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In vivo loss of slow potassium channel activity in individuals with benign familial neonatal epilepsy in remission

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Benign familial neonatal epilepsy is a neuronal channelopathy most commonly caused by mutations in *KCNQ2*, which encodes the $K_v7.2$ subunit of the slow K^+ channel. $K_v7.2$ is expressed in both central and peripheral nervous systems. Seizures occur in the neonatal period, often in clusters within the first few days of life, and usually remit by 12 months of age. The mechanism of involvement of $K_v7.2$ mutations in the process of seizure generation has not been established *in vivo*. In peripheral axons, $K_v7.2$ contributes to the nodal slow K^+ current. The present study aimed to determine whether axonal excitability studies could detect changes in peripheral nerve function related to dysfunction or loss of slow potassium channel activity. Nerve excitability studies were performed on eight adults with *KCNQ2* mutations and a history of benign familial neonatal epilepsy, now in remission. Studies detected distinctive changes in peripheral nerve, indicating a reduction in slow K^+ current. Specifically, accommodation to long-lasting depolarizing currents was reduced in mutation carriers by 24% compared with normal controls, and the threshold undershoot after 100 ms depolarizing currents was reduced by 22%. Additional changes in excitability included a reduction in the relative refractory period, an increase in superexcitability and a tendency towards reduced sub-excitability. Modelling of the nerve excitability changes suggested that peripheral nerve hyperexcitability may have been ameliorated by upregulation of other potassium channels. We conclude that subclinical dysfunction of $K_v7.2$ in peripheral axons can be reliably detected non-invasively in adulthood. Related alterations in neuronal excitability may contribute to epilepsy associated with *KCNQ2* mutations.

Keywords: epilepsy; channelopathy; nerve excitability; neuromyotonia; potassium channel

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Introduction

Patients with benign familial neonatal epilepsy usually develop seizures within the first few weeks of life, although they typically remit by the age of 12 months, thereby obviating the need for long-term anti-convulsant treatment. A proportion of patients (estimated at 15%) may continue to have seizures beyond the first year of life, reflecting sustained susceptibility to aberrant neuronal excitability (Plouin *et al*., 2005; Reid *et al*., 2009; Berg *et al*., 2010; Bellini *et al*., 2011). Most patients with benign familial neonatal epilepsy carry heterozygous mutations in the *KCNQ2* gene, which encodes the $K_v7.2$ subunit of low-threshold slowly activating K^+ channels (Bievert *et al*., 1998; Singh *et al*., 1998, 2003). Less commonly, benign familial neonatal epilepsy is due to mutations in the *KCNQ3* gene, which encodes the $K_v7.3$ subunit that co-assembles with $K_v7.2$ (Charlier *et al*., 1998; Dedek *et al*., 2003).

$K_v7.2$ is expressed in both the central nervous system (CNS) and peripheral nervous system (PNS) and is highly expressed at the nodes of Ranvier of peripheral myelinated nerve (Schwarz *et al*., 1995). $K_v7.2$-containing channels activate slowly upon depolarization and tend not to inactivate. Given that they remain open during prolonged depolarization, these channels serve to stabilize the nerve membrane and prevent repetitive firing, an effect sometimes referred to as a 'neuronal brake' (Maljevic *et al*., 2008). Blockade of slow K^+ channels using tetraethylammonium or XE991 has shown that loss of slow K^+ channel activity in peripheral nerve increases axonal excitability (Kocsis *et al*., 1983; Baker *et al*., 1987; Schwarz *et al*., 2006) and predisposes to repetitive discharges, particularly when fast K^+ channels are blocked by 4-aminopyridine (Eng *et al*., 1988). Although some rare *KCNQ2* mutations that affect channel activation kinetics are associated with myokymia (Dedek *et al*., 2001), most individuals with benign familial neonatal epilepsy do not have symptoms attributable to altered peripheral nerve excitability.

The aim of this study was to test whether a specific channel dysfunction can be detected in subjects with benign familial neonatal epilepsy in remission using nerve excitability studies, despite an absence of signs and symptoms of peripheral nerve dysfunction. This study reports the clinical, genetic and nerve excitability studies on eight subjects from three families with *KCNQ2* mutations and a history of benign familial neonatal epilepsy, now in remission, and provides insight into the effects of the mutation *in vivo*.

Materials and methods

Ethical approval was obtained for the studies from the Human Research Ethics Committees of the University of Sydney, Australia. All subjects provided written informed consent to participate and were recruited from a database held at the University of Melbourne Epilepsy Research Centre. Individuals were eligible for inclusion if they were over the age of 18, with a history of benign familial neonatal epilepsy and genetic confirmation of a KCNQ2 mutation. Eight mutation-positive subjects from three families were recruited.

