Prediction by Modeling That Epilepsy May Be Caused by Very Small Functional Changes in Ion Channels

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Objective: To use computer simulation to perform a "genetic sensitivity" analysis to predict which genes are best positioned to increase risk as well as to predict functionally how variants in these genes might increase network excitability.

Methods: A previously published, biophysically realistic model of the dentate gyrus that included mossy fiber sprouting between granule cells was used to model putative environmental changes associated with temporal lobe epilepsy. Properties of voltage-gated ion channels, either 1 at a time or in combinations, were varied systematically to determine their effect on network excitability.

Results: We found that the network is most sensitive to changes in steady-state voltage dependence of activation and relatively insensitive to changes in inactivation. Changes in sodium channels had the greatest effect on excitability, followed by changes in fast-delayed rectifier potassium channels and then N-type calcium channels. We also investigated the effects of simultaneous small changes in several ion channels, modeling a complex genetic background expected for common epilepsies. A combination of 2 or 3 simultaneous voltage shifts in steady-state activation as small as 2 mV could produce large changes in network excitability.

Conclusion: Statistical power calculations indicate that changes this small are effectively undetectable with current experimental practices, thus posing new challenges for the functional analysis and validation of epilepsy genes.

Arch Neurol. 2009;66(10):1225-1232

Many single-gene mutations that cause epilepsy have been identified but these cause only a small percentage of idiopathic epilepsies. In most cases, the inherited causes of epilepsy are believed to be additive or multiplicative effects of an unknown number of genes, sometimes interacting with environmental factors. Identifying genes that underlie complex diseases is challenging and, although both common and rare variants underlying generalized epilepsies have recently been discovered, these account for only a tiny fraction of the attributable genetic risk. Furthermore, though screening technology is improving and a number of projects are under way to collect data from large populations, there is still a problem of how to perform functional analyses of candidate susceptibility genes. Even in the case of monogenic mutations, functional analysis lags significantly behind mutation discovery. The initial functional analysis of mutations is usually done using patch clamp electrophysiology in heterologous expression systems, with reported functional changes being large and obvious; this, however, misses other, potentially important cell biological effects such as trafficking deficits and subtle changes in function that would escape detection. More informative techniques such as in vitro or in vivo viral transfections or knockin animal models are costly, time consuming, and cannot meet the demands of genetic screening studies. In the case of susceptibility genes, the interpretation of data is likely to be extremely difficult because, by definition, several susceptibility variants (as well as potential environmental influences) are required to cause a disease; the effects of any 1 variant are likely to be small. Furthermore, it is possible that phenotypic expression as an epilepsy is not simply an additive effect of several susceptibility polymorphisms or rare variants, but requires an epistatic interaction between them.
With few epilepsy susceptibility variants yet identified, it is not possible to say which classes of genes are predominant and what biophysical functions are affected. Common variants will have small physiological effects, and the current study addresses this issue by limiting the magnitude of parameter variation below that seen in monogenic epilepsies. Monogenic epilepsies almost exclusively involve ion channels, so it is a reasonable starting assumption that variants in ion channel genes will modify seizure susceptibility; this is supported by initial evidence in epilepsies with complex inheritance. It would be helpful to predict in which genes variants are likely to have a bigger effect and how large the biophysical changes are likely to be in the resulting proteins. The flexibility of biophysically realistic computer modeling can make these specific predictions. This can be done by performing a sensitivity analysis in which electrophysiological properties of ion channels are systematically varied and input/output relationships are used as an assay of network excitability. Environmental influences can similarly be introduced into the network, such as mossy fiber sprouting in the dentate gyrus (DG) that occurs in temporal lobe epilepsy. In this way, the interactions of multiple genetic variants, each with minor functional change, can be studied in isolation or in the presence of environmental factors.

