Increased Neuronal Firing in Computer Simulations of Sodium Channel Mutations That Cause Generalized Epilepsy With Febrile Seizures Plus

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Increased neuronal firing in computer simulations of sodium channel mutations that cause generalized epilepsy with febrile seizures plus. J Neurophysiol 91: 2040–2050, 2004. First published December 31, 2003; 10.1152/jn.00982.2003. Generalized epilepsy with febrile seizures plus (GEFS+) is an autosomal dominant familial syndrome with a complex seizure phenotype. It is caused by mutations in one of 3 voltage-gated sodium channel subunit genes (SCN1B, SCN1A, and SCN2A) and the GABA<sub>2</sub> receptor γ2 subunit gene (GGRG2). The biophysical characterization of 3 mutations (T875M, W1204R, and R1648H) in SCN1A, the gene encoding the CNS voltage-gated sodium channel α subunit Na<sub>1.1</sub>, demonstrated a variety of functional effects. The T875M mutation enhanced slow inactivation, the W1204R mutation shifted the voltage dependency of activation and inactivation in the negative direction, and the R1648H mutation accelerated recovery from inactivation. To determine how these changes affect neuronal firing, we used the NEURON simulation software to design a computational model based on the experimentally determined properties of each GEFS+ mutant sodium channel and a delayed rectifier potassium channel. The model predicted that W1204R decreased the threshold, T875M increased the threshold, and R1648H did not affect the threshold for firing a single action potential. Despite the different effects on the threshold for firing a single action potential, all of the mutations resulted in an increased propensity to fire repetitive action potentials. In addition, each mutation was capable of driving repetitive firing in a mixed population of mutant and wild-type channels, consistent with the dominant nature of these mutations. These results suggest a common physiological mechanism for epileptogenesis resulting from sodium channel mutations that cause GEFS+.

INTRODUCTION

Generalized epilepsy with febrile seizures plus (GEFS+) is a familial syndrome with a complex clinical seizure phenotype ranging from febrile seizures that persist beyond 6 yr of age to generalized epilepsies such as absences, myoclonic seizures, tonic-clonic seizures (ICEGTC) and severe myoclonic epilepsy of infancy (SMEI), a more severe form of GEFS+ (Claes et al. 2001, 2003; Fujiwara et al. 2003; Gennaro et al. 2003; Scheffer et al. 2001; Sugawara et al. 2002; Veggio et al. 2001; Wallace et al. 2003). SCN1A encodes the CNS voltage-gated sodium channel α subunit Na<sub>1.1</sub>, which is a 260-kDa transmembrane protein with 4 homologous domains, each with 6 transmembrane segments. Na<sub>1.1</sub> is one of 3 primary sodium channel α subunits (along with Na<sub>1.2</sub> and Na<sub>1.6</sub>) responsible for initiation and propagation of action potentials in the CNS. Na<sub>1.1</sub> mRNA is expressed during postnatal development predominantly in hippocampal pyramidal neurons, cerebellar Purkinje neurons, and spinal motor neurons (Black et al. 1994; Brysch et al. 1991; Furuyama et al. 1993; Novakovic et al. 2001), with the channel protein localized in a somatic distribution (Kaplan et al. 2001; Westenbroek et al. 1989).

We previously analyzed the effects of 3 Na<sub>1.1</sub> mutations that cause GEFS+ and we observed different biophysical effects on channel function for each mutation (Spampanato et al. 2001, 2003). T875M, located in the 4th transmembrane segment of the second domain, caused a negative shift in the voltage dependency of slow inactivation and a rapid entry into the slow inactivated state. W1204R, located in the cytoplasmic region between the 2nd and 3rd domains, caused a negative shift in the voltage dependency of both activation and inactivation. R1648H, located in the 4th transmembrane segment of the 4th domain, dramatically accelerated recovery from inactivation. To determine how these very different changes in sodium channel function affected neuronal activity, we designed a computational model using the experimentally defined characteristics of the mutant sodium channels and a generic delayed rectifier potassium channel. The computer simulations demonstrate that each mutation results in a greater propensity for the neuron to fire repetitive action potentials at a given stimulus intensity compared with neurons expressing the wild-type sodium channel.

METHODS

Computational model

To study the effect of changes in sodium channel kinetics in GEFS+ mutations, Hodgkin–Huxley type conductance-based models of spiking neurons were constructed using the NEURON simulation software.
software (Hines and Carnevale 1997). We constructed single-compartment models of neuronal soma that contained sodium channels and delayed rectifier potassium channels. Passive parameters similar to the passive properties of hippocampal principal cells (Spruston and Johnston 1992; Staley and Mody 1992) were adapted from previous models (Aradi and Soltesz 2002). The input resistance of the soma was 100 MΩ and the resting membrane potential was –60 mV.