Peripheral nerve excitability studies were performed on the median nerve using the Trond protocol (Kiernan *et al*., 2000), which is summarized below. Results were compared with measurements from 30 healthy volunteers previously recorded by the same operator (S.T.) at the Institute of Neurology, University College London [benign familial neonatal epilepsy subjects: 33.9 ± 9.5 years (mean $\pm$ SD), controls: 39.1 ± 13.2 years].

Statistical analysis was performed using unpaired *t*-tests. Student's *t*-test is sensitive to the assumptions of normality and equality of variance when sample values are small. However, of the 18 excitability variables in Table 1, only one failed the Lilliefors test of normality, which is no more than expected by chance, and for this variable, TEh^{40} (90–100 ms) in Table 1, the non-parametric Mann–Whitney U-test indicated lack of statistical significance ($P > 0.05$). Three of the 20 variables exhibited a significantly higher variance in the patient group (i.e. stimulus for 50% compound muscle action potential, rheobase and hyperpolarizing current-voltage (I/V) slope in Table 1), but these variables were not significantly different between the two groups, whether Student's *t*-test, the Welch test for unequal variances or Mann-Whitney U-test was employed. A glossary of terms used in this study is available in the online Supplementary material.

Nerve excitability studies

The non-invasive nerve excitability tests are similar in application to the nerve conduction studies routinely used in clinical practice, but differing in the information obtained. Each study was well tolerated and took ~15 min to complete. The median nerve was stimulated at the wrist, with the anode placed 10 cm more proximally over muscle, off the course of the median nerve. The compound muscle action potential was recorded over the abductor pollicis brevis. The reference electrode was placed distally near the interphalangeal joint of the thumb. In all experiments, temperature was monitored at the stimulus site and was kept at ~33°C (mean $\pm$ SEM: controls 33.2 ± 0.2°C; mutation carriers 33.1 ± 0.2°C).

Stimuli were delivered from a laptop computer running the QtracS program (©UCL Institute of Neurology). They were converted to voltage waveforms by a data acquisition board (National Instruments; NI USB 6221) and then to currents by a DS5-isolated linear bipolar constant-current stimulator (Digitimer). The Trond protocol uses the principle of 'threshold tracking', which involves determining the effects of conditioning stimuli on the threshold current required to produce a target response. The target response was set to 40% of the maximal compound muscle action potential, determined by first recording a stimulus–response function. Nerve excitability was then tracked by making continuous automated trial and error adjustments to the stimulus current to keep the amplitude of the target response constant, using the slope of the stimulus–response function to optimize the stimulus adjustments. The Trond protocol measures nerve excitability in the following ways.

Strength–duration properties

As stimulus duration increases, a smaller current is required to produce a compound muscle action potential of constant size. Threshold current measurements were made for stimuli lasting 0.2, 0.4, 0.6, 0.8 and 1.0 ms. Rheobase and strength duration time constant (τ_{sd}) were then calculated from a plot of stimulus charge against stimulus duration. Strength–duration measurements reflect properties of the nodes of Ranvier (Mogyoros *et al*., 2000), particularly persistent Na^+ currents (Bostock and Rothwell, 1997).

Table 1 Nerve excitability measurements

Variable	Controls Mean ± SE (*n* = 30)	BFNE Mean ± SE (*n* = 8)	*t* (df = 36)	*P*
Stimulus response and strength–duration properties				
Stimulus for 50% Compound muscle action potential (CMAP) (mA)	4.29 ×/÷ 1.04†	4.47 ×/÷ 1.14†	0.44	0.66
Strength–duration time constant (ms)	0.481 ± 0.018	0.493 ± 0.047	0.29	0.77
Rheobase (mA)	2.80 ×/÷ 1.04†	2.94 ×/÷ 1.14†	0.51	0.88
Threshold electrotonus (%)				
TEd^{40}(10–20 ms)	68.69 ± 0.74	69.33 ± 0.96	0.41	0.64
TEd^{40}(40–60 ms)	50.66 ± 0.67	55.98 ± 1.25	3.68	0.00085***
TEd^{40}(90–100 ms)	43.2 ± 0.66	50.09 ± 1.36	4.20	0.00021***
TEh^{40}(90–100 ms)	−114.5 ± 3.5	−127.0 ± 6.5	1.83	0.075
TEd^{40} undershoot	−18.78 ± 0.60	−14.65 ± 1.55	2.93	0.0058**
TEh^{40} overshoot	14.06 ± 0.60	10.18 ± 1.75	2.67	0.011*
S2 accommodation	24.21 ± 0.53	18.61 ± 1.30	4.60	0.000073****
Accommodation half time (ms)	40.1 ± 0.78	38.8 ± 1.15	0.82	0.42
Current–voltage relationship				
Depolarizing I/V slope	1.317 ± 0.026	1.145 ± 0.030	3.27	0.0025**
Resting I/V slope	0.607 ± 0.014	0.561 ± 0.033	1.43	0.16
Minimum I/V slope	0.246 ± 0.008	0.228 ± 0.018	0.99	0.33
Hyperpolarizing I/V slope	0.341 ± 0.011	0.355 ± 0.037	0.49	0.63
Recovery cycle				
Relative refractory period (ms)	2.95 ×/÷ 1.02†	2.68 ×/÷ 1.05†	2.14	0.038*
Superexcitability (%)	−23.05 ± 0.93	−27.83 ± 1.03	2.54	0.015*
Sub-excitability (%)	14.40 ± 0.66	12.84 ± 1.15	1.12	0.27

