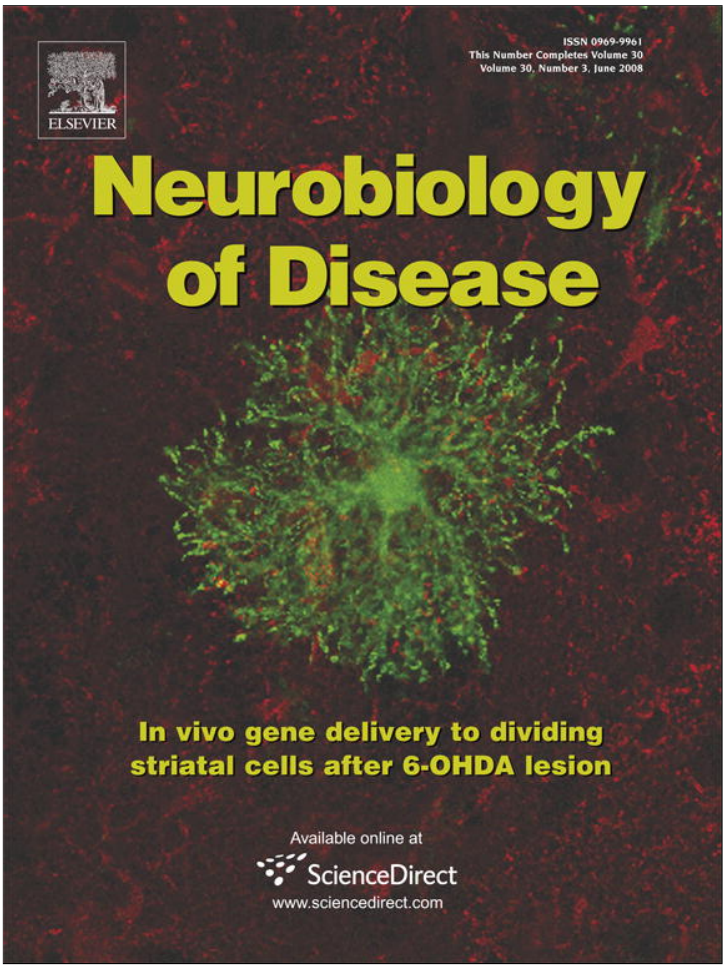

ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 30 (2008) 323–330

# A seizure-induced gain-of-function in BK channels is associated with elevated firing activity in neocortical pyramidal neurons

Sonal Shruti,[1] Roger L. Clem,[1] and Alison L. Barth*

*Department of Biological Sciences, Carnegie Mellon University, Pittsburgh PA 15213, USA*
*Center for the Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh PA 15213, USA*

Received 11 September 2007; revised 4 January 2008; accepted 6 February 2008
Available online 20 February 2008

**A heritable gain-of-function in BK channel activity has been associated with spontaneous seizures in both rodents and humans. We find that chemoconvulsant-induced seizures induce a gain-of-function in BK channel current that is associated with abnormal, elevated network excitability. Action potential half-width, evoked firing rate, and spontaneous network activity in vitro were all altered 24 h following picrotoxin-induced seizures in layer 2/3 pyramidal cells in the neocortex of young mice (P13–P16). Action potential half-width and firing output could be normalized to control values by application of BK channel antagonists in vitro. Thus, both inherited and acquired BK channel gain-of-functions are linked to abnormal excitability. Because BK channel antagonists can reduce elevated firing activity in neocortical neurons, BK channels might serve as a new target for anticonvulsant therapy.**


*Keywords:* Epilepsy; Excitability; Potassium channel; BK channel; Neocortex; Chemoconvulsant seizure; Paxilline; Picrotoxin; Anticonvulsant

## Introduction

Seizure activity can have profound consequences on neuronal firing properties, and seizure-dependent changes in ion channel function have been linked to abnormal excitability in experimental models of seizure disorders (Chen et al., 2001; Brewster et al., 2002; Su et al., 2002; Bernard et al., 2004; Shah et al., 2004). Although mutations in a large number of different ion channels have been linked to familial seizure disorders, so far there has been little overlap between the group of ion channels modified by activity or seizures and those that are mutated in inherited forms of epilepsy (Mulley et al., 2003; Noebels, 2003). A common link between genetic and acquired forms of epilepsy might suggest a useful point of intervention for anticonvulsant therapy. Furthermore, if seizure activity itself could result in the same sort of changes in ion channel function found in the inherited epilepsies, this might indicate that seizure history could sensitize an individual to future seizures, a controversial hypothesis (Gower, 1881; Hauser and Lee, 2002).

The large-conductance, $Ca^{2+}$- and voltage-gated $K^+$ channel BK (MaxiK, slo, $K_{Ca}1.1$, KCNMA1) is broadly expressed through the CNS and shapes neuronal firing output. A gain-of-function in BK channels was recently shown to underlie a heritable epilepsy and paroxysmal movement disorder in humans (Du et al., 2005), and mice that had been genetically engineered to have a gain-of-function in BK channel also developed spontaneous, recurrent seizures (Brenner et al., 2005). In this study, we sought to determine whether chemoconvulsant-induced seizures could induce a gain-of-function in BK channel properties, and whether BK channel antagonists might reduce abnormal post-seizure activity.

BK channels localize to both neuronal soma and processes (Hu et al., 2001; Misonou et al., 2006). Gated both by voltage and by $Ca^+$, they not only repolarize the membrane after an action potential and shape the fast after-hyperpolarization (fAHP) (Vergara et al., 1998; Faber and Sah, 2002), but can also influence rhythmic firing patterns and bursting output (Shao et al., 1999; Jin et al., 2000; Gu et al., 2007). In addition, in some neural systems, BK channels may modulate neurotransmitter release (Robitaille et al., 1993; Hu et al., 2001; Pattillo et al., 2001; Raffaelli et al., 2004; Xu and Slaughter, 2005). Thus, a change in BK channel function can influence firing rate both cell autonomously and at the network level.

Here we show that chemoconvulsant-induced seizures enhance BK channel function in layer 2/3 pyramidal neurons. This gain-of-function was manifested during the action potential (AP) as a faster decay time, and application of BK channel antagonists increased AP half-width to control values. Whole-cell BK channel currents were significantly larger after seizure. Evoked firing output was increased after seizure compared to seizure naïve, control animals, but can be reduced by BK channel antagonists. In addition, spontaneous firing activity in semi-intact cortical networks was increased more than two-fold after seizure and could be normalized to control values by application of BK channel antagonists. These data indicate that a gain-

* Corresponding author. Fax: +1 412 268 8423.
*E-mail address:* barth@cmu.edu (A.L. Barth).
[1] These authors contributed equally to this work.
**Available online on ScienceDirect (www.sciencedirect.com).**

0969-9961/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2008.02.002

of-function in BK channels can occur after chemoconvulsant-induced seizures, that this gain-of-function is linked to abnormal and elevated excitability, and that BK channel antagonists are effective at normalizing firing rates to control levels *in vitro*.

