>)	G protein-coupled inward rectifier (GIRK2)	Adult	+	Patil et al., 1995
GluRB	Q/R site editing of glutamate receptor Subunit	2 Weeks	+	Brusa et al., 1995
Overexpression				
GAP-43	Neural growth-associated protein	>4 Weeks	-	Aignier et al., 1995
<i>Plp</i>	Myelin proteolipid protein	<4 Weeks	+	Kagawa et al., 1994
No seizures, increased threshold for epileptogenesis				
<i>fyn</i>	Tyrosine kinase receptor			Cain et al., 1995
TPA	Tissue plasminogen activator			Tsirka et al., 1995
BDNF (-/+)	Brain-derived neurotrophin			Kokaia et al., 1995

Targeting Intrinsic Excitability: Ion Channel Genes

K⁺ channel blockers are potent convulsants; the deletion of mKv1.1, 1 of 16 murine homologs of the *Shaker* gene, eliminates a delayed rectifier K⁺ channel, resulting in a lethal epilepsy phenotype (Smart et al., 1995, Soc. Neurosci., abstract). The mKv1.1 subunit is expressed widely in the neuraxis, with a subcellular localization that includes the presynaptic axon terminal. Thus, loss of this repolarizing current should potentiate transmitter release. Once the extent of other potential patterns of activity-dependent abnormal gene expression is defined and correlated with seizure onset, this mutant may provide one of the most parsimonious experimental models of cellular epileptogenesis.

The gene for the *weaver* (*wv*) mutant has been found to be a brain G protein-coupled inward rectifier K⁺ channel (GIRK2) containing a missense mutation replacing a Gly with a Ser at residue 156 (Patil et al., 1995). The substitution lies in a conserved transmembrane domain of GIRK2 where permutations occlude or alter channel conductance, but the effects on cellular membrane properties are still unknown. The expression of GIRK2 in neocortex is compatible with the appearance of seizures in *wv* heterozygotes but does not explain their late onset. The severe cellular migration defect seen in *wv* homozygotes may signal the contribution of secondary developmental network defects; however, even striking architectonic rearrangements are not necessarily epileptogenic; *reeler* mice, which have a severe migration defect in cortical structures, show no evidence of behavioral or electrographic seizures (Noebels, 1995).

Targeting the Synapse: Release Mechanisms and Transmitters

Vesicle proteins mediate the probability of transmitter release and provide a novel site and mechanism for inherited epilepsy. Deletion of the synapsin I and II genes leads to nonlethal spontaneous seizures in mice; the double mutant shows the most severe disorder (Rosahl et al., 1995). Vesicle numbers are evenly reduced in both symmetric and asymmetric cortical synapses, providing

ultrastructural evidence against the interesting possibility that seizures might arise through preferential loss of inhibitory transmitter release in the neocortex.

Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) phosphorylates an array of proteins critical to neuronal signaling, including the synapsins, and is abundant in neocortex and hippocampus. Mice homozygous for a null mutation of the CaMKII α subunit show spontaneous seizures generalizing from a limbic EEG discharge and lethal stimulus-induced amygdalar seizures (Butler et al., 1995). The hippocampal hyperexcitability is associated with the reduction of two forms of long-term plasticity at these synapses. One point of convergence for the synapsin and CaMKII null mutants involves their participation in the plasticity of evoked transmitter release. Interestingly, synapsin deletion reduces hippocampal posttetanic potentiation with no effect on long-term potentiation, while CaMKII deletion shows the opposite behavior. Whether either of these patterns contributes to the underlying mechanism of epileptogenesis has not been resolved.

Reduction in GABAergic neurotransmission produces seizures; this mechanism was triggered from a distance by targeting pyridoxal phosphate (plp) metabolism with a deletion of the gene for tissue nonspecific alkaline phosphatase (Waymire et al., 1995). Absence of the enzyme (which converts plp to pl, the nonphosphorylated form of vitamin B6 that crosses cell membranes, where it is subsequently reconstituted by pyridoxal kinase) results in a 20-fold elevation of plp serum levels and large reductions in intracellular brain plp levels. Plp is an essential cofactor for many enzymes, catalyzing reactions involving α amino acids, including glutamic acid decarboxylase, which synthesizes GABA. Significant brain GABA reductions are present at 2 weeks postnatal in the TNAP^{-/-} mutant, followed by lethal tonic-clonic convulsions. Rescue of the seizure phenotype and correction of GABA levels can be achieved with daily pyridoxal treatment. This mutant provides a highly instructive genetic model of vitamin B6-deficient seizures.

Targeting Synaptic Receptors: Excitatory and Neuromodulatory

Two new mutants provide unexpected examples of how mutations in the ionic selectivity or permeability of a postsynaptic receptor can lead to a progressive epilepsy syndrome. One mutation targets the enzyme that mediates the posttranscriptional conversion of a genomically encoded Gln codon to an Arg codon in the mRNA of the GluRB receptor. The wild-type conversion results in a charged Arg residue within the second hydrophobic domain of the GluRB (AMPA-type) receptor subunit, the element responsible for the low divalent cation permeability of the heteromeric AMPA-sensitive glutamate receptor. The intron immediately downstream is essential for accurate editing. When this site is eliminated, the mutant mouse expresses reduced levels of edited GluRB subunits; the resultant increase in Ca^{2+} permeability through AMPA channels produces altered patterns of secondary immediate-early gene expression (Brusa et al., 1995). Beginning in the third postnatal week, heterozygous mutants show spontaneous recurrent behavioral seizures suggestive of a limbic origin; all mice die by postnatal day 20 and exhibit neuronal degeneration within this region.