†These values expressed as geometric mean and geometric SE, since they were log converted to normalize before applying *t*-test; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. BFNE = benign familial neonatal epilepsy; CMAP = compound muscle action potential; TEd = depolarizing threshold electrotonus; TEh = hyperpolarizing threshold electrotonus.

Threshold electrotonus

During threshold electrotonus, subthreshold conditioning currents of pre-set intensities were applied for 100 ms, and the change in threshold was tested at different time points during and up to 100 ms after the 100-ms conditioning current. The conditioning currents were applied in both hyperpolarizing (TEh) and depolarizing (TEd) directions and were set at strengths of ±20 and ±40% of the unconditioned threshold current, which was monitored continuously. Threshold electrotonus provides information regarding the state of membrane polarization and ion channel activity, including nodal Na^+ channels, fast and slow K^+ channels and hyperpolarization-activated, cyclic nucleotide-gated channels (I_h current).

Current/threshold relationship

The current/threshold relationship measured the change in threshold 200 ms into a conditioning current lasting 220 ms, and, thereby, assessed the rectifying currents responsible for the accommodation to the polarizing current. The prolonged hyperpolarizing currents evoked inward rectification due to the activation of I_h. Depolarizing currents evoked outward rectification due to outward K^+ currents (Trevillion *et al*., 2007). In both cases, greater accommodation would provide an increase in the slope of the curve (analogous to conductance).

Recovery cycle

The recovery cycle was measured by giving a supramaximal conditioning stimulus and tracking the change in threshold at time points up to 200 ms afterwards. Recovery cycles comprise the relative refractory period (reflecting recovery of nodal Na^+ channels from inactivation), superexcitable period (due to the depolarizing afterpotential, which is affected by the activity of fast K^+ channels) and the late sub-excitable period (reflecting hyperpolarization due to slow K^+ channels activated by the conditioning stimulus and whose activity outlasts the depolarizing afterpotential).

Mathematical modelling

The 'Bostock' mathematical model of the human motor axon, recently described in detail by Howells *et al*. (2012), was used to assess the likely biophysical basis of the altered nerve excitability properties in subjects with benign familial neonatal epilepsy. This two-compartment (node + internode) model is incorporated in the MEMFIT function of the QtracP (©UCL Institute of Neurology) data analysis program (Bostock, 2006). MEMFIT uses an iterative procedure to minimize the 'discrepancy' (normalized least squares difference) between simulated excitability measures and all the recorded excitability measurements of the Trond protocol, by adjusting single parameters or combinations of parameters of the model (Kiernan *et al*., 2005*a*; Farrar *et al*., 2011).

Results

Patient phenotype and genotype

Clinical and genetic information for the eight mutation carriers is summarized in Table 2 (Biervert *et al*., 1998; Richards *et al*., 2004; Heron *et al*., 2007). Family 1 carried a heterozygous point mutation c.1910T>G resulting in the Leu637Arg mutation that lies in the cytoplasmic C-terminal domain of the channel. Previous *in vitro* studies of the mutant channel demonstrated an increase in calmodulin binding to the C-terminus by ~5-fold, probably due to a conformational change in the protein (Richards *et al*., 2004).

Table 2 Clinical and genetic features of patients with *KCNQ2* mutations

Family, mutation	Sex; age (years)	Onset of seizures	Phenotype	Medication	Offset of seizures
Family 1, c.1910T>G p.L637R (Richards *et al*., 2004)	M; 38	Neonatal period	Apnoeic spells, convulsions	PHB; stopped by 3 years	First few months of life
	F; 26	Neonatal period	Apnoeic spells, convulsions	PHB; stopped by 1 year	First few months of life
Family 2, c.1684insGCCCT p.Y562fsX566 (Biervert *et al*., 1998)	M; 50	First few weeks of life	Convulsions	None known	First few weeks of life
	M; 29	Day 5	Apnoeic spells, convulsions	None known	Day 10
	F; 27	Day 3	Convulsions; FC at 2 months	PHB (briefly)	Age 2 months
	F; 27	Day 3	Convulsions; FC at 2 months	PHB (briefly)	Age 2 months
Family 3, deletion of exons 1–5 (Heron *et al*., 2007)	F; 58	5 days	Convulsions	PHB, PHT (stopped at 13 years)	Age 12 years
	F; 30	4 months	Apnoeic spells; Convulsions	PHB (stopped at 18 months)	5 months

F = female; FC = febrile convulsions; M = male; PHB = phenobarbitone; PHT = phenytoin.