## Materials and methods

### *In vivo injections*

P13–P16 C57bl6 mice were injected with picrotoxin (2 mg/kg body weight) i.p. and visually monitored for seizure activity using a modification of the scale developed by (Racine, 1972; Pinel and Rovner, 1978) where the number and duration of only tonic–clonic seizures (class 8 on Pinel's modified scale) were scored. Only animals that had at least one tonic–clonic seizure were used for subsequent experiments. Animals were monitored for 3 h post-injection, and those that died within this observation window or did not have a single class 8 event were not included for further analysis.

### *Electrophysiology*

P13–P16 C57bl6 mice were anesthetized with isoflurane and decapitated. Brains were sectioned at 350–400 μm in 2–6 °C artificial cerebrospinal fluid (ACSF) composed of (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 $NaH_2PO_4$, 26.2 $NaHCO_3$, and 11 glucose equilibrated with 95/5% $O_2/CO_2$. Slices were maintained and recordings were performed in room temperature (21–24 °C) ACSF. Somata of layer 2/3 pyramidal neurons in primary somatosensory (barrel) cortex were targeted for whole-cell recording with borosilicate glass electrodes with a resistance of 6–8 MΩ. Electrode internal solution was composed of (in mM): 116 potassium gluconate, 6 KCl, 8 NaCl, 4 Mg-ATP, and 0.4 Na-GTP, at pH 7.25–7.35, and 290 mOsm. Cell identity was verified by pyramidal soma shape and the presence of dendritic spines after filling with the red fluorescent dye Alexa 568 during the course of recording. Iberiotoxin (Ibtx, 50 nM; Sigma) or paxilline (10 nM; Sigma) were bath applied as indicated, and 1,2-Bis (2-amino-5-bromophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid (BAPTA; 10 mM) was included in the electrode internal solution where indicated. Data were acquired using Multiclamp 700A (Axon Instruments, Foster City, CA) and a National Instruments acquisition interface, filtered at 1–2 kHz, digitized at 5–10 kHz and collected custom software designed for Igor Pro (Wavemetrics, Lake Oswego, Oregon).

### *AP analysis*

APs were elicited by delivering square pulses of rheobase current (minimal current required to elicit a single spike) from a resting potential of −70 mV, uncorrected for junction potential. APs elicited by short depolarizing current steps (3 ms) from −50 mV were also examined and were similar to APs during long pulses (data not shown). AP half-width (width at half-height, measured from threshold to AP peak) in the presence of Ibtx, paxilline or BAPTA was measured at 10 min after application, when effects had saturated. Parameters of AP waveforms were analyzed using peak detection software (Synaptosoft). For all experiments, cells were included in the analysis if the resting membrane potential was $<-55$, if input resistance was $>200$, and series resistance was $<40$ MΩ and did not change by more than 20% during the course of the experiment.

Statistical comparisons between conditions were carried out using AP waveforms averaged over 2–4 traces, and values were compared using unpaired *t*-tests. All values are mean±s.e.m. unless otherwise indicated.

### *Whole-cell BK channel currents*

$K^+$ channel currents were isolated by holding layer 2/3 pyramidal cells at −80 mV in the presence of 0.5 μM TTX (Sigma) to block $Na^+$ channels. An 80–160 ms voltage step of 20–180 mV was applied before and after paxilline application (10 nM; Sanchez and McManus, 1996). At least 10 min elapsed between initial measurements and measurements after drug application. In most cases, at least four traces were averaged per holding potential. Traces from before and after paxilline application were subtracted to identify BK channel current and currents were leak subtracted using the P/4 method. Currents were fully blocked by application of 10 mM TEA,

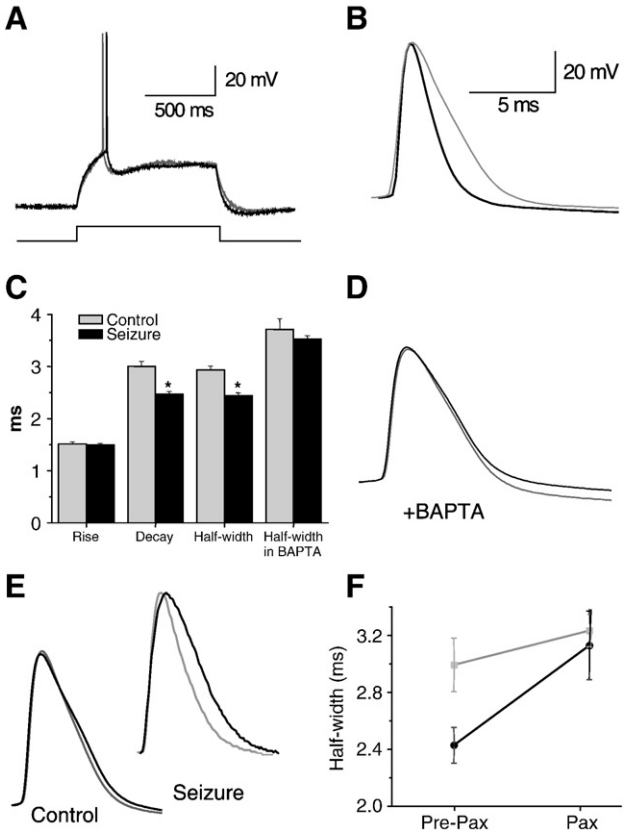


Fig. 1. Seizure increases BK channel contribution to AP waveform. (A) Minimal current injection (1 s duration) elicits a single AP from a representative control cell (gray) and 24 h post-seizure cell (black). (B) Overlay of AP waveforms aligned to threshold from a control cell (gray) and post-seizure cell (black). (C) Analysis of AP waveform. Rise times (threshold to peak) were not affected by prior seizure ($p>0.5$) while decay times (peak to baseline) were reduced ($p<0.001$). Half-width was likewise reduced after seizure (see text). (D) Internally-applied BAPTA (10 mM) normalized AP waveforms aligned to threshold from control (gray) and post-seizure animals (black). Inset shows quantitative comparison between control and seizure cells. (E) Paxilline (10 nM) significantly broadened APs in post-seizure ("seizure") neurons ($p<0.05$) but had a negligible effect in control cells ($p>0.3$). Traces from single representative cells before paxilline, gray; after paxilline application, black (within cell comparisons). (F) Paxilline (Pax) significantly broadened only APs from post-seizure animals and had no effect on AP rise times (data not shown). Scale bar in (B) applies to (D) and (E).

