

# Gabapentin Is a Potent Activator of KCNQ3 and KCNQ5 Potassium Channels<sup>§</sup>

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## ABSTRACT

Synthetic gabapentinoids, exemplified by gabapentin and pregabalin, are in extensive clinical use for indications including epilepsy, neuropathic pain, anxiety, and alcohol withdrawal. Their mechanisms of action are incompletely understood, but are thought to involve inhibition of  $\alpha_2\delta$  subunit-containing voltage-gated calcium channels. Here, we report that gabapentin is a potent activator of the heteromeric KCNQ2/3 voltage-gated potassium channel, the primary molecular correlate of the neuronal M-current, and also homomeric KCNQ3 and KCNQ5 channels. In contrast, the structurally related gabapentinoid, pregabalin, does not activate

KCNQ2/3, and at higher concentrations ( $\geq 10 \mu\text{M}$ ) is inhibitory. Gabapentin activation of KCNQ2/3 ( $\text{EC}_{50} = 4.2 \text{ nM}$ ) or homomeric KCNQ3\* ( $\text{EC}_{50} = 5.3 \text{ nM}$ ) channels requires KCNQ3-W265, a conserved tryptophan in KCNQ3 transmembrane segment 5. Homomeric KCNQ2 or KCNQ4 channels are insensitive to gabapentin, whereas KCNQ5 is highly sensitive ( $\text{EC}_{50} = 1.9 \text{ nM}$ ). Given the potent effects and the known anticonvulsant, antinociceptive, and anxiolytic effects of M-channel activation, our findings suggest the possibility of an unexpected role for M-channel activation in the mechanism of action of gabapentin.

## Introduction

Gabapentin (Neurontin) and pregabalin (Lyrica) are synthetic antiepileptic and antinociceptive gabapentinoid compounds originally designed as analogs of the neurotransmitter  $\gamma$ -aminobutyric acid (GABA), and both are in widespread clinical use (Calandre et al., 2016). However, the mechanisms of action of gabapentinoids, exemplified by gabapentin and pregabalin, are incompletely understood. [<sup>3</sup>H]-Gabapentin binding was first described in membrane fractions from rat brain homogenates, and the target protein was identified as the  $\alpha_2\delta$  subunit of voltage-gated calcium ( $\text{Ca}_v$ ) channels. The findings were later recapitulated using porcine brain tissue, heterologously expressed  $\alpha_2\delta$ , and also with pregabalin; binding was found to be exclusive to  $\alpha_2\delta 1$  and 2 isoforms (Gee et al., 1996; Brown and Gee, 1998; Field et al., 2006; Fuller-Bicer et al., 2009). Gabapentin and pregabalin are generally considered inactive against canonical GABA<sub>A</sub> and GABA<sub>B</sub> receptors despite their structural similarity to GABA (Taylor, 1997; Stringer and Lorenzo, 1999; Lanneau et al., 2001; Jensen et al., 2002; Ben-Menachem, 2004), although some investigators contend that there are some subtype-specific effects on GABA<sub>B</sub> receptors (Ng et al., 2001; Bertrand et al., 2003a; Parker et al., 2004). Binding of gabapentin and pregabalin to  $\alpha_2\delta$  is suggested to act therapeutically via impairment of  $\text{Ca}_v$  channel activity, thus reducing neuronal calcium

currents (Stefani et al., 1998, 2001), although others observed no evidence for gabapentin-induced changes in neuronal  $\text{Ca}_v$  activity (Rock et al., 1993; Schumacher et al., 1998).

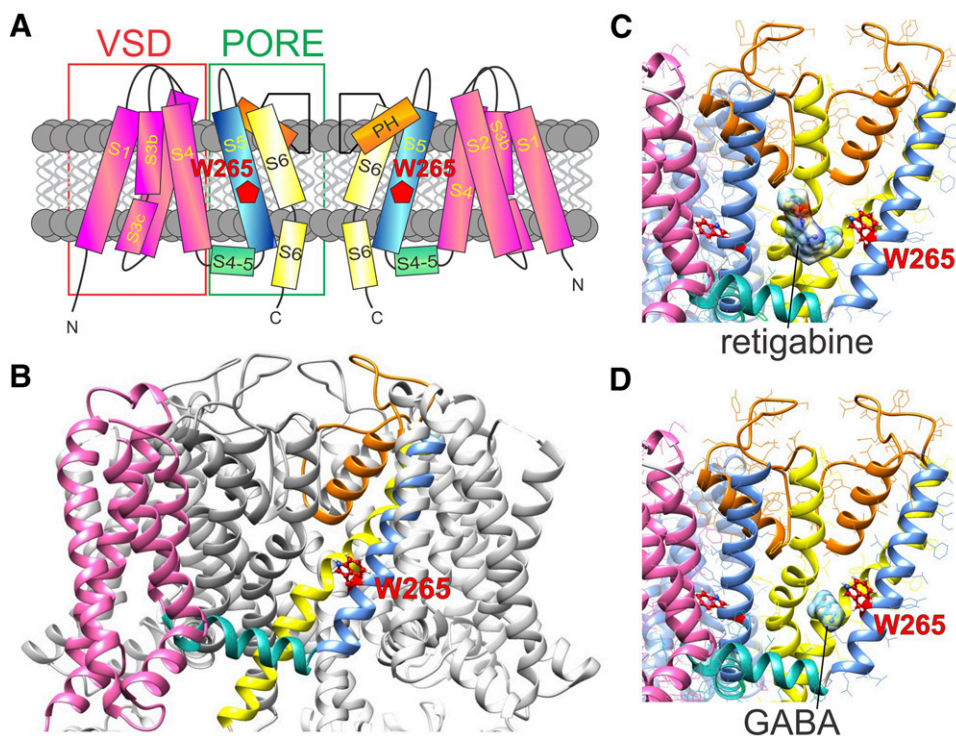
We recently made the unexpected discovery that GABA can activate voltage-gated potassium ( $\text{K}_v$ ) channels composed of heteromeric assemblies of KCNQ2 ( $\text{K}_v 7.2$ ) and KCNQ3 ( $\text{K}_v 7.3$ ) pore-forming  $\alpha$  subunits (Manville et al., 2018). KCNQ ( $\text{K}_v 7$ ) channels comprise tetramers of  $\alpha$  subunits, each containing six transmembrane (S) segments, organized into the voltage-sensing domain (S1–S4) and the pore module (S5 and S6) (Fig. 1, A and B). In vertebrate nervous systems, KCNQ2/3 ( $\text{K}_v 7.2/3$ ) heteromers are the primary molecular correlate of the M-current, a muscarinic-inhibited  $\text{K}_v$  current essential for regulating excitability of a wide range of neurons throughout the nervous system (Brown and Adams, 1980; Marrion et al., 1989; Wang et al., 1998). We found that, like the anticonvulsant retigabine (Schenzer et al., 2005; Kim et al., 2015), GABA binds to a conserved tryptophan (W265) on KCNQ3 to activate KCNQ3 homomers and KCNQ2/3 heteromers (Manville et al., 2018) (Fig. 1, B–D).