The model neurons included either control sodium channels (Na\textsubscript{v} 1.1) or one of the 3 mutations T875M, W1204R, or R1648H, for which we had previously characterized the electrophysiological properties (Spampanato et al. 2001, 2003). In all cases, the modeled sodium channel kinetics represented those that were determined during coexpression of the β1 subunit. The model also included delayed rectifier potassium channels with kinetics similar to those used previously (Aradi and Holmes 1999). Although the interaction of the wild-type and mutant sodium channels with other potassium channels could vary depending on the type of potassium channels, we have chosen to use a simplified model in which only delayed rectifier channels are present so that any differences in the firing of action potentials between the mutant and wild-type cells could be attributed exclusively to changes in the sodium channel conductances. The sodium currents were described with activation and fast and slow inactivation as follows

\[ I_{\text{Na}} = g_{\text{Na}}(V - E_{\text{Na}}) \]

in which

\[ g_{\text{Na}} = g_{\text{Na}}^{\text{max}} \text{ mS/cm}^2 \]
\[ E_{\text{Na}} = 50 \text{ mV} \]
\[ g_{\text{Na}}^{\text{max}} = 200 \text{ mS/cm}^2 \]

The steady-state activation and inactivation curves were fitted with Boltzmann functions as described previously (Spampanato et al. 2001, 2003) and the parameters of the fits are shown in Table 1. The fast and slow inactivation kinetics were fitted with the 3-parameter Gaussian function

\[ \tau(V) = a \exp[\{(V - V_0)/b]\} \]

in which \( V \) is the voltage at any given time, \( V_0 \) is the mean voltage around which the Gaussian is positioned, \( b \) is the SD, and \( a \) is the height. The parameters of the Gaussian functions are shown in Table 2.

The following equations specifically determined the steady-state voltage dependencies and the time-dependent kinetics of the wild-type Na\textsubscript{v} 1.1 channels. The equations describing \( m, h, \) and \( s \) were used to calculate the voltage dependency of conductance; and the equations describing \( \tau_a, \tau_h, \) and \( \tau_s \) were used to provide time dependency to the conductance (Johnston and Wu 1995).

### TABLE 1. Voltage-dependent activation and inactivation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Activation ( V_{1/2} ) (mV)</th>
<th>Activation ( S )</th>
<th>Fast Inactivation ( V_{1/2} ) (mV)</th>
<th>Fast Inactivation ( S )</th>
<th>Slow Inactivation ( V_{1/2} ) (mV)</th>
<th>Slow Inactivation ( S )</th>
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<td>Na\textsubscript{v} 1.1</td>
<td>–21.2</td>
<td>4.9</td>
<td>–39.7</td>
<td>7.7</td>
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<td>T875M</td>
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<td>–39.7</td>
<td>7.7</td>
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<td>–26.3</td>
<td>4.9</td>
<td>–45.4</td>
<td>7.7</td>
<td>–46.1</td>
<td>5.4</td>
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<td>R1648H</td>
<td>–21.2</td>
<td>4.9</td>
<td>–39.7</td>
<td>7.7</td>
<td>–46.1</td>
<td>5.4</td>
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The steady-state activation and inactivation data were fitted with Boltzmann functions determined by the half-maximal voltage \( (V_{1/2}) \) and the slope, as previously described (Spampanato et al. 2001, 2003).

### TABLE 2. Kinetics of activation and inactivation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Activation ( a ) (msec)</th>
<th>Fast Inactivation ( \tau_h ) (msec)</th>
<th>Fast Inactivation ( a ) (msec)</th>
<th>Slow Inactivation ( \tau_s ) (msec)</th>
<th>Slow Inactivation ( a ) (msec)</th>
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<td>20.1</td>
<td>61.4</td>
<td>32.7</td>
<td>1,067 × 10^5</td>
<td>52.7</td>
</tr>
<tr>
<td>T875M</td>
<td>0.15</td>
<td>20.1</td>
<td>61.4</td>
<td>32.7</td>
<td>3,633 × 10^5</td>
<td>89.7</td>
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<tr>
<td>W1204R</td>
<td>0.15</td>
<td>20.1</td>
<td>61.4</td>
<td>32.7</td>
<td>1,067 × 10^5</td>
<td>52.7</td>
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<tr>
<td>R1648H</td>
<td>0.15</td>
<td>11.8</td>
<td>57.4</td>
<td>28.8</td>
<td>1,067 × 10^5</td>
<td>52.7</td>
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The fast and slow inactivation kinetic data were fitted with 3-parameter Gaussian functions in which \( a \) is the height of the Gaussian, \( V_0 \) is the mean voltage around which the Gaussian is positioned, and \( b \) is the standard deviation, as described in the METHODS.