Table 1
Passive and active membrane properties of control and post-seizure cells

| | Control | 24 h post-seizure | *p* value |
|---|---|---|---|
| Resting potential (mV) | −71.18±3.51 (*n*=20) | −69.29±0.68 (*n*=26) | 0.07470 |
| Input resistance (MΩ) | 378.84±79.87 (*n*=20) | 395.40±20.19 (*n*=26) | 0.55536 |
| Rheobase (pA) | 117.10±41.28 (*n*=19) | 108.58±6.17 (*n*=26) | 0.43562 |
| AP threshold (mV) | −27.59±0.86 (*n*=17) | −28.62±0.70 (*n*=25) | 0.35815 |
| AP waveform: | | | |
| Height (mV) | 74.71±1.00 (*n*=26) | 76.89±1.10 (*n*=25) | 0.14875 |
| Half-width (ms) | 2.93±0.07 (*n*=26) | 2.44±0.06 (*n*=25) | 0.00003 |
| Rise time (ms) | 1.52±0.04 (*n*=26) | 1.50±0.03 (*n*=25) | 0.69828 |
| Decay time (ms) | 3.00±0.10 (*n*=26) | 2.46±0.06 (*n*=25) | 0.00002 |
| Area (mV*ms) | 204.22±4.97 (*n*=26) | 177.42±2.81 (*n*=25) | <0.00001 |

suggesting that we had isolated $K^+$ channel currents. Series and input resistance of cells from control and post-seizure groups were similar and did not change after drug application.

*Spontaneous firing activity*

Spontaneous activity in cortical slices was recorded using a modified ACSF solution with 0.5 mM $Mg^{2+}$ and 1 mM $Ca^{2+}$ (Maffei et al., 2004) and current was injected to maintain an interspike membrane potential of −50 mV. When recording spontaneous firing from slices, Ibtx or paxilline was bath applied for 15 min prior to initiation of whole-cell recording.

*PPF experiments*

The paired-pulse ratio (PPR) of EPSCs was assessed in normal ACSF in the presence of 50 μM picrotoxin to eliminate IPSCs. A monopolar stimulating electrode of 50 μm diameter was constructed from borosilicate glass, and both stimulating and recording electrodes were positioned within layer 2/3 of the adjacent cortical column, approximately 100 mm apart. Stimulus strength was adjusted to be slightly larger than the minimum current required to elicit an EPSC, in order to increase the reliability of the response. Recording electrode internal was composed of (in mM): 130 cesium gluconate, 10 HEPES, 0.5 EGTA, 8 NaCl, 4 Mg-ATP, and 0.4 Na-GTP, at pH 7.25–7.3, and 290 mOsm. Interstimulus interval was 50 ms. Only cells that showed a synaptic response 1) that showed a fixed latency 3–4 ms post-stimulus, 2) that was consistent between trials, and 3) that was stable for at least 5 min of recording prior to drug application were used in this analysis. To measure PPR, the mean amplitude of 10–30 traces during the baseline recording period and 10 min after the application of 10 nM paxilline were calculated. A ratio of current amplitude of the second EPSC/first EPSC was calculated to yield the PPR and statistically compared using Student's *t*-test.

## Results

*Seizure reduces AP half-width via BK channels*

Seizure-induced changes in AP waveform were analyzed using whole-cell recording of layer 2/3 cortical pyramidal neurons from young (P13–P16) mouse somatosensory cortex. The $GABA_A$ receptor antagonist picrotoxin was injected in mice to induce generalized seizures, a type that has a strong neocortical component (Willoughby et al., 1995).

Comparison of AP waveform in cells from control and 24 h post-seizure animals ("control" and "post-seizure" groups) showed a significant change in AP half-width (control: 2.93±0.07 ms, *n*=25 cells; post-seizure: 2.44±0.06 ms, *n*=26 cells; $p<0.001$) that could be

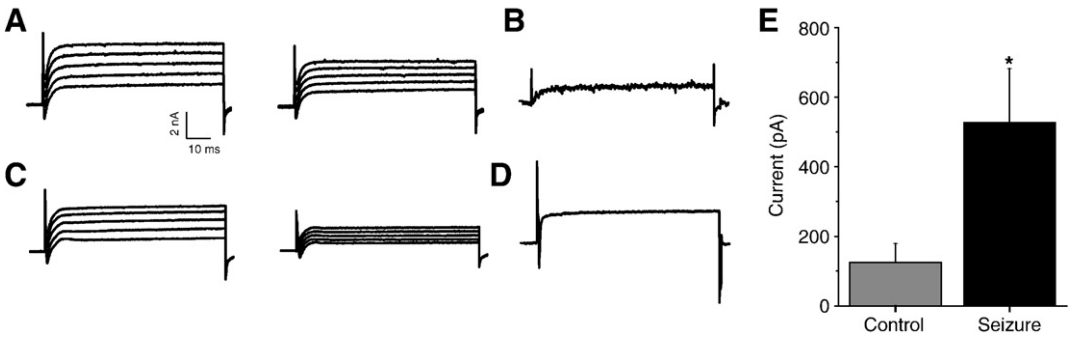


Fig. 2. BK channel currents are altered after seizure. (A) Representative potassium channel currents from a control neuron (P14) before (left) and after (right) paxilline application. Cells were held at −80 mV and an 80–160 ms voltage step (+100 to +180 mV in 20 mV increments) was applied. Scale is the same for (A–D). (B) Subtracted traces at 100 mV holding potential before and after paxilline application yield the BK channel current for control. (C) The same as (A) but in a post-seizure neuron. (D) Subtracted paxilline-sensitive current from a post-seizure neuron. (E) Amplitude of paxilline-sensitive steady-state current (measured at the end of an 80 ms pulse) in post-seizure neurons was greater than control. *$p<0.05$.

entirely attributed to decrease in decay time (Figs. 1A–C), suggesting the involvement of $K^+$ channels. Analysis of other intrinsic and active membrane properties did not show any significant differences between control and post-seizure neurons (Table 1).

Inclusion of the intracellular $Ca^{2+}$ chelator BAPTA normalized the difference in AP decay and half-width (Figs. 1C, D), consistent with the activation of a $Ca^{2+}$-gated $K^+$ channel (half-width in control in BAPTA 3.71±0.21 ms, $n=5$; seizure in BAPTA 3.52±0.07 ms, $n=5$; $p>0.3$ for BAPTA in control vs. post-seizure). Application of the BK channel antagonists paxilline or Ibtx induced a significant increase in AP half-width in post-seizure, but not control neurons (Figs. 1E, F; Ibtx data not shown), indicating that after seizure, BK channels have a prominent role in shaping the AP (half-width in control: 2.97±0.18 ms before paxilline versus 3.2±0.11 ms after paxilline, $n=5$, $p>0.3$. post-seizure: 2.43±0.12 ms before paxilline versus 3.1±0.23 ms after paxilline, $n=6$, $p<0.05$). In no case did we observe that drug application significantly altered input resistance; thus we conclude that BK channel currents are not a significant source of currents at resting membrane potentials.