Because of the structural similarities between gabapentinoids and GABA, and the known influence of the M-current in many of the disease states responsive to gabapentinoids (epilepsy, pain, anxiety, and alcohol withdrawal) (Blackburn-Munro et al., 2005; Kang et al., 2017; Mason et al., 2018), we hypothesized that gabapentinoids might modulate KCNQ2/3 channels. Here, using electrostatic surface mapping, in silico docking studies, cellular electrophysiology, and site-directed mutagenesis, we examined whether the two gabapentinoids in widespread clinical use (gabapentin and pregabalin) can modulate KCNQ2/3 channel function.

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**ABBREVIATIONS:**  $\text{Ca}_v$ , voltage-gated calcium; DRG, dorsal root ganglion; GABA,  $\gamma$ -aminobutyric acid;  $\text{K}_v$ , voltage-gated potassium.



**Fig. 1.** KCNQ3 contains a conserved neuro-transmitter binding pocket. (A) Topological representation of KCNQ3 showing two of the four subunits, without domain swapping for clarity. Pentagon, approximate position of KCNQ3-W265; VSD, voltage-sensing domain. (B) Chimeric KCNQ1/KCNQ3 structural model (red, KCNQ3-W265). Domain coloring as in (A). (C and D) Close-up tail currents were plotted versus prepulse voltage and fitted with a single Boltzmann function:

## Materials and Methods

**Channel Subunit cRNA Preparation and *Xenopus laevis* Oocyte Injection.** cRNA transcripts encoding human KCNQ2, KCNQ3, KCNQ4, and KCNQ5 ( $K_v7.2-7.5$ ) were generated by in vitro transcription using the T7 polymerase mMessage mMachine kit (Thermo Fisher Scientific, Waltham, MA) after vector linearization from cDNA subcloned into plasmids incorporating *Xenopus laevis*  $\beta$ -globin 5' and 3' untranslated regions flanking the coding region to enhance translation and cRNA stability. cRNA was quantified by spectrophotometry. Mutant KCNQ2 and KCNQ3 cDNAs were generated with site-directed mutagenesis using a QuikChange kit according to the manufacturer's protocol (Stratagene, San Diego, CA), and corresponding cRNAs were prepared as described previously. Defolliculated stage V and VI *Xenopus laevis* oocytes (Ecocyte Bioscience, Austin, TX) were injected with KCNQ channel  $\alpha$  subunit cRNAs (5–10 ng). The oocytes were incubated at 16°C in Barth's saline solution (Ecocyte Bioscience) containing penicillin and streptomycin, with daily washing, for 2–5 days prior to two-electrode voltage-clamp recording.

**Two-Electrode Voltage Clamp.** Two-electrode voltage-clamp recording was performed at room temperature using a OC-725C amplifier (Warner Instruments, Hamden, CT) and pClamp8 software (Molecular Devices, Sunnyvale, CA) 2–5 days after cRNA injection as described in the previous section. The oocytes were placed in a small-volume oocyte bath (Warner Instruments) and viewed with a dissection microscope. Unless otherwise stated, chemicals were sourced from Sigma (St. Louis, MO). The bath solution contained 96 mM NaCl, 4 mM KCl, 1 mM  $MgCl_2$ , 1 mM  $CaCl_2$ , and 10 mM HEPES (pH 7.6). Gabapentin and pregabalin were stored at  $-80^\circ\text{C}$  as 1 M stocks in molecular grade  $H_2O$  and diluted to working concentrations on each experimental day. The drugs were introduced into the recording bath by gravity perfusion at a constant flow of 1 ml per minute for 3 minutes prior to recording. Pipettes were of 1 to 2 M $\Omega$  resistance when filled with 3 M KCl. Currents were recorded in response to pulses between  $-80$  and  $+40$  mV at 20 mV intervals, or after a single pulse to  $+40$  mV from a holding potential of  $-80$  mV to yield current-voltage relationships and current magnitude, and to quantify the activation

rate. Deactivation was recorded at  $-80$  mV after a single pulse to  $+40$  mV from a holding potential of  $-80$  mV. Electrophysiology data analysis was performed with Clampfit (Molecular Devices) and Graphpad Prism software (GraphPad, San Diego, CA); values are stated as mean  $\pm$  S.D. Raw or normalized tail currents were plotted versus prepulse voltage and fitted with a single Boltzmann function:

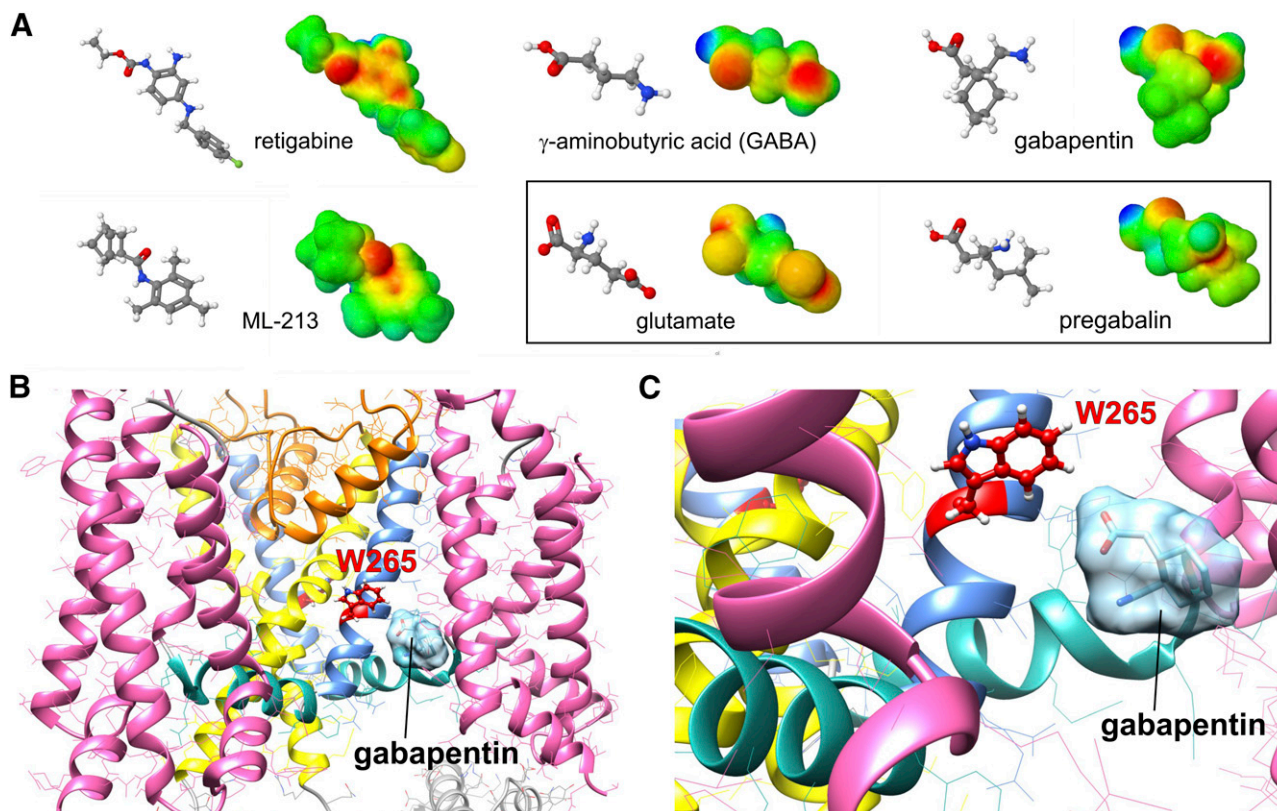
$$g = \frac{(A_1 - A_2)}{\{1 + \exp[V_{1/2} - V_s]\}y + A_2} \quad (1)$$

where  $g$  is the normalized tail conductance;  $A_1$  is the initial value at  $-\infty$ ;  $A_2$  is the final value at  $+\infty$ ;  $V_{1/2}$  is the half-maximal voltage of activation; and  $V_s$  the slope factor. Activation and deactivation kinetics were fitted with single exponential functions.

**Chemical Structures and Silico Docking.** Chemical structures and electrostatic surface potentials were plotted and viewed using Jmol, an open-source Java viewer for chemical structures in three dimensions (<http://jmol.org/>). For in silico ligand docking predictions, the *Xenopus laevis* KCNQ1 cryo-electron microscopy structure (Sun and MacKinnon, 2017) was first altered to incorporate KCNQ3/KCNQ5 residues known to be important for retigabine and ML-213 binding, and their immediate neighbors, followed by energy minimization as previously described (Manville et al., 2018) using the GROMOS 43B1 force field (van Gunsteren, 1996) in DeepView (Johansson et al., 2012). Thus, *Xenopus laevis* KCNQ1 amino acid sequence LITLYIGF was converted to LITAWYIGF; the underlined W being W265 in human KCNQ3 and the italicized residues being the immediate neighbors in KCNQ3/KCNQ5. In addition, *Xenopus laevis* KCNQ1 sequence WWGVVTVTTIGYGD was converted to WWGLITLATIGYGD; the underlined L being Leu314 in human KCNQ3 and the italicized residues being the immediate neighbors in KCNQ5 and/or KCNQ3. Surrounding nonmutated sequences are shown to illustrate the otherwise high sequence identity in these stretches. No other KCNQ1 residues were changed in the model. Unguided docking of gabapentin and pregabalin to predict native binding sites was performed using SwissDock with CHARMM force fields (Grosdidier et al., 2011a,b).

**Statistical Analysis.** All values are expressed as mean  $\pm$  S.D. One-way analysis of variance was applied for all other tests; if multiple





**Fig. 2.** Gabapentin is predicted to bind to KCNQ3-W265. (A) Electrostatic surface potentials (red, electron-dense; blue, electron-poor; green, neutral) and structures calculated and plotted using Jmol. (B and C) Long-range (B) and close-up (C) side views of KCNQ1/3 chimera model structure showing results of SwissDock unguided in silico docking of gabapentin. Domain colors as in Fig. 1.