1) Sodium current voltage-dependent activation and kinetics of activation

\[ m_{\text{Na}}(V)_{Na1.1} = \frac{1}{1 + \exp[-(V + 21.2)/4.9]} \]

\[ \tau_a(V)_{Na1.1} = 0.15 \text{ ms} \text{ (Aradi and Soltesz 2002)} \]

2) Sodium current voltage-dependent fast inactivation and kinetics of fast inactivation

\[ h_{\text{Na}}(V)_{Na1.1} = \frac{1}{1 + \exp[(V + 39.7)/7.7]} \]

\[ \tau_i(V)_{Na1.1} = 20.1 \exp(-0.5[(V + 61.4)/32.7]) \]

3) Sodium current voltage-dependent slow inactivation and kinetics of slow inactivation

\[ s_{\text{Na}}(V)_{Na1.1} = \frac{1}{1 + \exp[(V + 46.1)/5.4]} \]

\[ \tau_s(V)_{Na1.1} = 1,000(0.67 \exp(-0.5[(V + 52.7)/18.3])) \]

The T875M mutation altered the parameters for steady-state slow inactivation and the time constant of slow inactivation, as follows (Fig. 1, A and B)

\[ s_{\text{Na}}(V)_{T875M} = \frac{1}{1 + \exp[(V + 61.6)/3.7]} \]

\[ \tau_s(V)_{T875M} = 1,000(0.62 \exp(-0.5[(V + 89.7)/31.3])) \]

The W1204R mutation altered the parameters for steady-state activation and steady-state fast inactivation, as follows (Fig. 1C)

\[ m_{\text{Na}}(V)_{W1204R} = \frac{1}{1 + \exp[-(V + 26.3)/4.9]} \]

\[ h_{\text{Na}}(V)_{W1204R} = \frac{1}{1 + \exp[(V + 45.4)/7.7]} \]

The R1648H mutation altered the time constant of fast inactivation, as follows (Fig. 1D)

\[ \tau_{\text{Na}}(V)_{R1648H} = 11.8 \exp(-0.5[(V + 57.4)/28.8]) \]

In all simulations, the noninactivating potassium channel kinetics were the same as those used previously (Aradi and Soltesz 2002; Yuen and Durand 1991)

\[ g_K = g_{\text{K}}^{\text{max}} \]
\[ I_K = g_K(V - E_K) \]

where \( E_K = -80 \text{ mV} \), \( g_{\text{K}}^{\text{max}} = 16 \text{ mS/cm}^2 \), and

\[ \alpha_s = -0.07(V - 47)/(1 - \exp[(V - 47)/-6]) \]
\[ \beta_s = 0.264 \exp[(V - 22)/4] \]

Use-dependent sodium currents were recorded from the model neurons at 40 Hz using a 17.5 ms depolarizing potential from a holding potential of –100 mV to –10 mV. The protocol lasted for 1.5 s, and the currents were normalized to the initial peak current.
amplitude. This protocol is identical to the experimental procedure used to originally determine the effects of each GEFS+ mutation on sodium channel use dependency in Xenopus oocytes (Spampanato et al. 2001, 2003). Current-clamp simulations were carried out to study action potential (AP) threshold, AP shape, and repetitive firing properties in control and mutant model neurons.

**Electrophysiological recording**

Mutant channel characteristics were determined as described previously (Spampanato et al. 2001, 2003) by expression in Xenopus oocytes followed by electrophysiological analysis using both the 2-electrode voltage clamp and the cut-open oocyte voltage clamp. Wild-type and mutant α subunits were coexpressed with the β1 subunit to produce faster channel kinetics that more closely resembled those observed in neurons. Use dependency was analyzed at a frequency of about 40 Hz using 17.5 ms depolarizations to −10 mV from a holding potential of −100 mV. The protocol was carried out for more than 1.5 s, which was longer than necessary for the current to reach an equilibrium value in each case. Data were analyzed using a baseline subtraction method in which the average current amplitude recorded during the last 1 ms of the final test pulse was subtracted from the peak current amplitude of each test pulse before normalization. Peak current amplitudes were normalized to the peak current amplitude during the first depolarization and plotted against time.

**RESULTS**

**Sodium channel kinetics and use dependency**

The properties that are altered by each of the 3 GEFS+ mutations (T875M, W1204R, and R1648H) are shown in Fig. 1. The graphs show the fits to the experimental data (Spampanato et al. 2001, 2003), as described in METHODS. The voltage dependencies of activation [m(V)], fast inactivation [h(V)], and slow inactivation [s(V)] were fitted with a 2-state Boltzmann equation and the kinetics of fast inactivation (τ_h) and slow inactivation (τ_s) were fit with a 3-parameter Gaussian function. T875M produced a hyperpolarized shift in the voltage dependence of slow inactivation (V_{1/2} of −62 mV compared with −46 mV for wild-type Na_1.1) and accelerated the rate of entry into the slow-inactivated state compared with the wild-type Na_1.1 channels. Data were fit with a 3-point Gaussian function to describe the kinetics of slow gating. Parameters of the fits are listed in Table 2.