BAPTA appeared to have a slightly larger effect on increasing AP half-width compared to either paxilline or Ibtx in both control and post-seizure cells (Fig. 1C). Other voltage-gated conductances, such as voltage-gated $Ca^{2+}$ channels, or $Ca^{2+}$-gated conductances, such as IK or SK channels, that might be activated during the repolarizing phase of the AP could be responsible for the relative increase in spike half-width in BAPTA, though activation of these conductances is typically not observed during the AP itself. Another possibility is that under our experimental conditions, BAPTA provides a more complete blockade of BK channels than either paxilline or Ibtx alone.

The reduction in AP half-width required the induction of prior seizures, since administration of a subconvulsive dose of picrotoxin (1 mg/kg, no seizures observed) did not significantly change spike width (half-width after subconvulsive dose 3.19±0.13 ms; $n=14$ cells; $p>0.5$ vs. control).

### *Whole-cell BK channel currents are increased after seizure*

To further verify that BK channel currents were enhanced following seizures, we pharmacologically isolated BK channel currents from both control and post-seizure cells using paxilline. The amplitude of steady-state BK currents were examined by comparing currents after a 180 mV voltage step applied from an initial holding potential of −80 mV, where averaged traces were subtracted before and after paxilline application to yield the BK channel current. On average, total $K^+$-channel currents were not significantly larger 24 h after picrotoxin-induced seizures compared to control (control 1294±312 pA, $n=9$ versus post-seizure 2144±429 pA, $n=9$; $p>0.1$).

We found that BK current amplitude was highly variable amongst layer 2/3 pyramidal neurons in both control and post-seizure conditions. However, on average, BK current amplitudes significantly increased after seizure (Fig. 2; amplitude of paxilline-sensitive current in control 133.5±61.1 pA, $n=9$; post-seizure 547.78±195.5 pA, $n=9$, $p<0.05$). In BK channel currents from some cells, there appeared to be a clear reduction in activation kinetics after seizure (see for example, Figs. 2B, D). However, this finding was not consistent between cells within a group and failed to reach statistical significance in a comparison between control and post-seizure cells ($p>0.5$). These data indicate that concurrent with the seizure-dependent reduction in AP half-width, somatic BK channel currents are also enhanced, and that $K^+$ influx via BK channels may comprise a larger fraction of total cellular $K^+$-currents.

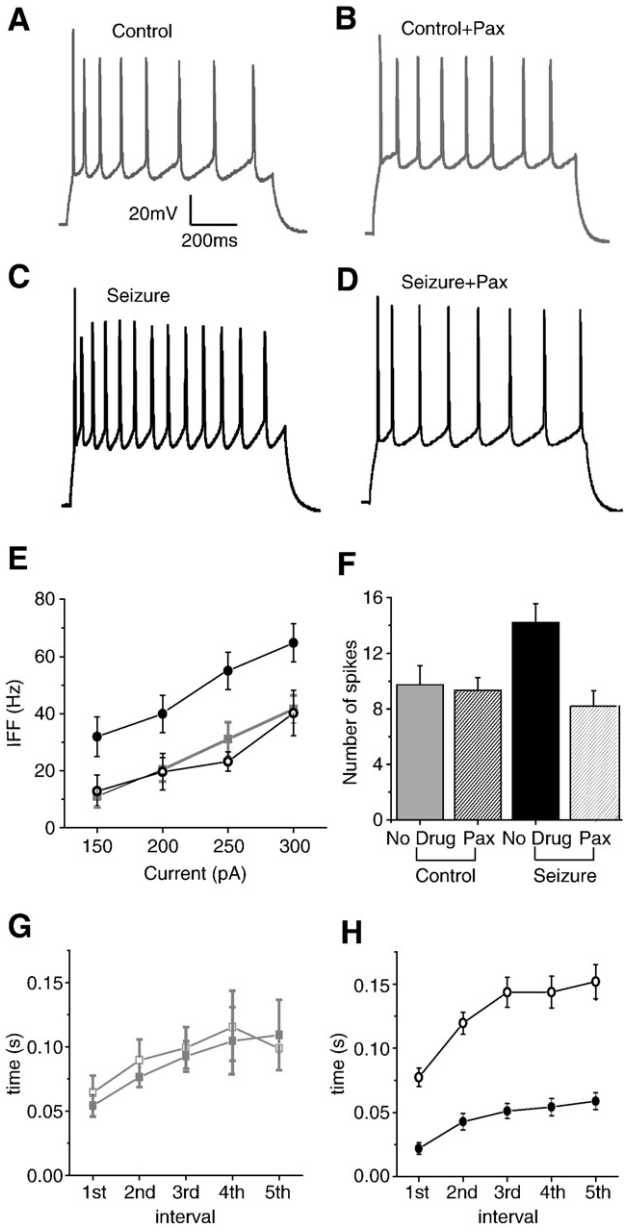


Fig. 3. Evoked firing is increased after seizure in a BK channel-dependent manner. (A) Evoked firing during a 200 pA current injection (1 s) in control neurons. (B) Same as (A) but after paxilline application. (C) Evoked firing as in (A) but in a post-seizure neuron. (D) Same as (C) but after paxilline application. (E) IFF for different amplitudes of current injection. In control, IFF at all current amplitudes (closed gray squares) is not altered after paxilline application (data not shown). After seizure, IFF is elevated (closed black circles) and can be reduced to control levels by paxilline application (open circles). (F) Total number of spikes elicited during a 200 pA current injection (see (A–D) above) show that total spike output is increased in post-seizure neurons and can be reduced to control levels by paxilline application, $p<0.05$ vs. seizure, No Drug. (G) ISIs for later spikes in the spike train are not affected by BK channel antagonists in control (closed gray squares, control No Drug; open gray squares, control+paxilline). (H) After seizure, all ISIs are reduced and can be increased by BK channel antagonists (post-seizure No Drug, closed black circles; post-seizure+paxilline, open black circles). All statistical comparisons were carried out by ANOVA between cell groups.