Family 2 members carry a 5 bp insertion resulting in the frameshift mutation Y562fsX566. This mutation lies in the calmodulin binding region and translates to a premature stop codon, truncating the protein in the C terminus by 300 amino acids. *In vitro* expression studies in this family found that no current was carried by mutant channels in functional expression studies performed in *Xenopus* oocytes (Biervert *et al*., 1998). When co-expressed with wild-type channels, a dominant negative effect was not seen.

Family 3 carried a deletion of exons 1–5 detected by multiplex ligase-dependent probe amplification. Although *in vitro* expression data are not available, the deletion segregated with benign familial neonatal epilepsy in affected family members was not detected in control DNA (Heron *et al*., 2007).

All patients studied from the present cohort had typical features of benign familial neonatal epilepsy. Seizures remitted in infancy in seven out of eight mutation carriers. The eighth patient experienced seizures until the age of 8 years. All patients had been seizure free for >25 years and had stopped anti-convulsant treatment for a minimum of 20 years before testing. No subject had clinical evidence of neuromyotonia.

Nerve excitability studies

The measures most affected by the activity of slow K^+ channel activity include the response to depolarizing conditioning stimuli during threshold electrotonus (Fig. 1A) and the degree of accommodation to prolonged depolarizing stimuli (Table 1). There were no significant differences in stimulus-response or strength-duration properties (Table 1).

Threshold electrotonus

Increased nerve excitability in mutation carriers was detected during threshold electrotonus in response to depolarizing conditioning stimuli when compared with controls (Fig. 1). During depolarizing threshold electrotonus, the extent of threshold reduction in response to conditioning stimuli normally reaches a peak at ~20 ms after the onset of the current pulse. As the conditioning stimulus continues, the threshold starts to return slowly towards the control level. This lessening of the degree of depolarization is due to activation of hyperpolarizing slow K^+ channels and is referred to as 'S2 accommodation' (Baker *et al*., 1987; Bostock and Baker, 1988; Baker and Bostock, 1989). In subjects with benign familial neonatal epilepsy, there was a reduction in this accommodative response to long-lasting depolarizing stimuli so that excitability at TEd^{40} (90–100 ms) (Arrow 1) was increased in benign familial neonatal epilepsy by 12% ($P = 0.006$). The degree of accommodation to the 40% conditioning stimulus was reduced in benign familial neonatal epilepsy by an average of 24% ($P = 7 \times 10^{-5}$) compared with healthy controls (Table 1). The threshold undershoot when the 100-ms depolarizing current ended (Arrow 2) was 22% less in mutation carriers ($P = 0.006$). During hyperpolarizing threshold electrotonus (Fig. 2), there was a non-significant trend towards a greater increase in threshold in affected individuals with the stronger −40% current (Arrow 1), but on release of hyperpolarization, there was an overshoot (Arrow 2) that was significantly less in benign familial neonatal epilepsy subjects.

Current–threshold relationship

At 200 ms into long subthreshold polarizing currents, the threshold approximates to a steady state, related to the membrane potential change, and the slope of this relationship (change in current/change in threshold) provides a threshold analogue of input conductance, which increases on depolarization due to outward rectification by potassium channels and also on hyperpolarization due to inward rectification (Fig. 3). Consistent with a reduction in G_{Ks} in the subjects with benign familial neonatal epilepsy, there was a 13% reduction in depolarizing I/V slope (Fig. 3B and Arrow 1 in Fig. 3A; $P = 0.0025$), but no significant abnormality in hyperpolarizing I/V slope (Fig. 3C and Arrow 2 in Fig. 3A).

Recovery cycle

The relative refractory period was 9% shorter in patients compared with controls (Fig. 4B; $P = 0.037$). Excitability during the superexcitable period was increased by 17.2% (Fig. 4C; $P = 0.015$). The mean hyperpolarizing threshold change during the late sub-excitable period was ~12% smaller in individuals with benign familial neonatal epilepsy during the recovery cycle compared with normal controls, but this was not statistically significant ($P = 0.27$).