To test the model’s ability to accurately predict sodium channel behavior, we simulated a rapid series of depolarizations similar to the use-dependency protocol that we used to acquire data in our previous studies (Spampanato et al. 2001, 2003). The model neuron was rapidly depolarized from a holding potential of −100 mV to a test potential of −10 mV at a frequency of 40 Hz to elicit use-dependent sodium currents. The pulse train was maintained for about 1.5 s and the recorded sodium currents during each depolarization were normalized to the current amplitude during the first depolarization and plotted against time (Fig. 2). In each panel, the train of currents was generated using the model and the inset shows the first and last (after 1.5 s) traces recorded experimentally from the mutant channels. The W1204R channels displayed a level of use dependency that was similar to that of the wild-type channel, with the peak current reaching an equilibrium level <60% of the initial peak current. The T875M mutant displayed the largest use-dependent decrease with an equilibrium level <40% of the initial peak current. The R1648H channels displayed the least use dependency with an equilibrium level <80% of the initial peak current. In each case, the model correctly predicted the experimentally determined results (Spampanato et al. 2001, 2003), verifying the design of the model and validating its use in predicting the effects of the

**FIG. 1. Functional properties of mutant sodium channels.** Functional parameters for the mutant sodium channels are identical to those used for wild-type Na_1.1 except for the specific differences determined experimentally for each mutation (Spampanato et al. 2001, 2003). Equations used to fit the original data are listed in METHODS. A: T875M mutation produced a hyperpolarized shift in the voltage dependency of slow inactivation. Two-state Boltzmann fit of the original data are shown for T875M mutant (gray line) and Na_1.1 wild-type (black line) channels. Parameters of the fits are listed in Table 1. B: R1648H mutation (gray line) accelerated entry into the slow-inactivated state compared with the wild-type Na_1.1 channels (black line). Data were fit with a 3-point Gaussian function to describe the kinetics of slow gating. Parameters of the fits are listed in Table 2. C: W1204R mutation resulted in a 5 mV hyperpolarized shift in the voltage dependency of channel activation and fast inactivation. Two-state Boltzmann equations are shown for both the W1204R mutant (gray lines) and wild-type Na_1.1 (black lines) channels. Parameters of the fits are listed in Table 1. D: R1648H mutation accelerated recovery from inactivation. A 3-point Gaussian function was fitted to the experimentally defined kinetics of fast gating for both the R1648H mutant (gray line) and the wild-type Na_1.1 (black line) channels. Parameters of the fits are listed in Table 2.
mutations on neuronal properties that we cannot experimen-
tally determine at this time.

Action potential threshold

Changes that result in neuronal hyperexcitability, such as a
decrease in the threshold for generating action potentials, can
be the primary cause of seizure activity. It seemed likely that
one or more of the changes in voltage-dependent activation,
fast inactivation, and slow inactivation could alter the action
potential threshold. To determine the minimum stimulus that
could initiate an action potential, model neurons expressing
pure populations of either mutant or wild-type channels were
injected with increasing amplitudes of current using the cur-
rent-clamp feature of the NEURON simulator. A 50 ms ad-
justment period was applied first to allow the membrane con-
ductance to settle to a steady state, after which a stimulus of
100 pA was applied and maintained for 200 ms. The resulting
membrane potential of the neuron was calculated and plotted
against time (Fig. 3). The amplitude of the injected current was
increased by 25 pA each successive time until the cell fired an
action potential. The model neuron expressing the wild-type
Na\textsubscript{v}1.1 channel fired an action potential after injection of 250
pA of current. This threshold level was not significantly altered
by the R1648H mutation. In contrast, the W1204R mutation
produced the most excitable model neuron, with an action
potential generated in response to a 175 pA current injection,
and the T875M mutation produced the least excitable model
neuron, requiring 275 pA of current to elicit a single action
potential.