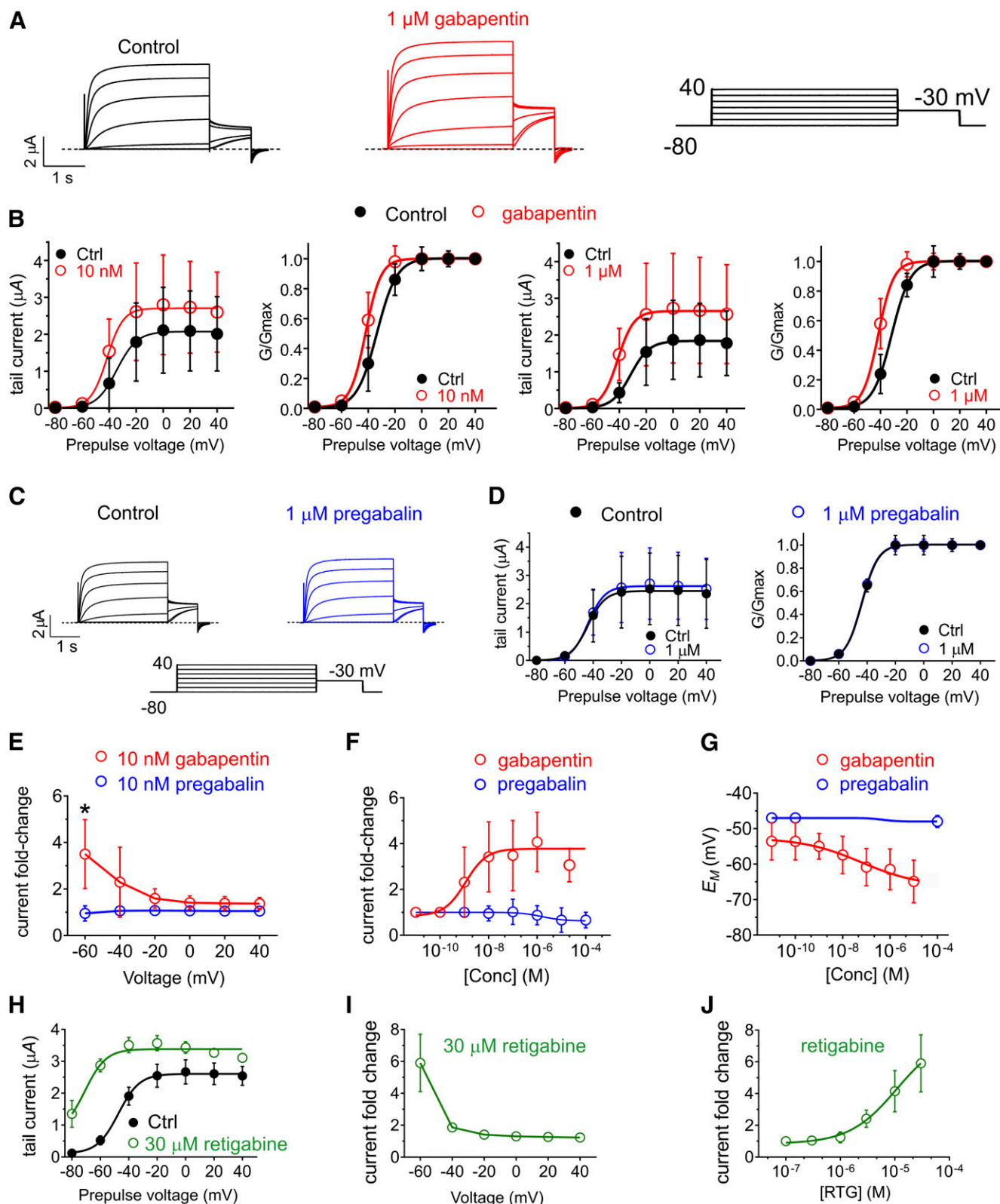
comparisons were performed, a post-hoc Tukey's honestly significant difference test was performed following analysis of variance. All  $P$  values were two-sided. Statistical significance was defined as  $P < 0.05$ .

## Results

Synthetic anticonvulsants such as retigabine and ML-213 exhibit negative electrostatic surface potential near their carbonyl oxygen moieties, a chemical property thought to be important for activation of KCNQ2/3 channels (Kim et al., 2015). We previously found that GABA also possesses this chemical property, whereas the excitatory neurotransmitter glutamate (which cannot open KCNQ2/3 channels) does not (Manville et al., 2018). Here, we found that gabapentin exhibits a similar negative electrostatic surface potential pattern to that of GABA, whereas pregabalin does not (Fig. 2A). Using SwissDock, we performed unbiased docking prediction analysis for gabapentin and pregabalin to a model of KCNQ3 (Manville et al., 2018) based on the recent cryo-electron microscopy-derived KCNQ1 structure (Sun and MacKinnon, 2017). Strikingly, gabapentin was predicted to bind to KCNQ3-W265 (Fig. 2, B and C), whereas pregabalin failed to dock to KCNQ3-W265.

We next tested the predictions using the *Xenopus laevis* oocyte expression and two-electrode voltage-clamp electrophysiology. Gabapentin potently activated heteromeric KCNQ2/3 potassium channels, even at low nanomolar concentrations (Fig. 3, A and B). In contrast, pregabalin had no augmenting effect on KCNQ2/3 activity, even at 1  $\mu$ M (Fig. 3, C and D). Thus, the experimental

data matched the docking predictions. Gabapentin efficacy was highest at  $-60$  to  $-40$  mV, leading to a  $-9$  mV shift in the voltage dependence of KCNQ2/3 activation (1  $\mu$ M gabapentin), but gabapentin also augmented currents at positive membrane potentials (Fig. 3, B and E). Dose-response studies showed that at  $-60$  mV, gabapentin exhibited an  $EC_{50}$  value for KCNQ2/3 activation of  $4.2 \pm 0.13$  nM ( $n = 5-7$ ); at 10 nM, gabapentin increased KCNQ2/3 current 3.5-fold at  $-60$  mV (Fig. 3F; Supplemental Fig. 1; Supplemental Table 1). The ability to activate KCNQ2/3 at subthreshold potentials enabled gabapentin to shift the membrane potential ( $E_M$ ) of KCNQ2/3-expressing oocytes by  $> -10$  mV ( $EC_{50}$ , 4.2 nM) (Fig. 3G). Parallel studies showed that pregabalin failed to activate KCNQ2/3 even at 1  $\mu$ M, and began to inhibit KCNQ2/3 at 10  $\mu$ M and above (Fig. 3, E and F; Supplemental Fig. 2; Supplemental Table 2). Pregabalin likewise failed to shift the oocyte  $E_M$  (Fig. 3G). Compared with the established KCNQ2/3 opener and anticonvulsant retigabine, gabapentin acted as a potent partial agonist. Thus, retigabine (30  $\mu$ M) shifted the voltage dependence of KCNQ2/3 activation by  $-30$  mV (Fig. 3H) and increased current at  $-60$  mV by 6-fold (Fig. 6I), however, the  $EC_{50}$  value for retigabine was in the micromolar (not nanomolar) range (Fig. 3J), which is  $\sim 1000$ -fold less potent than gabapentin (see Supplemental Fig. 3; Supplemental Table 3). In comparison, we recently found that GABA, which also acts at KCNQ3-W265, activates KCNQ2/3 with an  $EC_{50}$  value of  $0.85 \mu$ M at  $-60$  mV, increasing the current by 4-fold (Manville et al., 2018). Thus, gabapentin and GABA exhibit similar efficacy but gabapentin is 200-fold more potent.