*BK channels increase evoked firing frequency*

Does a gain-of-function in BK channels influence firing output and network excitability after seizure? It has been shown experimentally that BK channel antagonists can reduce high firing rates in both neocortical (Jin et al., 2000) and hippocampal pyramidal neurons (Gu et al., 2007). A gain-of-function in BK channels could increase spike output directly by reducing early interspike intervals (ISIs) during a period of strong synaptic activity, or indirectly by increasing $Na^+$ channel deinactivation that occurs during the fast after-hyperpolarization (fAHP) and thus enhancing the availability of $Na^+$ channels to participate in later APs during high frequency firing (Gu et al., 2007).

To examine whether enhanced BK channel activity after seizure might increase spike output at the level of an isolated neuron, we compared spike trains elicited by current injection in control and post-seizure cells in the presence or absence of the BK channel antagonist paxilline (Figs. 3A–D). Instantaneous firing frequency (IFF; inverse of the first ISI) was significantly increased in post-seizure neurons (Fig. 3E), despite comparable minimal current required for a single AP and input resistance values (see Table 1). Paxilline application to post-seizure neurons normalized IFF to control values, suggesting that this was primarily due to BK channels (Figs. 3E, F).

The post-seizure increase in IFF was accompanied by an increase in the total number of spikes elicited by a sustained 200 ms current injection (Figs. 3C, F. Number of spikes elicited by a 200 pA current injection: control 9.75±1.38, $n=5$; control in paxilline 9.33±0.92, $n=6$; seizure 14.20±1.36, $n=6$; seizure in paxilline 8.2±1.11, $n=6$). This increase was sensitive to BK channel antagonists, since paxilline application reduced firing output in post-seizure cells (Figs. 3F, H). Paxilline induced a significant increase in ISI for post-seizure neurons, but had no effect on total spike output, ISI (Figs. 3F, G), or IFF (data not shown) for control cells. This finding is consistent with results from Fig. 1, where BK channel antagonists had no significant effect on AP half-width in control neurons and support the conclusion that BK channels do not significantly influence AP half-width and firing output in layer 2/3 pyramidal neurons in slices from control animals, at least under our recording conditions. Furthermore, these results indicate that BK channels can increase spike output in a cell-autonomous manner, independent of changes in synaptic drive that may occur after seizure.

*Increased BK channel function does not alter neurotransmitter release*

Under some conditions, BK channel activity can reduce neurotransmitter release at excitatory synapses in the hippocampus by reducing the duration of depolarization at the axon terminal (Hu et al., 2001; Stewart and Foehring, 2001; Raffaelli et al., 2004). A seizure-dependent gain-of-function in BK channels at the axon terminal could conceivably result in reduced neurotransmitter release and thus decrease cortical excitability. In contrast, activation of BK channels in some cell types can enhance neurotransmitter release (Pattillo et al., 2001; Xu and Slaughter, 2005).

To more directly examine the contribution of BK channel to regulating neurotransmitter release, we examined the amplitude of paired-pulse-evoked EPSCs at excitatory synapses in layer 2/3. One measurement of neurotransmitter release efficacy is to examine how two closely timed stimuli affect vesicle release, and thus, the amplitude of the post-synaptic response. Excitatory synapses in this layer exhibit paired-pulse facilitation (PPF), indicating that an initial stimulus primes neurotransmitter release to a second, closely spaced stimulus. If a gain-of-function in BK channel was apparent at axon terminals after seizure, PPF might be changed as release probability to the initial stimulus was altered.

In order to more directly examine a role for BK channels in mediating neurotransmitter release after seizures, we examined the paired-pulse ratio (PPR) at excitatory synapses within layer 2/3 in both control and post-seizure cells. We found that under control conditions, BK channels have little effect in mediating the PPR, since bath application of paxilline did not alter the PPR (amplitude of 2nd/1st synaptic response) in control neurons (baseline: 1.26±0.09 pA; post-paxilline: 1.35±0.02 pA; $n=4$ cells, $p>0.3$). After seizure, PPR ratios were similar to control and were not altered by paxilline application (baseline: 1.21±0.04 pA; post-paxilline 1.25±0.03 pA; $n=4$ cells, $p>0.4$), suggesting that a BK channel gain-of-function does not change neurotransmitter release properties at synapses after

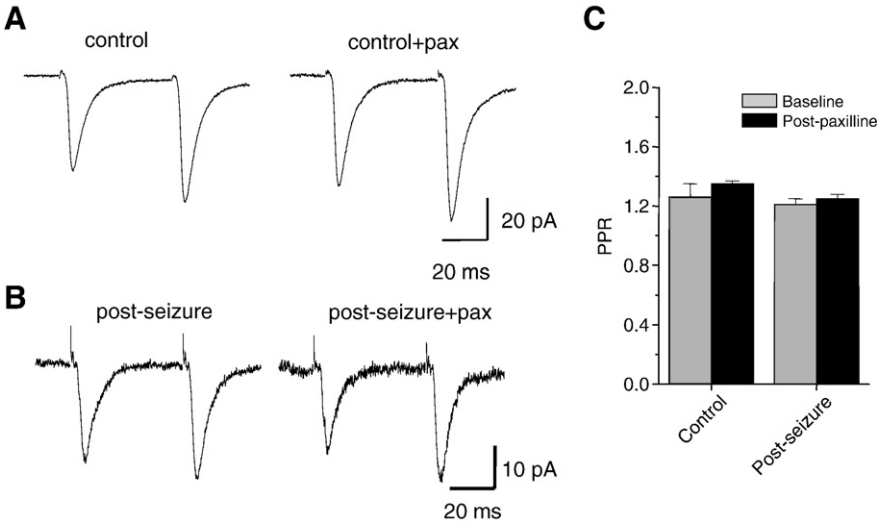


Fig. 4. BK channels do not contribute to short-term facilitation. (A, B) Paired pulse synaptic currents evoked by layer 2/3 stimulation (interstimulus interval=50 ms) in the presence of 50 μM picrotoxin before (baseline) and after bath application of 10 nM paxilline (post-paxilline), for control animals (A) and 24 h post-seizure (B). (C) Averaged PPR before (gray) and after paxilline application (black) for control and post-seizure cells.

seizure (Fig. 4). Thus, we find that a seizure-dependent gain-of-function in BK channels does not influence glutamate release at synapses onto layer 2/3 pyramidal neurons, at least as determined under our recording conditions.

*BK channel antagonists reduce spontaneous firing after seizure*

We analyzed spontaneous firing activity of single neurons in acute brain slices, a preparation that approximates the intact cortical network *in vivo* (Sanchez-Vives and McCormick, 2000). Spontaneous activity under these conditions is the sum of many different variables, such as total inhibitory and excitatory drive and intrinsic excitability; as such, it can be a useful indicator of seizure-dependent changes in average network activity. Furthermore, whole-cell recording in acute brain slices provides excellent pharmacological access and enables the examination of the role of specific ionic conductances on network excitability. Since a gain-of-function in BK channels enhanced spike output at the level of a single cell, we expected that these effects might be magnified in a semi-intact network in brain slices, resulting in an overall increase in firing rates.