Repetitive firing and action potential frequency

Neurons fire repetitively in response to larger incoming
stimuli, and an increased frequency of firing in response to a
given stimuli can result in hyperexcitability and seizures. Neu-onal burst firing is a known physiological mechanism of
seizure generation (Dichter 1991) and antiepileptic drugs that
target sodium channels are known to reduce the capability of a
neuron to fire repetitive action potentials (Dichter 1991;
Köhling 2002). Therefore we evaluated the effects of each of
the mutations on repetitive neuronal firing. The amount of
current injected was increased stepwise beyond threshold for
each model neuron and the number of action potentials gener-
ated during the 200 ms stimulus was counted and plotted
against the stimulus strength (Fig. 4). The wild-type Na\textsubscript{v}1.1
model neuron, which generated a single-action potential at 250
pA, required 375 pA to fire repetitively. The large window for

FIG. 2. Sodium current use dependency. Sodium currents were recorded from model neurons expressing either wild-type Na\textsubscript{v}1.1
or mutant T875M, W1204R, or R1648H channels in response to a train of 17.5 ms depolarizations to −10 mV from a holding
potential of −100 mV at a frequency of 40 Hz. In each case, the currents were normalized to the initial peak current and plotted
against time as a successive train of inward sodium currents with each peak resulting from a separate depolarization. Inset:
experimental data showing the first and last trace recorded from Xenopus oocytes expressing each channel, using the same
voltage-clamp protocol as for the model neurons. In each case, the 2 traces were normalized to the peak current of the first trace
to compare the peak sodium current during the first and last depolarizations. Each model neuron displayed a similar level of use
dependency to what was observed in recordings from Xenopus oocytes expressing the wild-type and GEFS+ mutant sodium
channels.
a single action potential may be a mechanism by which the neuron can buffer its output without firing multiple action potentials in relation to varying intensities of incoming stimuli. All 3 of the mutations decreased the window. The T875M mutant generated repetitive action potentials in response to a lower stimulus of 350 pA compared with wild-type Na\textsubscript{v} 1.1, despite the fact that this mutant had an elevated threshold for firing a single action potential (Fig. 4, up triangles). The W1204R mutant, which displayed the lowest single action potential frequency was determined for model neurons expressing either wild-type Na\textsubscript{v} 1.1 or mutant T875M, W1204R, or R1648H channels were injected with increasing amounts of current starting with a 100 pA stimulation for 200 ms. Intensity of the stimulation was increased by 25 pA until an action potential was recorded. Each step shown is in response to a 50 pA increase in stimulation. Minimum current necessary to elicit an action potential was 250 pA for Na\textsubscript{v} 1.1, 275 pA for T875M, 175 pA for W1204R, and 250 pA for R1648H.

Closer inspection of the action potential train generated by the T875M mutant model neuron at 400 pA reveals that the action potentials are not only faster but also slightly larger than those of wild-type Na\textsubscript{v} 1.1 (Fig. 5A). The experimental characterization of sodium currents produced by T875M demonstrated 2 distinct effects: a negative shift in steady-state slow inactivation and a more rapid entry into the slow-inactivated state (Spampanato et al. 2001). To determine which of these 2 alterations resulted in the faster and larger action potentials, separate simulations in which each of these changes was individually incorporated were performed. The negative shift in steady-state slow inactivation had little effect on the rate of action potential generation over the 100 ms time period shown and had no effect on the height of the action potentials (Fig. 5B). In contrast, the more rapid entry into the slow-inactivated state resulted in larger and faster action potentials in the model neuron (Fig. 5C).

Trace comparisons of the action potential train generated by the W1204R model neuron shows that, although the frequency is greatly increased, there is no difference in the size or shape of the action potentials compared with wild-type Na\textsubscript{v} 1.1 (Fig. 6A). The W1204R mutation also produced 2 separate effects on sodium channel function when characterized experimentally: a 5 mV hyperpolarized shift in the voltage dependency of activation and a similar shift in the voltage dependency of fast inactivation (Spampanato et al. 2003). To determine what effect each of these properties had on the generation of action potentials, 2 separate models were generated. The negative shift in the voltage dependency of activation produced a large increase in the rate of action potential firing and a slight increase in the magnitude of each action potential (Fig. 6B). In contrast, the negative shift in steady-state inactivation slowed the rate of action potential generation and reduced the height of the action potentials (Fig. 6C). The shift in inactivation had a much smaller effect on the firing properties of the neuron than did the shift of the same magnitude in activation, suggesting that the shift in activation is more significant with respect to AP firing.

The R1648H model neuron also produced a larger magnitude and shorter duration of action potentials compared with wild-type Na\textsubscript{v} 1.1 (Fig. 7). Because there was only one effect of this mutation (accelerated recovery from inactivation), these changes must be caused by that effect. This result is consistent with a phenotype of hyperexcitability and is similar to previous observations using a simulation of mechanoreceptor sensory neuron firing properties (Torkkeli and French 2002).

**Dominance of the GEFS\textsuperscript{+} mutations**

Although the mutant channels were studied as homogeneous populations to determine their functional characteristics, neurons contain a heterogeneous mixture of wild-type and mutant channels because GEFS\textsuperscript{+} is an autosomal dominant disorder (Scheffer and Berkovic 1997; Singh et al. 1999). Therefore it is important to determine how a heterogeneous population of neurons...
channels affects the physiology of a neuron to mimic the in vivo situation. The computational model makes it possible to assign both mutant and wild-type channels to a single neuron and thus examine the behavior of a mixed population of channels. Figure 8A shows trace overlays of the first 3 action potentials generated in response to a 200 ms injection of 400 pA for neurons containing all mutant channels (gray lines), a 50/50 mix of mutant and wild-type channels (+/−, dashed black lines), and pure wild-type channels (solid black lines).