The specific network that we studied is the DG because insult models of temporal lobe epilepsy such as kainic acid kindling or head trauma cause hyperexcitability, possibly because of mossy fiber sprouting. Using a published network model, we have previously studied the effects of changes in sodium channel electrophysiological properties similar to those seen in monogenic epilepsies. In the absence of mossy fiber sprouting, mutation-like effects reduce the ability of the network to block the transmission of ictal activity to deeper hippocampal structures. Electrophysiological changes caused by sodium channel mutations interact with mossy fiber sprouting to reduce the degree of sprouting required to cause seizure-like activity in the DG. However, in common cases of epilepsy, several genetic changes are required, each of which is insufficient to cause symptoms individually. Here we used the same model DG network, with and without mossy fiber sprouting, as an assay to predict which combinations of genetic variants cause hyperexcitability and to estimate the magnitude of these differences.

METHODS

We used a previously published and extensively studied model of the DG that contains morphologically realistic models of the predominant cell type, granule cells, and other excitatory and inhibitory neurons. The network consisted of 500 granule cells, 6 basket cells, 15 mossy cells, and 6 hilar perforant-path associated cells (HIPP). Granule and mossy cells are excitatory (α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate [AMPA] transmission), while basket and HIPP cells are inhibitory (transmission to γ-aminobutyric acid [GABA] type A receptors). Neuron models had between 9 and 17 compartments describing the dendritic arbor and realistic conductances including the fast sodium and potassium channels that directly form the action potential, an A current, L-, N-, and T-type calcium channels, hyperpolarization-activated, cyclic nucleotide-gated (HCN) current, and slow-voltage and calcium-gated potassium channels. Network connectivity is as follows: each HIPP cell contacts 160 granule cells, 4 mossy cells, and 4 basket cells. Each basket cell contacts 100 granule, 3 mossy, and 2 other basket cells. Each mossy cell contacts 200 granule, 3 other mossy, 1 basket, and 2 HIPP cells. In the absence of mossy fiber, sprouting granule cells contact 1 mossy, 1 basket, and 3 HIPP cells. In networks with mossy fiber, sprouting granule cells make spatially localized contacts with other granule cells. The degree of mossy fiber sprouting is quantified as a percentage, with 100% corresponding to 100 connections from each granule to other granule cells. Mossy fiber connections were generated randomly for each run.

The model was mathematically identical to model No. 51781, available from ModelDB (http://senselab.med.yale.edu/modeldb/). To mimic the effect of epilepsy-causing mutations, channel gating was modified by shifting the activation or inactivation curves (ie, $V_{1/2}$ → $V_{1/2} + \Delta V_{1/2}$, where $V_{1/2}$ is the voltage of half activation or inactivation). Curves were shifted by ±1 to 5 mV individually. In addition, all curves were shifted by ±1 to 2 mV in conjunction with a similar shift in activation of sodium channels. To constrain parameter space searching, we have limited combinations of shifts to values smaller than those noted for monogenic epilepsies. Models were simulated using our own software (http://www.evan-thomas.net/parplex), and code is available from ModelDB (http://senselab.med.yale.edu/modeldb/).

RESULTS

CONTROL NETWORKS

In the absence of both altered channel gating and mossy fiber sprouting, the network was relatively unexcitable (Figure 2, A and B), consistent with experimental observations. Perforant path stimulation applied to the central 100 neurons generated a single action potential in each stimulated neuron but did not generate subsequent action potentials nor action potentials in other neurons. As mossy fiber sprouting increases, neurons in the stimulus region were able to fire several action poten-
As sprouting continued to increase, activity was able to propagate out of the stimulus regions. At 20% sprouting, activity propagated throughout the network and neurons fired for about 50 to 75 milliseconds at each location (Figure 2, C and D). This reproduces the observations of Santhakumar et al.24

NETWORK SENSITIVITY TO CHANGES IN A SINGLE GATING PARAMETER

Variants are likely to differ in a number of gating parameters simultaneously; however, our previous modeling has shown that neurons are much more sensitive to changes in steady-state voltage dependence of activation and inactivation than they are to changes in gating rates.16,17 To understand the sensitivity of the network to hyperpolarizing or depolarizing shifts in voltage dependence, we systematically shifted the $V_{1/2}$ of activation and inactivation curves individually for each voltage-gated channel type by ±1 to 5 mV uniformly in all neuron types. This determines where the voltage sensitivity of these processes lies without affecting the rate of that process. Shifting the voltage sensitivity in the negative direction increases the probably that channels will enter that state for a given membrane potential.