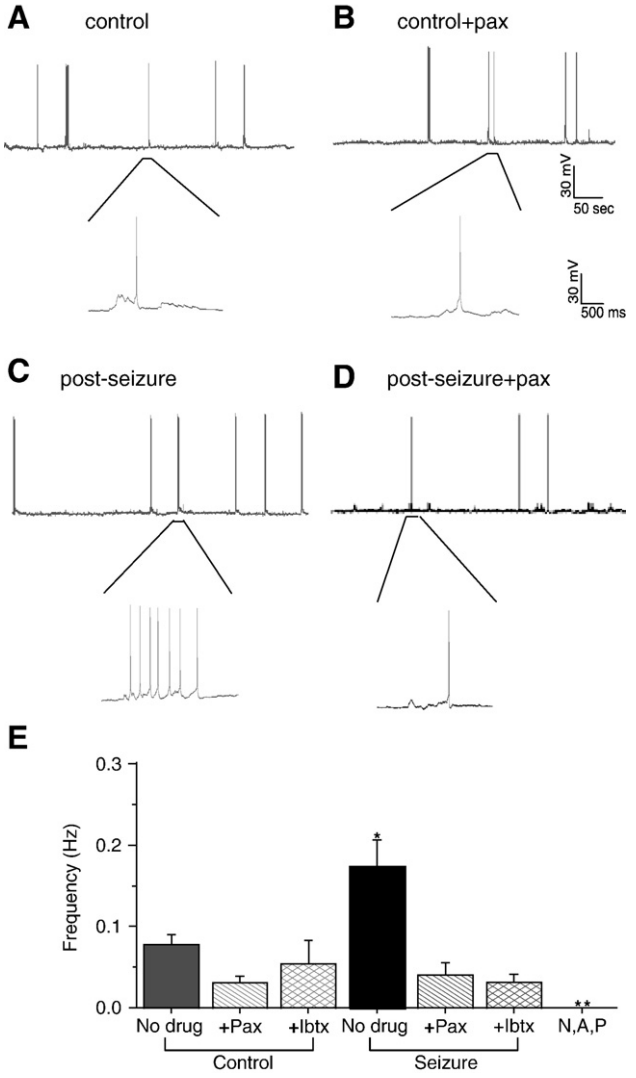


Fig. 5. BK channel antagonists reduce spontaneous activity after seizure. (A) Spontaneous firing activity over the course of ~8 min from a representative control cell. (B) Representative example of spontaneous firing in the presence of paxilline in a control cell. (C) Representative example showing increased spontaneous firing in a post-seizure cell. (D) Representative example of spontaneous firing in the presence of paxilline in a post-seizure cell. (E) Summary of spontaneous firing rates in control and post-seizure cells in either modified ACSF or with Ibtx or paxilline application. Bath application of glutamate and $GABA_A$ antagonists (N, A, P; last column in (E)) abolished all firing. *$p<0.05$ and **$p<0.01$ for seizure versus all other groups by ANOVA.

Because spontaneous firing rates are extremely low (<0.01 Hz) in the presence of ACSF containing elevated $Ca^{2+}$ and $Mg^{2+}$, we used a solution with lower $Ca^{2+}$ and $Mg^{2+}$ that more closely resembles CSF *in vivo* (Fishman, 1992; Sanchez-Vives and McCormick, 2000) to examine spontaneous firing activity in acute brain slices. Under these conditions, spontaneous firing was observed in slices from control animals (Figs. 5A, E). Twenty-four hours after the initial seizure, we observed a significant increase in firing activity (Figs. 5C, E; control 0.078±0.012 Hz, $n=13$; post-seizure 0.173±0.033, $n=14$, $p<0.05$ between post-seizure versus all other groups by ANOVA) suggesting that seizures may initiate a cascade of changes that result in an increase in network activity in the cortex.

Elevated spontaneous firing rates in post-seizure neurons could be reduced to near control levels by application of BK channel antagonists (Figs. 5D, E; post-seizure in paxilline 0.040±0.015 Hz, $n=7$; post-seizure in iberiotoxin 0.031±0.010 Hz, $n=8$). Although BK channel antagonists induced a reduction in firing rates when applied to control slices, this difference was not significant (Figs. 5B, E; control in paxilline 0.031±0.008 Hz, $n=7$; control in iberiotoxin 0.054±0.030 Hz, $n=11$).

Firing activity was not due to intrinsic bursting of layer 2/3 neurons but was dependent on synaptic transmission, since bath application of the AMPA receptor antagonist NBQX (10 μM), the NMDA receptor antagonist D-APV (50 μM), and the $GABA_A$ receptor antagonist picrotoxin (100 μM) eliminated all firing (Fig. 5E, $n=4$, $p<0.01$ versus all other groups by ANOVA). These data indicate that antagonism of BK channels is sufficient to reduce abnormal firing activity after chemoconvulsant-induced seizures in semi-intact cortical networks.

## Discussion

Here we present evidence that chemoconvulsant-induced seizures result in a BK channel gain-of-function, and that this effect increases spike output and firing activity *in vitro*. The data presented here indicate that the gain-of-function in BK channels is a seizure-induced, acquired channelopathy that results in abnormal and elevated neuronal excitability in the neocortex *in vitro*.

It may be counterintuitive to find that a gain-of-function in BK channel current is linked to elevated, not reduced, excitability. For example, loss-of-function in other $K^+$ channels that have been associated with epilepsy include the M-current mediated by KCNQ channels (Biervert et al., 1998; Singh et al., 1998) which help set resting membrane potential and are a target for anticonvulsant therapy (Peretz et al., 2005). However, our findings are consistent with the role of BK channels in shaping firing output both for single spikes and during repetitive firing, possibly by providing hyperpolarization that resets $Na^+$ channels or activates HCN currents to facilitate firing at short latencies (Shao et al., 1999; Gu et al., 2007).

*Molecular mechanisms for increased BK channel currents*

What could be the molecular mechanism behind this gain-of-function in BK channels after seizure? The simplest explanation

would be an increase in the number of BK channels in the cell. Alternatively, there might be a change in the expression of the regulatory β subunits associated with the channel that influence channel opening (Brenner et al., 2005), inactivation (Lorenz et al., 2007), or cellular localization (Toro et al., 2006; Zarei et al., 2007). BK channels are closely associated with intracellular $Ca^{2+}$ channels (Sun et al., 2003; Grunnet and Kaufmann, 2004), and it is possible that a seizure-induced increase in local $Ca^{2+}$ concentration might more effectively gate existing BK channel currents. Reduced intracellular calcium buffering might also have a similar effect.