The T875M+/− model neuron displayed very small effects compared with wild-type Na,1.1, although it was able to fire a third action potential slightly faster than either pure population. This effect was not maintained over a longer period of time (data not shown), so the overall effect of the T875M+/− mixed population was an increased propensity to fire repetitively, similar to the effect of a pure population of T875M mutant channels. The W1204R+/− model neuron was very similar to the pure W1204R mutant model neuron, which displayed the lowest threshold for single action potential generation and the most rapid entry into a state of repetitive firing. This mutation results in a gain-of-function that is dominant over the wild-type channels. The R1648H+/− model neuron displayed a mixed phenotype with action potentials generated at intervals halfway between the faster pure R1648H population and the slower wild-type Na,1.1 population. Although the R1648H mutation was not completely dominant over the wild-type channels, the mixed population still demonstrated an increased propensity to fire repetitive action potentials.

In the previous experiments, the ratio of GEFS+ mutant channels to wild-type Na,1.1 channels was fixed at 1:1. However, a real neuron may not uniformly express an equal number of wild-type and mutant channels, and slight variations in the ratio between mutant and wild-type channels could alter neuronal excitability. Therefore we examined the effects of different ratios of GEFS+ mutant channels to wild-type Na,1.1 channels on neuronal excitability by analyzing repetitive firing after a 350 pA stimulus that does not elicit repetitive firing for the wild-type channels alone (Fig. 8B). Action potentials generated during a 200 ms stimulus were counted and plotted against the percentage of mutant channels in the total population. Each of the GEFS+ mutant channels was capable of driving the model neuron into a state of repetitive firing when the mutant channels made up ≈40% of the total population. The W1204R mutant channels were the most effective, requiring only 5% mutant channels for the model neuron to fire repetitively (squares). The W1204R mutant also produced an “all-or-nothing” effect in that the model neuron fired either a single action potential or a repetitive burst of action potentials at close to the maximum rate, with little change from 5 to 100% of the sodium channels being mutant. Both the T875M

![FIG. 5. Repetitive firing behavior of the T875M mutant model neuron. Action potentials were generated in response to a 400 pA current injection for mutant T875M (gray line) and wild-type Na,1.1 (black line) model neurons. A: the T875M model neuron fires larger action potentials more rapidly than the wild-type Na,1.1 model neuron. The mutant model neuron fired one extra action potential compared with wild-type for the short duration of this protocol. Insert: enlargement of the time scale of a single action potential fired by both the mutant T875M and wild-type Na,1.1 model neurons between 46 and 54 ms, demonstrating that the T875M neuron fired a slightly larger and earlier but similarly shaped action potential compared with the wild-type neuron. B: a model neuron expressing sodium channels with only the negative shift in the voltage dependency of slow inactivation that was observed for T875M, with no change in the kinetics of slow inactivation, fires action potentials at a frequency comparable to that of a neuron with the wild-type channel. Negative shift in the voltage dependency of slow inactivation is not sufficient to produce the effect demonstrated in A for the T875M mutation. C: a model neuron expressing sodium channels with only the more rapid entry into the slow-inactivated state (τ_slow) that was observed for T875M, with no shift in the voltage dependency of slow inactivation, produces larger and more frequent action potentials than a neuron with the wild-type channel. The mutation’s effects on the kinetics of slow inactivation are sufficient to produce the results demonstrated in A.](http://jn.physiology.org/)

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Frequent action potentials than wild-type Na\textsubscript{v} 1.1. The mutation dependency of activation that was observed for W1204R, with no shift in the voltage dependency of inactivation, produces more wild-type Na\textsubscript{v} 1.1 model neuron.

Demonstrating that the mutant W1204R model neuron could result in the same clinical epileptic syndrome.

To address this question, we used the model neuron to determine how different biophysical changes affected action potential threshold, shape, size, and frequency.

The effects of the mutations on the threshold for action potential initiation were generally consistent with predictions based on the biophysical properties of the mutant channels. A negative shift in the voltage dependency of slow inactivation combined with an increased entry into the slow-inactivated state produced by the T875M mutation resulted in a model neuron with an increased threshold for generation of a single action potential. This is easily explained by the fact that the negative shift in activation produced a decreased threshold for action potential generation as a result of a negative shift in both the voltage dependency of activation and fast inactivation. The negative shift in activation produced a much greater effect on channel excitability than the equal shift in inactivation and would account for the decreased threshold. Not surprisingly, the increased rate of recovery produced by the R1648H mutation had no effect on the model neuron’s single action potential threshold.