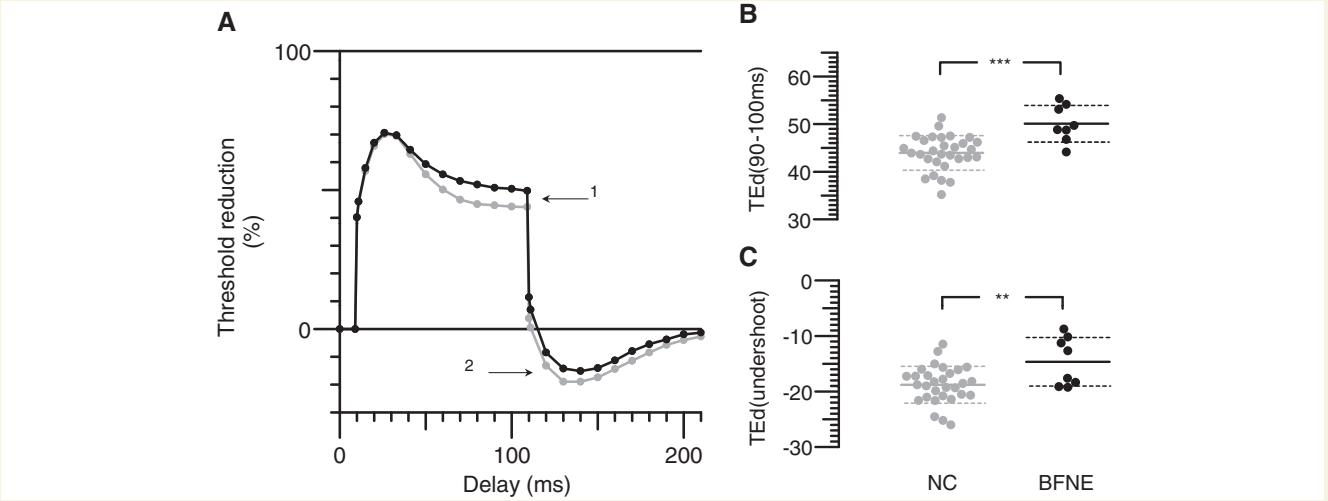

Figure 1 Depolarizing threshold electrotonus. (**A**) Changes in threshold during and after a 100 ms depolarizing current, set to 40% of unconditioned threshold. Grey lines and dots: mean of 30 normal control subjects (NC). Black lines and dots: mean of eight subjects with benign familial neonatal epilepsy (BFNE). (**B**) Distribution of threshold reductions at the end of depolarizing current, as indicated by Arrow 1 in **A**. (**C**) Peak threshold increases after the end of depolarizing current, as indicated by Arrow 2 in **A**. Asterisks indicate probability of such a threshold difference occurring by chance: $^{**}P < 0.01$, $^{***}P < 0.001$. In **B** and **C**, horizontal solid lines indicate means, and dashed lines indicate mean ± SD. Subjects with benign familial neonatal epilepsy show less accommodation to depolarizing currents and less undershoot, attributed to reduced activation of hyperpolarizing slow potassium currents.

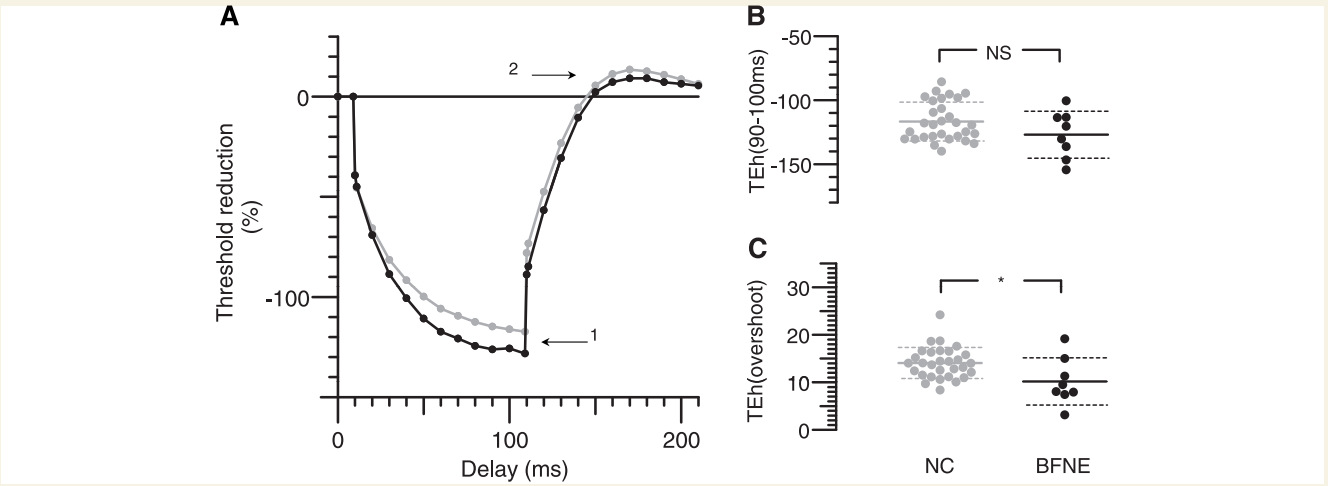

Figure 2 Hyperpolarizing threshold electrotonus. (**A**) Changes in threshold during and after a 100-ms hyperpolarizing current, set to −40% of unconditioned threshold current, presented as in Fig. 1. (**B**) Distribution of threshold changes at the end of hyperpolarizing current, as indicated by Arrow 1 in **A**. (**C**) Peak threshold decreases after the end of depolarizing current, as indicated by Arrow 2 in **A**. Asterisks indicate probability of such a threshold difference occurring by chance: $^{*}P < 0.05$; NS = $P > 0.05$. BFNE = benign familial neonatal epilepsy; NC = normal control.