Further investigation is required to distinguish between the many molecular mechanisms which could underlie the increase in BK channel currents. Indeed, these potential scenarios are not mutually exclusive, and more than one change in ion channel function or localization may be initiated by chemoconvulsant-induced seizures. For example, other acquired channelopathies (in different brains areas, at different ages; (Chen et al., 2001; Su et al., 2002; Bernard et al., 2004; Shah et al., 2004)) have previously been described, and it is well-established that seizures themselves induce a complex series of changes in gene expression programs (Sanchez et al., 2001; Brewster et al., 2002; Elliott and Lowenstein, 2004). Indeed, our data show that BK channel antagonists reduce firing to slightly lower frequencies than observed in control (though this difference was not significant), suggesting that there may be other conductances that are altered after seizure. However, since BK channel antagonists are sufficient to reduce excitability even in the face of these other changes, they may have an overriding role in controlling neural output that makes them attractive targets for anticonvulsant therapy.

### *BK channel function and mechanisms of increased network activity*

The circuit-level effect by which BK channels enhance spontaneous firing output may have several components: first, by increasing spike output in a cell-autonomous manner, and second by increasing excitatory drive within the network, a non-autonomous effect. In addition, it is possible that BK channel currents are augmented in inhibitory neurons. Such a possibility is mechanistically complicated to unravel, due to the large number of different types of interneurons in the neocortex as well as their specific circuit properties.

BK channels are also localized to axon terminals and may modulate neurotransmitter release under some conditions. Their role remains controversial and is likely to depend upon cell type as well as the firing frequency of the presynaptic cell. For example, increased BK channel current may reduce neurotransmitter release by shortening the window of depolarization at CA3 synapses onto CA1 pyramidal neurons in the hippocampus (Robitaille et al., 1993). However, such an effect is inconsistent with the post-seizure increase in spontaneous firing activity in superficial layers of the neocortex.

In photoreceptor cells, the hyperpolarization provided by BK channels has been shown to increase the driving force for $Ca^{+}$ entry, resulting in increased neurotransmitter release (Xu and Slaughter, 2005). Despite the fact that we did not observe a change in the PPR after seizures, nor did we find that BK channel antagonists altered this measurement in either control or post-seizure neurons, it remains formally possible that there is a BK channel gain-of-function that is manifested at the axon terminal as an increase in AP-driven neurotransmitter release under some conditions. Such a possibility would be consistent with the BK channel-related increase in network firing activity that we have observed.

### *BK channels and inherited seizure disorders*

Intriguingly, augmented BK channel activity has recently been associated with spontaneous seizures in both animal models and humans. For example, a rare human mutation that leads to a gain-of-function in BK channels is associated with an epilepsy and paroxysmal movement disorder (Du et al., 2005). Furthermore, knock-out of the regulatory β4 subunit which is known to reduce BK channel currents leads to spontaneous seizures in mice (Brenner et al., 2005). Taken together, these data suggest that abnormal function of BK channels is not restricted to a subgroup of rare, heritable epilepsies but may be more generally involved in idiopathic seizure disorders.

An important question in epilepsy research is whether "seizures beget seizures"; if a single seizure initiates changes in gene expression that promote abnormal excitability, this may facilitate the emerging pathogenesis of epilepsy. This progression has been suggested in other studies of seizure-dependent changes in synaptic and network function. For example, a seizure-dependent downregulation in HCN channels increases excitability in entorhinal cortex (Shah et al., 2004), and this increase in excitability has been proposed as an important step in epileptogenesis. Other investigators have observed seizure-dependent changes in glutamate or GABA receptor function that might facilitate epileptogenesis (see for example (Sanchez et al., 2005)). If seizures themselves initiate a gain-of-function in BK channels that is linked to abnormal and elevated excitability, it is possible that BK antagonists may represent new avenue for intervention in the early stages of seizure disorders.

## Acknowledgments

This work was supported by start-up funds from Carnegie Mellon University, the Sloan Foundation, the Milken Family Foundation, and NIH R01DA017188. Special thanks to Jesse Sheehan and David Beil for expert animal care.

## References

Bernard, C., Anderson, A., Becker, A., Poolos, N.P., Beck, H., Johnston, D., 2004. Acquired dendritic channelopathy in temporal lobe epilepsy. Science 305, 532–535.

Biervert, C., Schroeder, B.C., Kubisch, C., Berkovic, S.F., Propping, P., Jentsch, T.J., Steinlein, O.K., 1998. A potassium channel mutation in neonatal human epilepsy. Science 279, 403–406.

Brenner, R., Chen, Q.H., Vilaythong, A., Toney, G.M., Noebels, J.L., Aldrich, R.W., 2005. BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. Nat. Neurosci. 8, 1752–1759.

Brewster, A., Bender, R.A., Chen, Y., Dube, C., Eghbal-Ahmadi, M., Baram, T.Z., 2002. Developmental febrile seizures modulate hippocampal gene expression of hyperpolarization-activated channels in an isoform- and cell-specific manner. J. Neurosci. 22, 4591–4599.

Chen, K., Aradi, I., Thon, N., Eghbal-Ahmadi, M., Baram, T.Z., Soltesz, I., 2001. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. Nat. Med. 7, 331–337.

Du, W., Bautista, J.F., Yang, H., Diez-Sampedro, A., You, S.A., Wang, L., Kotagal, P., HO LD, Shi, J., Cui, J., Richerson, G.B., Wang, Q.K., 2005. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. Nat. Genet. 37 (7), 733–738.

Elliott, R.C., Lowenstein, D.H., 2004. Gene expression profiling of seizure disorders. Neurochem. Res. 29, 1083–1092.

Faber, E.S., Sah, P., 2002. Physiological role of calcium-activated potassium currents in the rat lateral amygdala. J. Neurosci. 22, 1618–1628.

Fishman, R.A., 1992. Cerebrospinal Fluid in Diseases of the Nervous System, 2nd edition. Elsevier Health Sciences, Philadelphia.

Gower, W., 1881. Epilepsy and Other Chronic Convulsive Disorders. Churchill, London.

Grunnet, M., Kaufmann, W.A., 2004. Coassembly of big conductance Ca2+-activated K+ channels and L-type voltage-gated Ca2+ channels in rat brain. J. Biol. Chem. 279, 36445–36453.

Gu, N., Vervaeke, K., Storm, J.F., 2007. BK potassium channels facilitate high-frequency firing and cause early spike frequency adaptation in rat CA1 hippocampal pyramidal cells. J. Physiol. 580, 859–882.