All 3 of the GEFS\textsuperscript{+} mutations increased the tendency for the model neuron to fire action potentials repetitively in response to a 400 pA current injection for mutant W1204R (gray line) and wild-type Na\textsubscript{v} 1.1 (black line) model neurons. A: The W1204R model neuron fires action potentials much more rapidly than the wild-type Na\textsubscript{v} 1.1 model neuron. The mutant model neuron fired 4 extra action potentials compared with the wild-type neuron during the short duration of this protocol. Insert: enlargement of the time scale of a single action potential fired by both the mutant W1204R and wild-type Na\textsubscript{v} 1.1 model neurons between 46 and 54 ms, demonstrating that the mutant W1204R model neuron fired an identically shaped action potential significantly earlier than the wild-type Na\textsubscript{v} 1.1 model neuron. B: A model neuron expressing sodium channels with only the negative shift in the voltage dependency of activation that was observed for W1204R, with no shift in the voltage dependency of inactivation, produces more frequent action potentials than wild-type Na\textsubscript{v} 1.1. The mutation’s effects on the voltage dependency of activation are sufficient to produce the results demonstrated in A. C: A model neuron expressing sodium channels with only the negative shift in the voltage dependency of inactivation that was observed for W1204R, with no change in the voltage dependency of activation, produces less frequent action potentials than wild-type Na\textsubscript{v} 1.1. A negative shift in the voltage dependency of inactivation is not sufficient to produce the effect demonstrated in A. The effects on action potential generation of the shift in the voltage dependency of inactivation are much smaller than those of the shift on the voltage dependency of activation.

DISCUSSION

Using a computational model neuron, we have shown that 3 GEFS\textsuperscript{+} mutations that alter sodium channel function in different ways all increase the propensity of a model neuron to fire repetitive action potentials. Each mutation produced biophysically unique sodium channels with kinetic and/or gating properties that differed from the wild-type channel as well as from each other (Spampanato et al. 2001, 2003), prompting the question of how these diverse differences in sodium channel function could result in the same clinical epileptic syndrome. To address this question, we used the model neuron to determine how the different biophysical changes affected action potential threshold, shape, size, and frequency.
response to an incoming stimulus that produced a single action potential only in the wild-type Na_v 1.1 model neuron. The wild-type model neuron produced a single action potential in response to a 250 pA stimulus and continued to fire a single action potential until a stimulus of 375 pA or greater was applied. This large buffer between single action potential generation and repetitive firing was not present in the GEFS/H11001 model neurons and may play an important role in modulating the excitability of the soma. The mutation with the most dramatic effect, W1204R, produced a model neuron that fired a single action potential in response to a 175 pA stimulus and quickly entered into a repetitive firing mode when the stimulus was 225 pA. This decrease and shift of the single action potential window to smaller stimuli can be attributed entirely to T875M.

FIG. 7. Repetitive firing behavior of the R1648H mutant model neuron. Action potentials were generated in response to a 400 pA current injection for mutant R1648H (gray line) and wild-type Na_v 1.1 (black line) model neurons. The R1648H model neuron fires larger action potentials much more rapidly than the wild-type Na_v 1.1 model neuron. The mutant model neuron fired 3 extra action potentials compared with the wild-type neuron during the short duration of this protocol. Inset: enlargement of the time scale of a single action potential fired by both the mutant R1648H and wild-type Na_v 1.1 model neurons between 60 and 68 ms, demonstrating that the mutant R1648H model neuron fired an action potential with a taller peak and significantly earlier compared with the wild-type model neuron.

FIG. 8. Dominance of the GEFS+ mutations. A: Model neurons were assigned a mixed population of 50% mutant and 50% wild-type sodium channels to determine whether the GEFS+ mutations maintained their physiological effects on action potential generation in the presence of the wild-type channels, as would be expected for an autosomal dominant disorder. Action potentials were elicited for pure populations of mutant channels (gray lines), wild-type channels (black lines), and a mixed population of channels (dashed black lines) in response to a 400 pA current injection after a 50 ms delay. The 1st, 2nd, and 3rd action potentials are shown for each experimental condition. B: to determine the extent of the dominance of each GEFS+ mutation, different ratios of mutant to wild-type channels were assigned to model neurons. Action potentials were elicited in response to a 350 pA stimulus and the number of action potentials generated in 200 ms was determined and plotted against the percentage of mutant (bottom axis) and wild-type (top axis) channels. T875M (up triangles) model neurons began to fire repetitively when ≥20% of the total channel population were mutant, reaching a maximum firing frequency when about 40% of the total channel population were mutant. W1204R (squares) model neurons began to fire repetitively when ≥5% of the total channel population were mutant, reaching a maximum firing frequency when about 20% of the total channel population were mutant. R1648H (down triangles) model neurons began to fire repetitively when ≥20% of the total channel population were mutant, reaching a maximum firing frequency when about 30% of the total channel population were mutant.
the shift in the voltage dependency of activation. The R1648H mutation also increased the propensity for the model neuron to fire repetitively in response to a stimulus of $\geq 325$ pA, resulting in a smaller window for single action potential generation. This result is consistent with previously published data suggesting that broad changes in sodium channel inactivation and recovery can shift the firing properties of spider mechanoreceptor neurons from single action potential generation to repetitive firing (Torkkeli and French 2002). The T875M mutation had the smallest effect but still resulted in a model neuron that fired repetitively at a stimulus of 350 pA with a smaller window for single action potential generation.