Modelling of nerve excitability changes

Table 3 lists the best fits obtained to the mean benign familial neonatal epilepsy excitability data obtained by altering either one or two membrane parameters. Reduction in the slow potassium conductance G_{Ks} on its own did not produce as good a fit to the recordings as an alteration in membrane potential alone. A much better fit was obtained, however, when a 38% reduction in G_{Ks} was combined with a 32% increase in the fast potassium conductance, G_{Kf}.

Discussion

This study documents for the first time changes in peripheral nerve, specifically reflecting loss of slow K^+ channel function in asymptomatic *KCNQ2* mutation carriers. Although seizures had remitted years ago in these adults and patients had no clinical features of peripheral nerve hyperexcitability, the functional effects of the mutations could still be demonstrated *in vivo*.

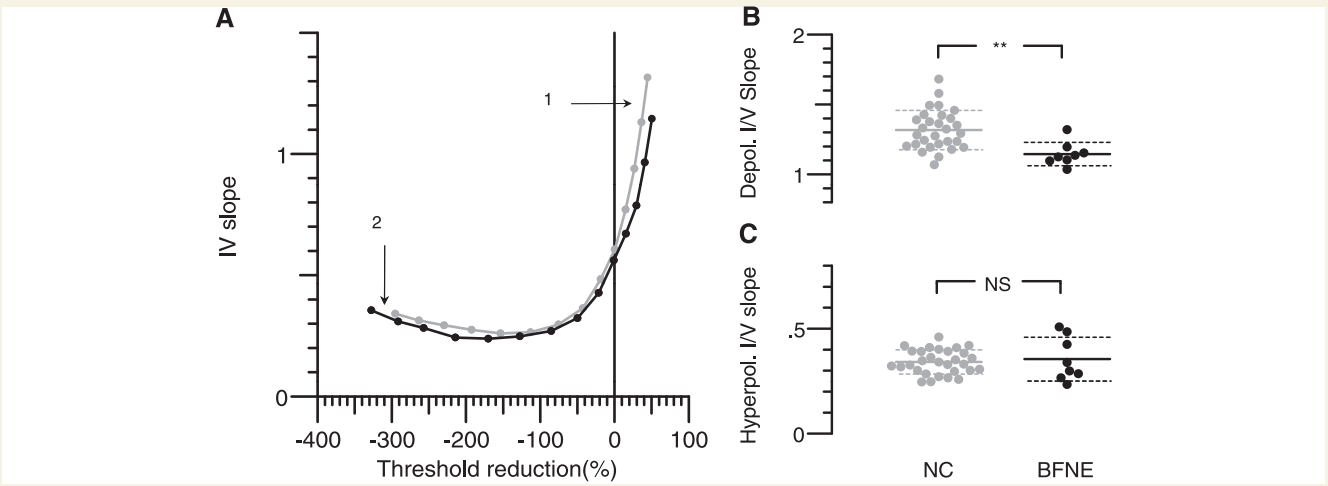

Figure 3 Current/threshold slope. (**A**) Slope of the current/threshold relationship, recorded 200 ms after application of polarizing currents, and representing a threshold analogue of input conductance. Mean control recordings are in grey, and subjects with benign familial neonatal epilepsy are in black. (**B**) Distributions of slopes at the depolarizing end of the recording, as indicated by Arrow 1 in **A**. (**C**) Distributions of hyperpolarizing slopes, indicated by Arrow 2 in **A**. Subjects with benign familial neonatal epilepsy (BNFE) show less increase in conductance on depolarization, attributed to reduced activation of slow potassium channels, but no significant difference on hyperpolarization. $^{**}P < 0.01$, NS = $P > 0.05$. NC = normal control.