NETWORKS WITHOUT MOSSY FIBER SPROUTING

Because networks without mossy fiber sprouting are unable to maintain sustained activity, we stimulated these networks with trains of constant frequency, random synaptic input from the perforant path. Each synaptic event generated an action potential in the target neuron. Plots of network activity are shown in Figure 3. The channel types in which changes had the largest effects on network excitability were sodium channels, slow delayed rectifier potassium channels, and N-type calcium channels. Shifts in activation in these channels as small as 1 mV were able to make measurable differences to network response. However, similar shifts in inactivation of sodium channels and N-type calcium channels and in activation or inactivation of other channels types did not have measurable effects on network responses.

In the case of sodium channels, a negative shift increased inward current, which drives the membrane potential in the positive direction. Shifting the voltage dependence of activation of potassium channels in the positive direction reduces outward current which, in turn, slows membrane hyperpolarization. In the case of calcium channels, the effect of shifting the voltage dependence of activation in the positive direction is to reduce

Figure 2. Responses in control networks with and without mossy fiber sprouting. A, Neurons 200 through 299 were stimulated in a network without mossy fiber sprouting, and the membrane potential trace for granule cell number 250 is shown. The action potential is a direct response to the stimulus at $t=3$ milliseconds. B, A raster plot of action potentials from the network without mossy fiber sprouting is shown. C and D, The same data as A and B are shown for a network with 20% mossy fiber sprouting.
the amount of calcium entry. Reduced calcium entry reduces activation of calcium gated potassium channels, again reducing outward current.

NETWORKS WITH MOSSY FIBER SPROUTING

We tested shifts of ±1 to 5 mV in voltage dependence of activation and inactivation in networks with varying degrees of mossy fiber sprouting. The network was stimulated with a single superthreshold perforant path input to the central 100 neurons of the network. Raster plots of some responses in networks with 20% sprouting are shown in Figure 4. Left shifts in sodium channel activation had the most profound effect on excitability, followed by right shifts in the voltage dependence of slow delayed rectifier potassium channels and N-type calcium channels. Increasing excitability increased the duration of the poststimulus activity. Changing the voltage dependence of inactivation of sodium channels or N-type calcium channels or changing either activation or inactivation of other channel types had a negligible effect on network excitability (not shown). These results are consistent with observations in networks lacking mossy fiber sprouting.

CUMULATIVE EFFECTS OF MULTIPLE SMALL CHANGES

We wanted to test how several small (2 mV) shifts in activation interacted. Networks were most sensitive to changes in sodium channel activation so we combined a 2-mV hyperpolarizing shift in sodium activation with a ±2-mV shift in activation or inactivation of other ion channels with varying degrees of mossy fiber sprouting. The combination of changes that produced the greatest network excitability is shown in Figure 5. In the case of networks lacking mossy fiber sprouting, the combination of shifts reduced the ability of the network to accommodate sustained input. Networks with mossy fiber sprouting showed greater excitability in both the duration of the response and the distance the response traveled in the network. As the shifts were applied cumulatively to the networks, the greatest change in excitability is caused by the shift in the voltage dependence of sodium channel activation followed by the shift in activation of the N-type calcium channels.