Targeted deletion of the 5-HT_{2c} gene, which encodes a G protein-coupled serotonin receptor, results in lethal spontaneous seizures in male hemizygotes beginning in the fifth postnatal week (Tecott et al., 1995). In wild-type mice, both agonists and antagonists of the 5-HT_{2c} receptor alter induced seizure parameters in a manner consistent with the null mutation; however, brief pharmacologic blockade of the receptor does not elicit spontaneous seizures, suggesting that the primary defect in serotonergic neuromodulation may still not be solely responsible for the epileptic trait.

Targeting the Network: Axon Terminal Growth, Synaptogenesis, and Glia

Gene overexpression mutants complement deletion strategies to alter the balance of excitability in neural circuits. Postnatal overexpression of the growth cone-associated protein GAP-43 induces mossy fiber axon terminal sprouting in hippocampus after 4 weeks of age, followed by the onset of spontaneous seizures (Aignier et al., 1995). While it is tempting to conclude that excess recurrent excitation in the hippocampus alone might provoke seizures, other synapses are undoubtedly affected; evidence for interneuron loss in the mutant suggests that hyperinnervation may lead to cell death and disinhibition in some circuits. Interestingly, the corresponding GAP-43 null mutant develops axon pathfinding defects that should also result in excitatory synapse imbalance; however, these mutants do not exhibit a spontaneous seizure phenotype (Strittmater et al., 1995).

Cytokines are important mediators in pathophysiologic processes, and astrocytosis is a prominent feature of human epileptic foci. Overexpression of interleukin-6 (IL-6), under the control of the glial fibrillary acidic protein promoter sequence, produces a degenerative seizure syndrome in mice (Campbell et al., 1993). Since inflammatory cytopathologic responses include the dramatic up-regulation of many cytokines, this strategy may provide a set of experimental gene models that isolate

specific steps in posttraumatic and postinfectious seizure disorders. Targeting oligodendroglia by overexpression of wild-type copies of the myelin proteolipid protein gene causes a demyelinating syndrome leading to tremor and convulsions (Kagawa et al., 1994). In these glial mutants, the clinical onset and neurological severity are tightly dependent on transgene copy number.

Human Epilepsy Mutations

New human epilepsy syndromes continue to be mapped (Ryan, 1995), including a recent report in *Neuron* (Eksioglu et al., 1996) of a gene locus for periventricular neuronal heterotopias with seizures, but the basis for aberrant synchronization in these lesions remains elusive. The first example of an autosomal gene defect involving a membrane excitability protein, the nicotinic acetylcholine receptor $\alpha 4$ subunit, has been identified by positional candidate cloning of a single pedigree with a dominant frontal lobe seizure syndrome beginning in childhood (Steinlein et al., 1995). The missense mutation replaces Ser with Phe at codon 248 in the second transmembrane domain, coincident with the position where other site-directed substitutions have impaired pore function. The subunit is expressed in frontal neocortex, but there is little evidence linking the inhibition of nicotinic receptors to paroxysmal bursting, and the widespread distribution of the subunit in the remainder of the brain does not explain the limited zone of hyperexcitability. Mice homozygous for a $\beta 2$ subunit gene deletion lack high affinity nicotine binding in the brain, and the neurons are insensitive to applied nicotine. These mutants have not been reported to show seizures, and based on behavioral testing in an associative memory task, appear, interestingly, to perform better without them (Picciotto et al., 1995).

These examples highlight the recurring concern of whether, even in the case of a missing single channel subunit, the excitability phenotype can be directly explained in terms of the mutant channel behavior without looking for new and potentially deleterious patterns of cellular plasticity. Like the molecular homeostatic response of supersensitivity that follows synaptic deafferentation, the targeted loss of an endogenous source of depolarization in the membrane may trigger the nucleus to invoke new mechanisms of maintaining excitability. A more clear understanding of the intervening steps between the loss of $\alpha 4$ nicotinic receptor subunits and paroxysmal EEG patterns awaits the construction of a homologous mutant mouse model.

From Target to Therapy

In medieval art it was traditional to show a demon emanating from a suffering epileptic at the conclusion of a seizure, with concern clearly evident regarding its inevitable reappearance. In this century, anticonvulsant drugs have been developed for their ability to control, rather than prevent or reverse, seizure disorders. Has the strategy for a rational search for definitive therapy of certain epilepsies finally unfolded, and is that goal within reach? What is clear is that few inherited epilepsies will be correctly interpreted in transgenic mutants without assessing specific compensatory pathways; these intermediate steps may provide the most realistic insight into modifying the disease process. Phenotypic rescue by replacement of a missing gene product after

the onset of seizures, where possible, provides an important a posteriori test to discriminate primary from secondary mechanisms in mice, but in clinical syndromes the damage may already be done.