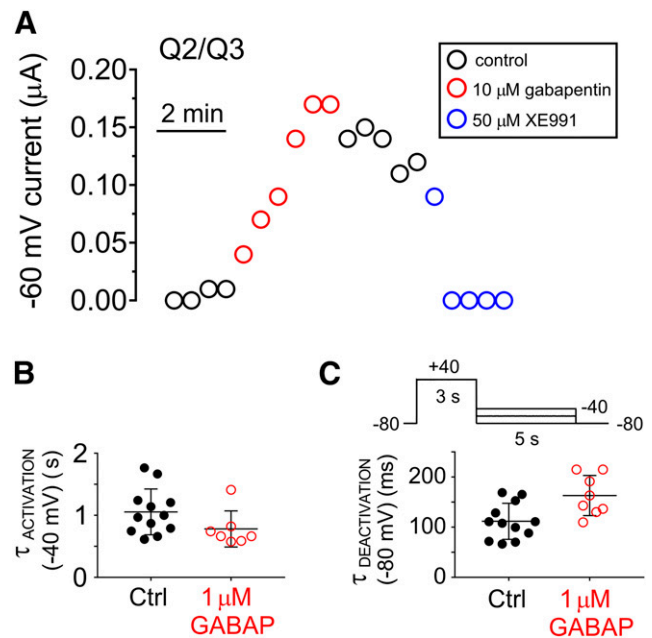
**Fig. 3.** Gabapentin is a potent activator of heteromeric KCNQ2/3 potassium channels. (A) Left, mean two-electrode voltage-clamp (TEVC) traces for KCNQ2/3 expressed in *Xenopus* oocytes in the absence (control) or presence of 10 nM gabapentin ( $n = 7$  to 8). Dashed line here and throughout, zero current level. Right, voltage protocol. (B) Mean tail current and normalized tail currents ( $G/G_{\max}$ ) vs. prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (red) of 10 nM or 1  $\mu$ M gabapentin as indicated ( $n = 7$  to 8). Error bars indicate S.D. Voltage protocol as in (A). (C) Mean TEVC traces for KCNQ2/3 expressed in *Xenopus* oocytes in the absence (control) or presence of 1  $\mu$ M pregabalin ( $n = 7$  to 8). Lower inset, voltage protocol. (D) Mean tail current and normalized tail currents ( $G/G_{\max}$ ) vs. prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (blue) of 1  $\mu$ M pregabalin as indicated ( $n = 5$ ). Error bars indicate S.D. Voltage protocol as in (C). (E) Voltage dependence of KCNQ2/3 current fold increase by gabapentin vs. pregabalin (10 nM), plotted from traces as in (A) and (C) ( $n = 5-8$ ). Error bars indicate S.D. \* $P < 0.05$  vs. pregabalin current at -60 mV. (F) Gabapentin and pregabalin dose responses



Gabapentin began to activate KCNQ2/3 immediately upon wash-in, with the current augmentation taking ~2 minutes to plateau. Gabapentin effects washed out relatively slowly (<50% washout after 2 minutes), but the gabapentin-augmented current was rapidly inhibited by washing in the KCNQ channel inhibitor, XE991 (50  $\mu$ M) (Fig. 4A). Gabapentin effects on KCNQ2/3 gating kinetics were suggestive of it stabilizing the open state and destabilizing the closed state; at 10 nM, gabapentin speeded up KCNQ2/3 activation and slowed down deactivation (Fig. 4, B and C; Supplemental Fig. 1; Supplemental Table 1).

We next examined the effects of gabapentin on homomeric channels formed by neuronal KCNQ isoforms. At 1  $\mu$ M, gabapentin activated KCNQ3\* (an expression-optimized KCNQ3-A315T mutant that ensures robust currents) (Zaika et al., 2008) and KCNQ5, especially at subthreshold potentials. In contrast, KCNQ2 and KCNQ4 were insensitive to 1  $\mu$ M gabapentin (Fig. 5, A–C; Supplemental Figs. 4–7; Supplemental Tables 4–7). Dose-response studies revealed that KCNQ3 and KCNQ5, like KCNQ2/3 channels, were activated at –60 mV even by 10 nM gabapentin, and that KCNQ3 exhibited similar gabapentin sensitivity and efficacy to that of KCNQ2/3 channels ( $EC_{50}$  = 5.3 nM; maximal 4-fold increase in current at –60 mV). In contrast, KCNQ5 channels exhibited higher sensitivity but lower efficacy ( $EC_{50}$  = 1.9 nM; maximal 3-fold increase in current at –60 mV) (Fig. 5D; Supplemental Figs. 4–7; Supplemental Tables 4–7).

Canonical GABA<sub>A</sub> and GABA<sub>B</sub> receptors are generally considered to be gabapentin insensitive (Taylor, 1997; Jensen et al., 2002); in addition, previous studies have concluded that *Xenopus laevis* oocytes do not express endogenous GABA<sub>A</sub> or GABA<sub>B</sub> receptors (Guyon et al., 2013). Furthermore, the gabapentin-activated currents in KCNQ2/3-expressing oocytes were completely inhibited by the KCNQ-specific inhibitor, XE991 (Fig. 4A). These data, combined with docking prediction studies, rapid onset of activation, the lack of effects of pregabalin, and the KCNQ isoform specificity of gabapentin (Fig. 5), are consistent with direct activation of KCNQ2/3 channels by gabapentin. This conclusion was further supported by two additional sets of experiments. First, gabapentin (10 nM) had no effect on endogenous currents or membrane potential in noninjected oocytes, discounting the possibility that gabapentin was activating endogenous currents (Fig. 6A–C). Second, substitution to leucine of KCNQ3-W265, the GABA binding site (Manville et al., 2018), and the *in silico* predicted docking site for gabapentin (Fig. 2), essentially eliminated the effects of gabapentin on KCNQ2/3 currents (Fig. 6, D and E); the double mutation of KCNQ2-W236L and KCNQ3-W265L in KCNQ2/3 channels had similar effects (Fig. 6, F and G). KCNQ2/KCNQ3-W265L channels were insensitive to gabapentin across the voltage range (Fig. 6H) and up to 100  $\mu$ M gabapentin (Fig. 6I; Supplemental Fig. 8; Supplemental Table 8). Double-mutant (WL/WL) KCNQ2/3 channels showed slight ( $\leq$ 50%) augmentation by gabapentin at –60 mV only at 1  $\mu$ M and higher gabapentin (Fig. 6, H and I; Supplemental Fig. 9; Supplemental Table 9).