EXPERIMENT NUMBERS NEEDED TO DETECT SMALL CHANGES

Previous studies in our laboratory measured the standard deviation of $V_{1/2}$ for sodium channel activation (118 cells) as 4.9 mV. To detect a 2-mV difference between 2 means using a $t$ test with a significance of .05 and a power of 0.8, 75 cells from each group would be needed and 300 cells from each group would be needed to detect a 1-mV difference.

COMMENT

Single-gene mutations cause only a tiny minority of epilepsies and, even in families with these mutations,
penetrance is rarely complete. It is well recognized that epilepsy involves multiple gene interactions and environmental influences.3,13 Discovering risk factor genes is currently challenging, but with new large-scale epilepsy genetic consortia and new sequencing technologies, it is likely that this will change dur-

Figure 4. Effects of changes in ion channel gating in networks with mossy fiber sprouting. In the raster plots, each dot represents an action potential. Networks have 20% mossy fiber sprouting. A brief synaptic input is applied to neurons 200 through 299. Manipulations to the $V_{1/2}$ of steady state activation are indicated for each plot.
ing the next few years. Even so, it will be helpful to develop techniques that can predict which genes have the most influence on network excitability and predict the mechanisms by which they alter excitability. Moreover, whether discovered by genome-wide association or deep sequencing strategies, new genetic variants require functional validation before their pathogenicity can be definitely accepted. Knowledge of the functional consequences of mutations in monogenic disorders and risk variants in polygenic forms remains a major bottleneck in fully understanding the genetics of epilepsy. Traditional experimental techniques will not resolve this problem. Making transgenic animals is extremely slow and costly. Furthermore, transgenic animals do not necessarily allow easy dissection of cause and effect in the epileptogenic process. Viral transfection of brain slices and cultures is limited by the size of the gene and potential confounding issues caused by overexpression of the introduced protein. Heterologous expression systems offer the best throughput but require modeling to extrapolate to neurons and networks. There are currently no proposed solutions to this problem and, as more polymorphisms are discovered, this situation will become more severe.

The genetic sensitivity analysis of the DG network with mossy fiber sprouting performed here addressed 3 questions: (1) what voltage-gated ion channel genes have the biggest influence on network excitability, (2) what changes in their electrophysiological properties have the biggest influence on network excitability, and (3) what is the magnitude of these changes likely to be? We found that the network is most sensitive to changes in sodium channel properties. Our previous modeling has shown that changes in activation of sodium channels can affect both firing threshold and firing frequency. We found that networks are more sensitive to changes in the sodium channels than in other channel types. Changes in sodium channel activation that increase availability enhance excitability by prolonging the duration of the response to a focal stimulus and increasing the spread across the network. The network was also sensitive to changes in rapidly activating potassium channels that influence action potential firing frequency. In the case of these channels, decreasing availability exacerbates excitability by increasing firing frequency. Changes to the steady-state voltage dependence of activation of N-type calcium channels had a paradoxical effect on network excitability. Increasing calcium current causes a depolarization that could potentially increase excitability but is countered by calcium-dependent activation of potassium channels that ultimately causes neuronal and network inhibition.

It is worth noting that DG hyperexcitability is associated with temporal lobe epilepsy, while most genetic data, either for monogenic epilepsy or susceptibility genes, relates to generalized epilepsy syndromes, which predominantly involve cortical and thalamic networks. The prediction that variants in sodium and delayed rectifier channels are likely to confer risk is consistent with findings that mutations in genes cod-
ing these channels can cause monogenic generalized epilepsies.\textsuperscript{1} These channels are highly expressed in all neurons and are essential for action potential firing. On the other hand, there are no reports of epilepsy risk being conferred by variants in N-type calcium channels. This may be that the expression profile of these channels means that they are not well positioned to cause generalized epilepsy. However, in addition to being in the right place to cause a disease, a risk-confering gene needs to be able to support variation. It may be that variants in certain genes are more likely to be fatal or otherwise prevent spread in the population. Alternative explanations are that variation in N-type channels is smaller than for other genes or that these biophysical changes may be relevant to familial forms of temporal lobe epilepsy rather than generalized epilepsies. Further, neurons in this network only express HCN channels at low levels and do not express M currents. To predict the effects of changes in these ion channels, simulations would need to be performed in other networks such as CA3 or cortical networks.