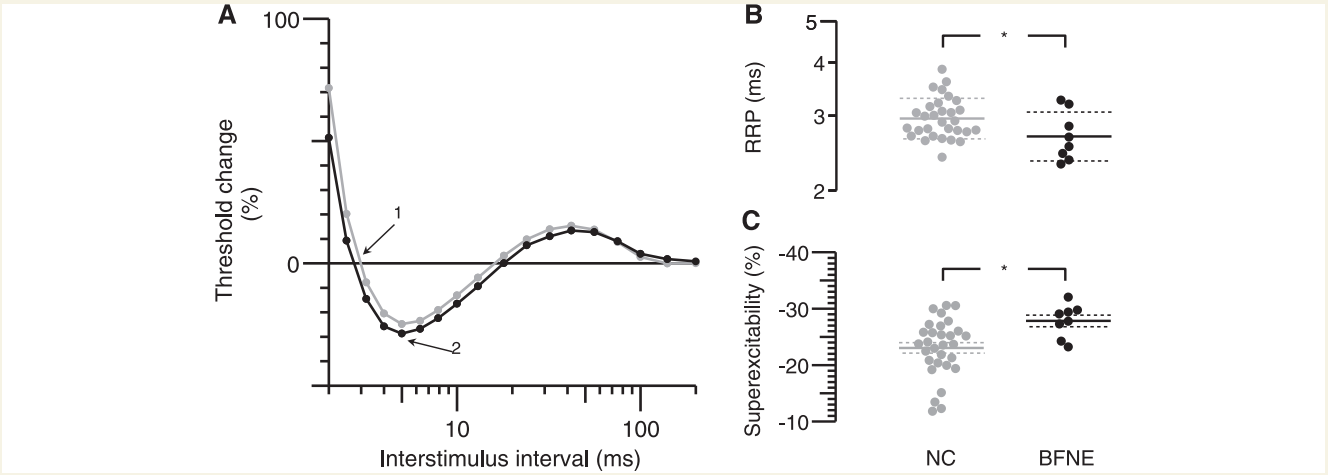

Figure 4 Recovery cycle. (**A**) Changes in threshold currents at different times after a supramaximal conditioning stimulus (note that logarithmic scales of interstimulus intervals are used). Means of 30 controls are in grey, and eight subjects with benign familial neonatal epilepsy are in black. (**B**) Distribution of relative refractory periods (RRPs), at which threshold returns to control value, is indicated by Arrow 1 in **A** (note logarithmic relative refractory period scale). (**C**) Distribution of maximum threshold reductions is indicated by Arrow 2 in **A**. Relative refractory period was slightly reduced and super-excitability increased in subjects with benign familial neonatal epilepsy. The small reduction in late sub-excitability was not significant. BFNE = benign familial neonatal epilepsy; NC = normal control.

Changes related to decreased slow K^+ channel function

The 'M' current was first identified in frog sympathetic neurons (Brown and Adams, 1980), and a similar slowly activating outwardly rectifying K^+ current was described in myelinated frog axons by Dubois (1981) and later in rat spinal roots (Baker *et al*., 1987), where blockade by tetraethylammonium or barium results in an increase in excitability to depolarization (see also Kocsis *et al*., 1983). Subsequent voltage clamp analysis of human nodal currents has revealed that the cumulative activation of this conductance limits repetitive activity (Reid *et al*., 1999). The M current is now known to be mediated by heterotetrameric K^+ channels containing subunits of the K_v7 family, including $K_v7.2$ and $K_v7.3$ (Wang *et al*., 1998; Selyanko *et al*., 2000).

Mutations in the *KCNQ2* gene encoding $K_v7.2$ were subsequently identified in benign familial neonatal epilepsy (Biervert *et al*., 1998; Singh *et al*., 1998). This was the first human genetic epilepsy to be attributed to a gene encoding a voltage-gated ion channel. XE991 and linopirdine are relatively specific blockers of

Table 3 Modelling the nerve excitability data from subjects with benign familial neonatal epilepsy

	Parameter	Change			Discrepancy reduction (%)
Best fits obtained by changing single parameters					
1	E_r	−0.7 mV			34.8
2	G_{Ks}	−12%			25.8
3	GBB	+7.4%			24.3
4	G_{Lk}	−19%			9.3
5	P_{Nap}	+5.9%			5.3
6	P_{Na}	+6.7%			3.1
7	G_{Kf}	0%			0
	Parameter 1	Change	Parameter 2	Change	Discrepancy reduction (%)
Best fits obtained by changing pairs of parameters					
1	**G_{Ks}**	**−38%**	**G_{Kf}**	**+32%**	**61.7**
2	G_{Ks}	−37.5%	P_{Nap}	−25%	48.5
3	G_{Ks}	−37.5%	P_{Na}	−16%	45.7
4	G_{Ks}	−5%	E_r	−0.3 mV	38.0
5	G_{Lk}	+21%	E_r	−1.1 mV	36.2

'Discrepancy' is scored as the weighted sum of the error terms: $[(x_m - x_n)/s_n]^2$, where x_m is the threshold of the model, x_n the mean and s_n the standard deviation of the threshold for the real nerves.
E_r = resting potential; G_{Ks} = slow potassium conductances (nodal and internodal); G_{Kf} = fast potassium conductances (nodal and internodal); GBB = 'Barrett–Barrett' conductance across myelin sheath; G_{Lk} = leak conductances (nodal and internodal); P_{Na} = sodium permeability (nodal); P_{Nap} = persistent sodium conductance (as % of P_{Na}).

the $K_v7.2$-containing channels, and these agents, as well as the non-specific blocker tetraethylammonium, reduced accommodation to 100 ms subthreshold depolarizing currents and reduced late sub-excitability (Schwarz *et al*., 2006).