Hauser, W.A., Lee, J.R., 2002. Do seizures beget seizures? Prog. Brain Res. 135, 215–219.

Hu, H., Shao, L.R., Chavoshy, S., Gu, N., Trieb, M., Behrens, R., Laake, P., Pongs, O., Knaus, H.G., Ottersen, O.P., Storm, J.F., 2001. Presynaptic Ca2+-activated K+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. J. Neurosci. 21, 9585–9597.

Jin, W., Sugaya, A., Tsuda, T., Ohguchi, H., Sugaya, E., 2000. Relationship between large conductance calcium-activated potassium channel and bursting activity. Brain Res. 860, 21–28.

Lorenz, S., Heils, A., Kasper, J.M., Sander, T., 2007. Allelic association of a truncation mutation of the KCNMB3 gene with idiopathic generalized epilepsy. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144 (1), 10–13.

Maffei, A., Nelson, S.B., Turrigiano, G.G., 2004. Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation. Nat. Neurosci. 7, 1353–1359.

Misonou, H., Menegola, M., Buchwalder, L., Park, E.W., Meredith, A., Rhodes, K.J., Aldrich, R.W., Trimmer, J.S., 2006. Immunolocalization of the Ca2+-activated K+ channel Slo1 in axons and nerve terminals of mammalian brain and cultured neurons. J. Comp. Neurol. 496, 289–302.

Mulley, J.C., Scheffer, I.E., Petrou, S., Berkovic, S.F., 2003. Channelopathies as a genetic cause of epilepsy. Curr. Opin. Neurol. 16, 171–176.

Noebels, J.L., 2003. The biology of epilepsy genes. Annu. Rev. Neurosci. 26, 599–625.

Pattillo, J.M., Yazejian, B., DiGregorio, D.A., Vergara, J.L., Grinnell, A.D., Meriney, S.D., 2001. Contribution of presynaptic calcium-activated potassium currents to transmitter release regulation in cultured *Xenopus* nerve-muscle synapses. Neuroscience 102, 229–240.

Peretz, A., Degani, N., Nachman, R., Uziyel, Y., Gibor, G., Shabat, D., Attali, B., 2005. Meclofenamic acid and diclofenac, novel templates of KCNQ2/Q3 potassium channel openers, depress cortical neuron activity and exhibit anticonvulsant properties. Mol. Pharmacol. 67, 1053–1066.

Pinel, J.P., Rovner, L.I., 1978. Experimental epileptogenesis: kindling-induced epilepsy in rats. Exp. Neurol. 58, 190–202.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294.

Raffaelli, G., Saviane, C., Mohajerani, M.H., Pedarzani, P., Cherubini, E., 2004. BK potassium channels control transmitter release at CA3–CA3 synapses in the rat hippocampus. J. Physiol. 557, 147–157.

Robitaille, R., Garcia, M.L., Kaczorowski, G.J., Charlton, M.P., 1993. Functional colocalization of calcium and calcium-gated potassium channels in control of transmitter release. Neuron 11, 645–655.

Sanchez-Vives, M.V., McCormick, D.A., 2000. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat. Neurosci. 3, 1027–1034.

Sanchez, M., McManus, O.B., 1996. Paxilline inhibition of the alpha-subunit of the high-conductance calcium-activated potassium channel. Neuropharmacology 35, 963–968.

Sanchez, R.M., Dai, W., Levada, R.E., Lippman, J.J., Jensen, F.E., 2005. AMPA/kainate receptor-mediated downregulation of GABAergic synaptic transmission by calcineurin after seizures in the developing rat brain. J. Neurosci. 25, 3442–3451.

Sanchez, R.M., Koh, S., Rio, C., Wang, C., Lamperti, E.D., Sharma, D., Corfas, G., Jensen, F.E., 2001. Decreased glutamate receptor 2 expression and enhanced epileptogenesis in immature rat hippocampus after perinatal hypoxia-induced seizures. J. Neurosci. 21, 8154–8163.

Shah, M.M., Anderson, A.E., Leung, V., Lin, X., Johnston, D., 2004. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. Neuron 44, 495–508.

Shao, L.R., Halvorsrud, R., Borg-Graham, L., Storm, J.F., 1999. The role of BK-type Ca2+-dependent K+ channels in spike broadening during repetitive firing in rat hippocampal pyramidal cells. J. Physiol. 521 (Pt 1:), 135–146.

Singh, N.A., Charlier, C., Stauffer, D., DuPont, B.R., Leach, R.J., Melis, R., Ronen, G.M., Bjerre, I., Quattlebaum, T., Murphy, J.V., McHarg, M.L., Gagnon, D., Rosales, T.O., Peiffer, A., Anderson, V.E., Leppert, M., 1998. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. Nat. Genet. 18, 25–29.

Stewart, A.E., Foehring, R.C., 2001. Effects of spike parameters and neuromodulators on action potential waveform-induced calcium entry into pyramidal neurons. J. Neurophysiol. 85, 1412–1423.

Su, H., Sochivko, D., Becker, A., Chen, J., Jiang, Y., Yaari, Y., Beck, H., 2002. Upregulation of a T-type Ca2+ channel causes a long-lasting modification of neuronal firing mode after status epilepticus. J. Neurosci. 22, 3645–3655.

Sun, X., Gu, X.Q., Haddad, G.G., 2003. Calcium influx via L- and N-type calcium channels activates a transient large-conductance Ca2+-activated K+ current in mouse neocortical pyramidal neurons. J. Neurosci. 23, 3639–3648.

Toro, B., Cox, N., Wilson, R.J., Garrido-Sanabria, E., Stefani, E., Toro, L., Zarei, M.M., 2006. KCNMB1 regulates surface expression of a voltage and Ca2+-activated K+ channel via endocytic trafficking signals. Neuroscience 142, 661–669.

Vergara, C., Latorre, R., Marrion, N.V., Adelman, J.P., 1998. Calcium-activated potassium channels. Curr. Opin. Neurobiol. 8, 321–329.

Willoughby, J.O., Mackenzie, L., Medvedev, A., Hiscock, J.J., 1995. Distribution of Fos-positive neurons in cortical and subcortical structures after picrotoxin-induced convulsions varies with seizure type. Brain Res. 683, 73–87.

Xu, J.W., Slaughter, M.M., 2005. Large-conductance calcium-activated potassium channels facilitate transmitter release in salamander rod synapse. J. Neurosci. 25, 7660–7668.

Zarei, M.M., Song, M., Wilson, R.J., Cox, N., Colom, L.V., Knaus, H.G., Stefani, E., Toro, L., 2007. Endocytic trafficking signals in KCNMB2 regulate surface expression of a large conductance voltage and Ca(2+)-activated K+ channel. Neuroscience 147, 80–89.