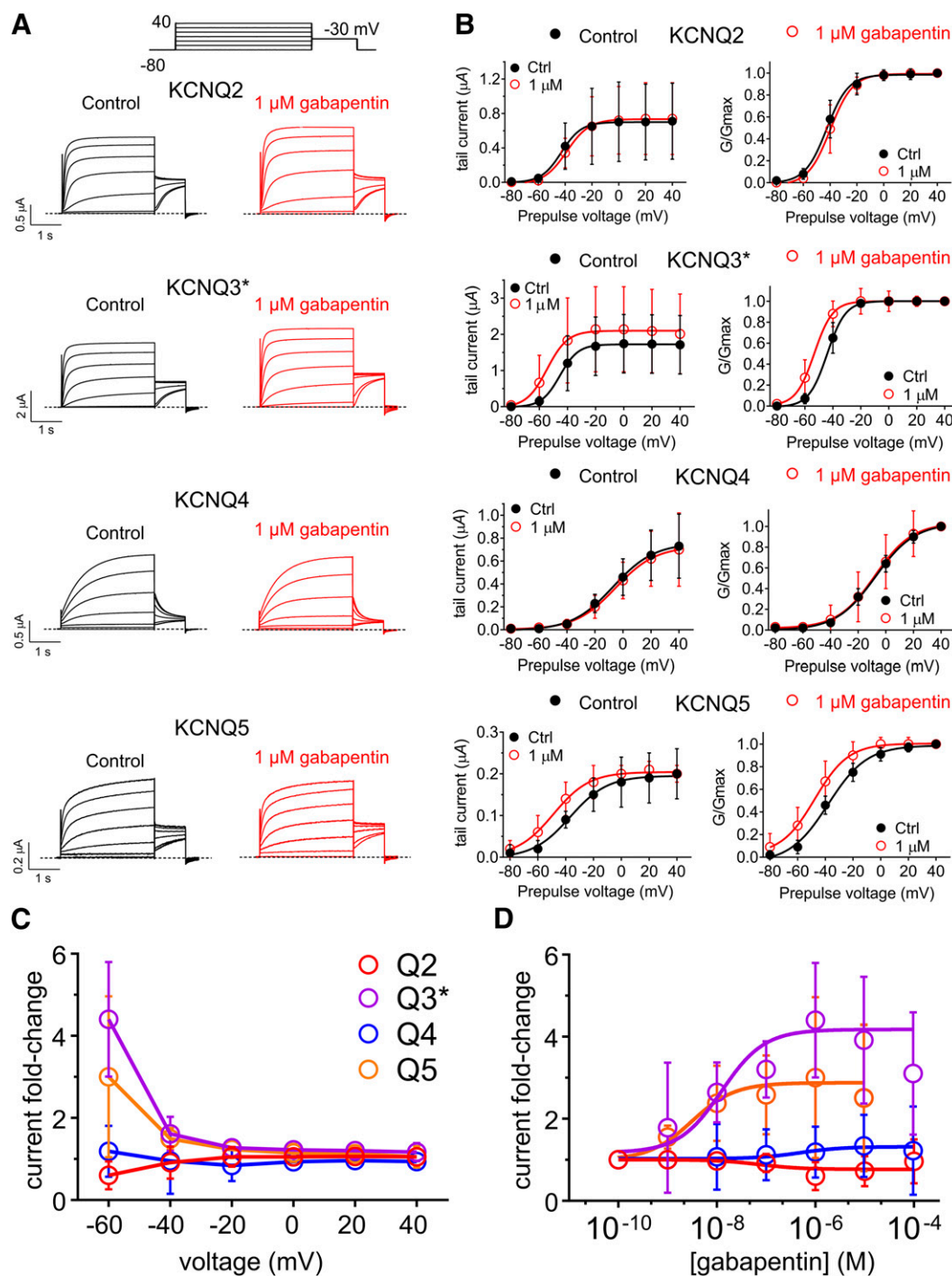


**Fig. 4.** Gabapentin-activated current is XE991 sensitive and exhibits altered gating kinetics. (A) Exemplar –60 mV KCNQ2/3 current before (left, black), during wash-in of gabapentin (red) and partial washout with bath solution in the absence of drug (black), and then wash-in of XE991 (blue). (B and C) Mean activation at +40 mV (B) and deactivation at –80 mV (C) rates for KCNQ2/3 before (control) and after wash-in of 1  $\mu$ M gabapentin (GABAP) ( $n$  = 7). Activation rate was quantified using the voltage protocol as in Fig. 3A. Deactivation rate was quantified using the voltage protocol shown here. Error bars indicate S.D.

## Discussion

**A Gabapentin Binding Site on KCNQ Channels.** We recently discovered that KCNQ3 and KCNQ5 are directly activated by the inhibitory neurotransmitter GABA, which binds close to the highly conserved S5 tryptophan, KCNQ3-W265 (Manville et al., 2018). In the current study, we show that gabapentin likewise activates KCNQ3 and KCNQ5, whereas the related gabapentinoid, pregabalin, does not. Substitution of KCNQ3-W265 with a leucine prevents activation by GABA and gabapentin and impairs GABA binding (Manville et al., 2018). KCNQ3-W265 (and its equivalent in KCNQ2, 4, and 5) is also very important for binding of retigabine and structurally related anticonvulsants (Schenzer et al., 2005). This is thought to be because small molecules with a strong negative electrostatic surface potential close to carbonyl/carbamate oxygen can hydrogen bond with the W265 (Kim et al., 2015). Indeed, here we found that pregabalin lacks this exposed negative surface potential and neither *in silico* docks with nor activates KCNQ3. Our *in silico* docking studies for gabapentin position it near to W265 and close to where retigabine (Kim et al., 2015) and GABA (Manville et al., 2018) are predicted to bind, but not necessarily overlapping—although no conclusions should be