The *in vivo* changes in peripheral nerve excitability described in this article in subjects with benign familial neonatal epilepsy reproduce some of the changes reported with pharmacological blockade of $K_v7.2$ and may therefore be reasonably ascribed to the effect of *KCNQ2* mutations on the slow K^+ channel, i.e. reduction in accommodation to depolarizing conditioning stimuli and a lesser threshold undershoot when the depolarizing current ends. The increase in superexcitability and reduction in relative refractory period are also consistent with the effects of XE991 on rat nerve (Schwarz *et al*., 2006), although these changes were only evident in rat after multiple conditioning stimuli. Interestingly, late sub-excitability was not significantly different in mutation carriers. This may seem surprising, because there is good evidence that late sub-excitability is due to G_{Ks}. However, it is clear from the electrotonus and recovery cycle recordings that there was only a partial reduction of G_{Ks} in the benign familial neonatal epilepsy patients. A partial reduction of G_{Ks} in the human motor axon model produces a membrane depolarization that causes a greater proportion of the maximum conductance to be activated by a nerve impulse so that late sub-excitability is maintained.

The homogeneity of the recordings from benign familial neonatal epilepsy patients, which were not significantly more variable than those from controls, contrasts with the much greater variability found previously in patients with episodic ataxia type 1 and *KCNA1* mutations (Tomlinson *et al*., 2010) and also with the heterogeneous, mutation-dependent recordings obtained from mutant $K_v7.2$ channels *in vitro*. This homogeneity may reflect the limited range of mutations amongst our patients. It would be surprising, for example, if the *KCNQ2* mutation described by Dedek *et al*. (2001), which causes myokymia as well as neonatal convulsions, did not result in different changes in peripheral nerve excitability.

The most striking finding is that the abnormalities remain demonstrable in peripheral nerve as a lasting signature of the mutation responsible for the epilepsy, even in subjects who have not suffered seizures for decades and have no symptoms attributable to peripheral nerve hyperexcitability. The lack of neuromyotonia in otherwise healthy subjects carrying the benign familial neonatal epilepsy mutation contrasts with its almost invariant occurrence in episodic ataxia type 1, due to fast K^+ channel mutations (Tomlinson *et al*., 2009, 2010), but this is not surprising given that in benign familial neonatal epilepsy the excitability changes are more subtle, possibly due in part to an adaptive increase in fast K^+ channel expression (see below).

Possible adaptive changes in other channels

The modelling suggests that there were also some adaptive changes in the axons in addition to the loss of slow potassium conductance. Reduction of G_{Ks} in the model by more than a limited amount caused the axon to fire during depolarizing electrotonus and produced a greater increase in superexcitability than observed in the subjects with benign familial neonatal epilepsy. The modelling further suggested that this hyperexcitability and superexcitability produced by loss of G_{Ks} may have been compensated by upregulation of G_{Kf}, the fast potassium conductance largely mediated by $K_v1.1$ (Tomlinson *et al*., 2010). The recordings were best matched by a 38% decrease in G_{Ks} coupled with a 32% increase in G_{Kf} (Table 3). Such an adaptive change could provide a simple explanation for the absence of symptoms of peripheral nerve hyperexcitability in these subjects, and if a similar adaptive change occurs in the central nervous system, it would provide an explanation for the remission of seizures despite the continued expression of mutant $K_v7.2$ channels. An alternative explanation for the remission of seizures in subjects despite detectable peripheral nerve changes may be that local cell responses to mutant channels (such as post-translational modification, differential splicing, altered regulation of channel expression or channel turnover) may differ between different neuronal cell types.

Clinical value

The finding of channel-specific changes in this cohort of asymptomatic individuals implies that nerve excitability studies may have clinical utility in screening patients with suspected but genetically undetermined abnormalities of ion channels in familial epilepsy syndromes. This would complement their established value in peripheral neuromuscular disorders (Krishnan *et al*., 2009; Krishnan, 2010). However, because such studies are not yet routine in most clinical neurophysiology units, this can be advocated only in centres where appropriate expertise is available.

Sequencing of *KCNQ2* is not readily available clinically and may only yield a result in 60% of typical cases. Furthermore, it is clear that the phenotype of *KCNQ2* mutations is broader than first thought; mutations are described in patients with epileptic encephalopathies as well as in cases with isolated peripheral nerve hyperexcitability syndromes (Steinlein *et al*., 2007; Weckhuysen *et al*., 2012). Nerve excitability testing can provide channel-specific information not only for the slow potassium channel but also for other Na^+ and K^+ channelopathies (Kiernan *et al*., 2005*b*; Tomlinson *et al*, 2010). The present findings confirm that excitability measurements can identify dysfunction of axonally expressed ion channels and auxillary proteins, even in the absence of peripheral nerve symptoms.

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Supplementary material

Supplementary material is available at *Brain* online.

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