Risk-confering polymorphisms must interact with other polymorphisms and often also with environmental influences. Our simulations, with simultaneous changes to multiple channel types, provide a concrete example of how this might work. In this case, 3 hypothetical polymorphisms that increase excitability are present. Mossy fiber sprouting, triggered by an environmental insult (or genetic cause), may trigger an epileptogenic cascade in individuals with this genetic background but may be benign in other individuals. If, on the other hand, mossy fiber sprouting is caused by excessive activity, individuals with the wrong genetic background are more likely to drive mossy fiber sprouting, thus starting a positive feedback epileptogenic process. Mossy fiber sprouting is generally believed to contribute conditionally to the emergence of recurrent excitation in the dentate gyrus,\textsuperscript{30} although there are contrary views that sprouting may enhance inhibitory circuits and that sprouting is not necessarily key to epileptogenesis. However, the model shows that networks with high levels of recurrent excitatory feedback, which include cortical networks, are sensitive to specific changes in ion channel gating. Thus, the principles presented here, generated from data on a dentate model, should generalize to other epilepsies arising in other networks.

The main finding of this study is how small the shifts in the voltage dependence of activation needed to be. Shifts as small as 3.2, or even 1 mV can have significant effects on network excitability. These differences are hard to detect experimentally using standard patch clamp or 2-electrode voltage clamp assays. Based on the variance seen in data from our laboratory, 150 cells would be needed to detect a 2-mV shift in activation and 600 cells to detect a 1-mV shift with a significance of $P=0.05$ and 80% power. Functional studies are not able keep pace with the rate of discovery of gene mutations for monogenic epilepsies in which gene effects are likely to be large. This problem is compounded in the study of polymorphisms or rare variants, where the rate of discovery is likely to exceed that of single gene mutations and the smaller gene effect size demands higher numbers of experiments. This combinatorial effect means that it is highly unlikely that current manual biophysical approaches will be able to meet these demands. However, high-throughput automation of functional assays is likely to permit significant progress to be made in our understanding of how subtle effects of variants contribute to the common epilepsy syndromes.

Accepted for Publication: June 9, 2009.

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Author Contributions: Study concept and design: Thomas, Reid, and Petrou. Acquisition of data: Thomas. Analysis and interpretation of data: Thomas, Reid, Berkovic, and Petrou. Drafting of the manuscript: Thomas. Critical revision of the manuscript for important intellectual content: Thomas, Reid, Berkovic, and Petrou. Statistical analysis: Thomas. Obtained funding: Thomas. Administrative, technical, and material support: Thomas.

Financial Disclosure: None reported.

Funding/Sponsor: This study was supported in part by an Australian National Health and Medical Research Council (NHMRC) Peter Doherty Fellowship (Dr Thomas); a University of Melbourne R. D. Wright Fellowship and NHMRC grant 454655 (Dr Reid); and NHMRC grant 400121.

REFERENCES


**Announcement**

New Initiatives: Clinical Trials and Videos. We have embarked on 2 new initiatives: Clinical Trials and video presentations. We welcome manuscripts that describe double-blind, randomized, placebo-controlled clinical trials as our primary area of interest. We plan on expediting the review process and time to publication and to include them online ahead of print as these studies are time sensitive and of direct benefit to our patients. We hope you will take advantage of this new initiative. Please refer to the Instructions for Authors when submitting a Clinical Trials paper, including the requirement to register the trial with an accepted clinical trials site.

We plan to utilize videos as part of published papers that highlight and provide convincing information about the observational and visual features of a patient’s neurologic findings. Please refer to Instructions for Authors for instructions on submitting video presentations.