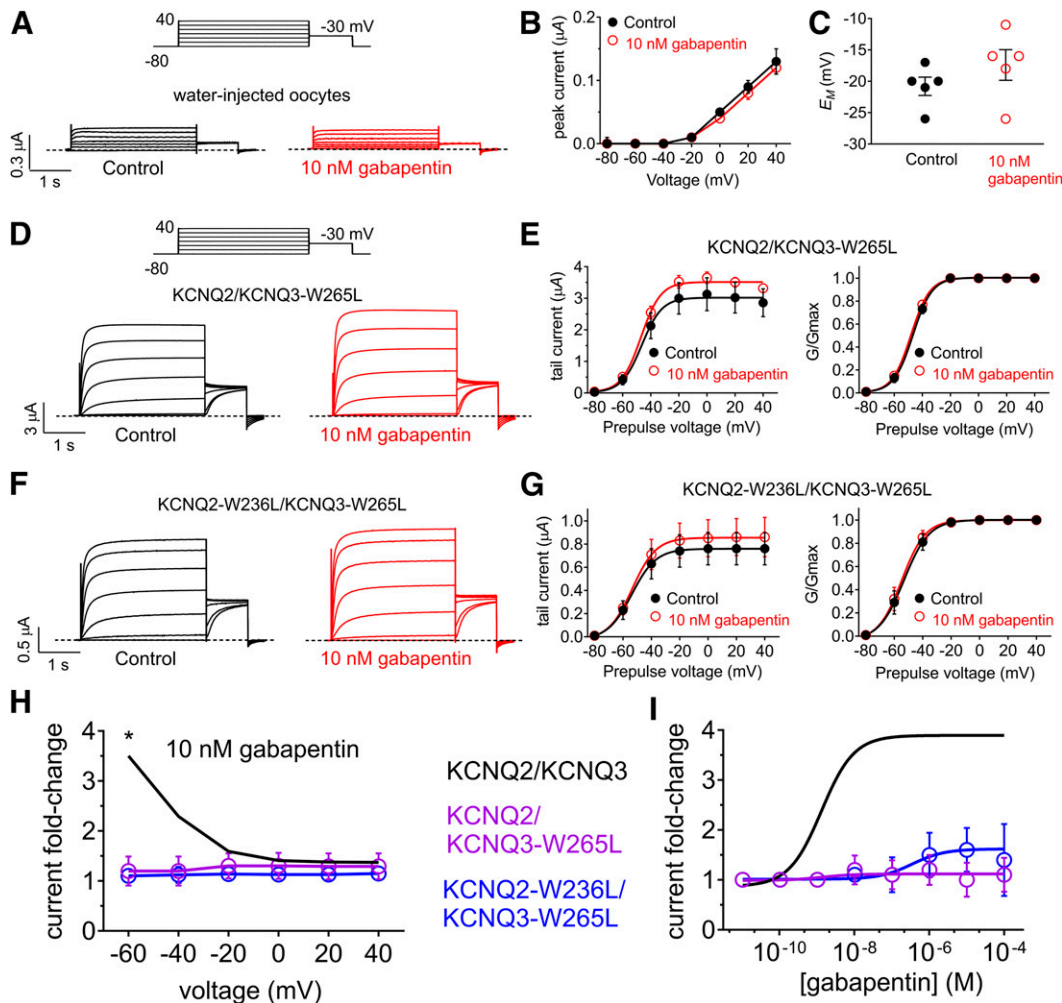
at –60 mV for KCNQ2/3 activation, quantified from data as in (A–E) ( $n$  = 7 to 8). Error bars indicate S.D. (G) Dose response for gabapentin and pregabalin effects on resting membrane potential ( $E_M$ ) of unclamped oocytes expressing KCNQ2/3 ( $n$  = 7 to 8). Error bars indicate S.D. (H) Mean tail current vs. prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing KCNQ2/3 channels in the absence (black) or presence (green) of 30  $\mu$ M retigabine as indicated ( $n$  = 4). Error bars indicate S.D. Voltage protocol as in (A). (I) Voltage dependence of KCNQ2/3 current fold increase by retigabine (30  $\mu$ M),  $n$  = 4. Error bars indicate S.D. (J) Retigabine dose responses at –60 mV for KCNQ2/3 activation, quantified from data as in (A–E) ( $n$  = 4). Ctrl, control. Error bars indicate S.D.



**Fig. 5.** Gabapentin is a potent activator of homomeric KCNQ3 and KCNQ5 potassium channels. (A) Mean two-electrode voltage-clamp (TEVC) traces for homomeric KCNQ2, 3\*, 4, or 5 channels (as indicated) expressed in *Xenopus* oocytes in the absence (control) or presence of 1  $\mu$ M gabapentin ( $n = 4-8$ ). Voltage protocol, upper inset. (B) Mean tail current (left) and normalized tail currents ( $G/G_{max}$ , right) vs. prepulse voltage relationships recorded by TEVC in *Xenopus* oocytes expressing homomeric KCNQ2, 3\*, 4, or 5 channels (as indicated) in the absence (black) or presence (red) of 1  $\mu$ M gabapentin as indicated ( $n = 4-8$ ). Error bars indicate S.D. (C) Voltage dependence of current fold increase by gabapentin (1  $\mu$ M) for homomeric KCNQ2, 3\*, 4, or 5 channels, plotted from traces as in (A) ( $n = 4-8$ ). Error bars indicate S.D. (D) Gabapentin dose responses at -60 mV for homomeric KCNQ2, 3\*, 4, or 5 channel activation, quantified from data as in (A) ( $n = 4-8$ ). Ctrl, control. Error bars indicate S.D.

drawn from the small differences in poses, and resolution of the exact pose would require structural analysis and/or further mutagenesis to map the entire binding site. We conclude that the W265-based binding site evolved to accommodate GABA and other endogenous metabolites and analogs of GABA, leading to sensitivity to modern synthetic

anticonvulsants including retigabine and gabapentin. Interestingly, KCNQ2-5 all bind GABA but only KCNQ3 and KCNQ5 are activated by GABA or gabapentin (Manville et al., 2018); retigabine activates all four (but not KCNQ1, which lacks the equivalent W) but KCNQ3 is the most sensitive (Tatulian et al., 2001).