An increase in the firing of repetitive action potentials as a cause of seizure activity is consistent with the effects of antiepileptic drugs used to treat generalized tonic-clonic seizures, such as valproate (Pugsley et al. 1999; Vreugdenhil and Wadamman 1999; Vreugdenhil et al. 1998), lamotrigine (Kuo 1998; Kuo and Lu 1997; Siel et al. 2002; Xie et al. 1995; Zona and Avoli 1997), and carbamazepine (Kuo 1998; Kuo et al. 1997; Pugsley et al. 1999; Reckziegel et al. 1999; Vreugdenhil and Wadamman 1999). These drugs increase the use dependency of sodium channel activity resulting in a decrease in repetitive action potential firing (Köhling 2002). We predict that application of these drugs to the GEFS+ mutant neurons would result in an elimination of their repetitive firing behavior.

The increased action potential frequency in the T875M mutant model neuron was unexpected because that mutation enhanced slow inactivation. The repetitive firing in our model resulted from rapid entry into the slow-inactivated state, which was modeled as a Gaussian function along with recovery from slow inactivation. The rates of recovery from slow inactivation and entry into slow inactivation were defined to be the same at potentials at which these 2 properties could not be experimentally separated. Therefore it is possible that the prediction of repetitive firing in the T875M mutant model neuron may not reflect the behavior of a real neuron. Alternatively, it is possible that the rapid entry into the slow-inactivated state gave the T875M mutant channels that were slow inactivated a “jump-start” on recovery, which occurred rapidly and similarly to wild-type in the first few seconds (Spamppanato et al. 2001). Experimental recordings from neurons expressing the T875M mutant will be necessary to distinguish between these 2 possibilities.

The model that we used included 2 simplifying assumptions. First, the model neuron represented only the neuronal soma without any processes. Second, Na$_{1.1}$ was the only sodium channel that was included, even though neurons often express more than one sodium channel isoform. We believe that these assumptions are a good first approximation because Na$_{1.1}$ is predominantly expressed in the soma of neurons (Kaplan et al. 2001; Westenbroek et al. 1989), with the other 2 CNS sodium channel isoforms (Na$_{1.2}$ and Na$_{1.6}$) present at the axon hillock and nodes of Ranvier (Boiko et al. 2003; Caldwell et al. 2000; Kaplan et al. 2001; Schaller and Caldwell 2003). In addition, Na$_{1.1}$ is expressed in regions that are likely to be involved in epileptogenesis, including hippocampal pyramidal, dentate granule, cerebellar Purkinje, and spinocerebellar motoneurons with expression generally beginning during postnatal development and increasing into adulthood (Black et al. 1994; Brysch et al. 1991; Furuyama et al. 1993; Novakovic et al. 2001; Schaller and Caldwell 2003).

Although action potential initiation in neurons of the CNS occurs outside of the soma (Colbert and Johnston 1996; Colbert and Pan 2002; Stuart and Sakmann 1994; Stuart et al. 1997), the reduction in action potential threshold produced by the W1204R mutation may enable the soma of affected neurons to initiate an action potential. The action potential would result from an otherwise subthreshold dendritic depolarization, resulting in a hyperexcitable neuron or one that could possibly initiate synchronization of a network of neurons, causing seizure activity. This hypothesis is supported by previous experimental and computational studies showing that axonal action potential initiation to somatic action potential initiation, resulting in premature action potential initiation in response to otherwise subthreshold stimuli.

In summary, we have shown that 3 sodium channel mutations that produce different biophysical effects on channel function all result in a lower threshold for the firing of repetitive action potentials in model neurons. This result suggests that, although there may be a number of different biophysical effects on sodium channel function produced by GEFS+ mutations, there may still be a similar physiological mechanism that produces neuronal hyperexcitability and seizure activity. If this prediction is correct, it suggests that a common therapeutic approach might be effective in treating patients with GEFS+, regardless of the specific underlying mutation.

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