Nevertheless, the extraordinary bounty of epilepsy models from geneticists' laboratories will continue to provide new and otherwise unobtainable parts of the puzzle. There are more such models to come, and they will both confirm predictions and yield surprises, such as the absence of an epileptic phenotype in mice lacking the Kv3.1 K⁺ channel (Ho and Joho, 1995, Soc. Neurosci., abstract), and the GABAR β 3 subunit (Culiat et al., 1995). Transgenic expression of human mutations in mouse models will allow critical epileptogenic steps to be traced and assigned to appropriate categories, while the mutants themselves serve as reproducible biological test systems for therapeutic discovery. Ultimately, these epilepsy models will not only help relieve the human condition but also provide insights into gene control of more subtle fluctuations of excitability, synchronization, and plasticity in neural networks.

Selected Reading

- Aignier, L., Arber, S., Kapfhammer, J.P., Laux, T., Schneider, C., Botteri, F., Brenner, H.-R., and Caroni, P. (1995). *Cell* 83, 269–278.
- Brusa, R., Zimmerman, F., Koh, D.-S., Feldmeyer, D., Gass, P., Sakmann, B., Seeberg, P.H., and Sprengel, R. (1995). *Science* 270, 1677–1680.
- Butler, L.S., Silva, A.J., Abeliovich, A., Watanabe, Y., Tonegawa, S., and McNamara, J.O. (1995). *Proc. Natl. Acad. Sci. USA* 92, 6852–6855.
- Cain, D.P., Grant, S.G.N., Saucier, D., Hargreaves, E.L., and Kandel, E.R. (1995). *Epilepsy Res.* 22, 107–114.
- Campbell, I.L., Abraham, C.R., Masliah, E., Kemper, P., Inglis, J.D., Oldstone, M.B., and Mucke, L. (1993). *Proc. Natl. Acad. Sci. USA* 90, 10061–10065.
- Culiat, C.T., Stubbs, L.J., Woychik, R.P., Russell, L.B., Johnson, D.K., and Rinchik, E. (1995). *Nature Genet.* 17, 344–346.
- Eksioglu, Y.Z., Scheffer, I.E., Cardenas, P., Knoll, J., DiMario, F., Ramsby, G., Berg, M., Kamuro, K., Berkovic, S.F., Duyk, G.M., Parisi, J., Huttenlocher, P.R., and Walsh, C.A. (1996). *Neuron* 16, 77–87.
- Kagawa, T., Ikenaka, K., Inoue, Y., Kuriyama, S., Tsujii, T., Nakao, J., Nakajima, K., Aruga, J., Okano, H., and Mikoshiba, K. (1994). *Neuron* 13, 427–442.
- Kokaia, M., Ernfors, P., Kokaia, Z., Elmer, E., Jaenisch, R., and Lindvall, O. (1995). Suppressed epileptogenesis in BDNF mutant mice. *Exp. Neurol.* 133, 215–224.
- Noebels, J.L. (1995). Gene Control of Cortical Excitability. In *The Cortical Neuron*, I. Mody and M. Gutnick, eds. (New York: Oxford University Press), pp. 210–229.
- Patil, N., Cox, D.R., Bhat, D., Faham, M., Myers, R.M., and Peterson, A.S. (1995). *Nature Genet.* 17, 126–129.
- Picciotto, M.R., Zoli, M., Lena, C., Bessis, A., Lallemand, Y., LeNovere, N., Vincent, P., Pich, E.M., Brulet, P., and Changeux, J.-P. (1995). *Nature* 374, 65–67.
- Rosahl, T.W., Spillane, D., Missler, M., Herz, J., Selig, D.K., Wolff, J.R., Hammer, R.E., Malenka, R.C., and Südhof, T.C. (1995). *Nature* 375, 488–493.
- Ryan, S.G. (1995). *Nature Genet.* 10, 4–6.
- Steinlein, O.K., Mulley, J.C., Propping, P., Wallace, R.H., Phillips, H.A., Sutherland, G.R., Scheffer, I.E., and Berkovic, S.F. (1995). *Nature Genet.* 11, 201–203.
- Strittmater, S.M., Fankhauser, C., Huang, P.L., Mashimo, H., and Fishman, M.C. (1995). *Cell* 80, 445–452.

- Tecott, L.H., Sun, M.S., Akana, S.F., Strack, A.M., Lowenstein, D.H., Dallman, M.F., and Julius, D. (1995). *Nature* 374, 542–545.
- Toth, M., Grimsby, J., Buzsaki, G., and Donovan, G.P. (1995). *Nature Genet.* 11, 71–75.
- Tsirka, S.E., Gualandris, A., and Amaral, D.G. (1995). *Nature* 377, 340–344.
- Waymire, K.G., Mahuren, J.D., Jaje, J.M., Guilarte, T.R., Coburn, S.P., and MacGregor, G.R. (1995). *Nature Genet.* 11, 45–51.