**Fig. 6.** Gabapentin activation of KCNQ2/3 requires KCNQ3-W265L. (A–C) Two-electrode voltage-clamp (TEVC) of water-injected *Xenopus laevis* oocytes showing no effect of gabapentin (10 nM) on endogenous currents or membrane potential ( $E_M$ ) ( $n = 5$ ). (A) Mean traces; (B) mean peak current; (C) mean  $E_M$ , in the absence (control) or presence of 10 nM gabapentin. Voltage protocol (A, upper inset). Error bars indicate S.D. (D and E) TEVC of *Xenopus laevis* oocytes showing effects of gabapentin (10 nM) on heteromeric KCNQ2/KCNQ3-W265L channels. (D) Mean traces; (E) mean tail current (left) and mean normalized tail current ( $G/G_{max}$ ; right).  $n = 5$ . Error bars indicate S.D. (F and G) TEVC of *Xenopus laevis* oocytes showing effects of gabapentin (10 nM) on heteromeric KCNQ2-W236L/KCNQ3-W265L channels. (F) Mean traces; (G) mean tail current (left) and mean normalized tail current ( $G/G_{max}$ ; right);  $n = 5$ . Error bars indicate S.D. (H) Mean tail current fold changes vs. prepulse voltages for channels as indicated; KCNQ2/KCNQ3 results (black line) from Fig. 3E shown for comparison;  $n = 5$ . Error bars indicate S.D. \* $P < 0.05$  vs. other groups at  $-60$  mV. (I) Mean dose responses for channels as indicated; KCNQ2/KCNQ3 results (black line) from Fig. 3F shown for comparison;  $n = 5$ . Error bars indicate S.D.

**Mechanisms of Therapeutic Action.** Gabapentin and pregabalin are in wide clinical use to treat a variety of disorders of the nervous system, including neuropathic pain and epilepsy. There is considerable overlap between the clinical indications for each drug (Sills, 2006; Calandre et al., 2016; Alles and Smith, 2018). This, together with the contrasting ability of gabapentin and pregabalin to activate neuronal KCNQ isoforms found herein, suggests that KCNQ activation cannot be the dominant mechanism of action for the majority of the therapeutic effects of gabapentin. Gabapentinoid binding to the  $\alpha_2\delta_1$  subunit reportedly inhibits  $\alpha_2\delta_1$ -containing  $Ca_v$  channels (Stefani et al., 1998, 2001), although others found that gabapentinoids have little effect on  $Ca_v$  channel activity or  $Ca_v$  channel-dependent neurotransmitter release at presynaptic nerve terminals (Rock et al., 1993; Schumacher et al., 1998; Brown and Randall, 2005; Hoppe et al., 2012).  $\alpha_2\delta_1$ -N-methyl-D-aspartate receptor complexes were recently discovered in human and rodent spinal cord; gabapentin inhibited  $\alpha_2\delta_1$ -dependent

potentiation of N-methyl-D-aspartate receptor activity and associated pain hypersensitivity, presenting a plausible mechanism for antinociceptive effects of gabapentin (Chen et al., 2018).

**Multiple Gabapentinoid Targets in Neurons—A Role for KCNQs?** In a study comparing pregabalin and gabapentin effects on cultured dorsal root ganglion (DRG) neurons from neonatal rats, pregabalin and gabapentin produced biphasic effects (acute inhibition, but longer-term augmentation) on endogenous  $K^+$  currents. The enhancing effect was attenuated by pertussis toxin or intracellular application of a synthetic cAMP analog, suggesting an indirect mechanism involving G protein activation (McClelland et al., 2004). Another group also found that effects of gabapentin on inward rectifier  $K^+$  and N-type  $Ca^{2+}$  channels were pertussis toxin sensitive (Bertrand et al., 2003b). Pertussis toxin is commonly used to inhibit the downstream effects of GABA<sub>B</sub> receptor activation since it inhibits some (but not all) of the G proteins involved in this process (Asano et al., 1985). However, others

have shown that GABA<sub>B</sub> receptors are insensitive to gabapentinoids (Lanneau et al., 2001), and GABA<sub>B</sub> receptor inhibitors did not alter the pregabalin-induced inhibition of Ca<sub>v</sub> currents in neonatal rat DRG neurons (Martin et al., 2002; McClelland et al., 2004). The most likely explanation for this apparent discrepancy is that gabapentinoids can activate pertussis-sensitive G proteins, but are independent of GABA<sub>B</sub> receptors (Martin et al., 2002).

With respect to the DRG neuron K<sup>+</sup> channel inhibition by pregabalin, it was apamin-sensitive, implying it involved small-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels (McClelland et al., 2004). The K<sup>+</sup> current enhancement did not begin until 10 minutes after initiation of administration of pregabalin, and the enhancement was apamin insensitive and faster when pregabalin was applied intracellularly, suggesting an intracellular signaling mechanism. The gating kinetics and voltage dependence of the DRG K<sub>v</sub> current described in the gabapentinoid study do not necessarily suggest against it containing an M-current component. Interestingly, KCNQ2 (which is gabapentin insensitive) expression precedes that of KCNQ3 (gabapentin sensitive) during human brain development (Tinel et al., 1998), and the effects of KCNQ channel inhibition upon depolarization-induced GABA release and action potential propagation also alter dramatically from P0 to P7 in rat (Okada et al., 2003). Thus, in some neurons M-current might be insensitive to gabapentin early in development (e.g., in the first week), unless KCNQ5 was appreciably expressed. Furthermore, in the study of gabapentinoid action on DRG neurons, K<sup>+</sup> channel activity was quantified at +40 mV, a voltage at which the activating effects of gabapentin (and most K<sub>v</sub> channel activators) are minimal. In addition, we find that pregabalin inhibits KCNQ2/3 channel activity at concentrations of 10 μM and above, suggesting that at the concentrations used in the prior study (250 μM) (McClelland et al., 2004) pregabalin would inhibit KCNQ2/3 channels and may have similar effects on other KCNQ isoforms that could be expressed in neonatal rat DRG neurons.

It is highly possible, given the somewhat pleiotropic actions of gabapentinoids, that the potent effects of gabapentin on KCNQ3 and KCNQ5 channels might be masked by other effects observed at higher doses, both experimentally and with respect to clinical mechanisms of action. Serum gabapentinoid concentrations may reach 100 μM in patients (although in the brain and spinal cord this concentration is likely to be lower) (Ben-Menachem et al., 1992, 1995; Berry et al., 2003), which is several orders of magnitude higher than the EC<sub>50</sub> values for gabapentin activation of KCNQ2/3, KCNQ3, and KCNQ5 channels, but as noted previously this is within the range for pregabalin inhibition of KCNQ2/3.

Gabapentin has also been found to augment K<sub>ATP</sub> currents in rat hippocampal and human neocortical slices (but not, incidentally, in rat DRG neurons) (Freiman et al., 2001), and to inhibit the hyperpolarization-activated, cyclic nucleotide-gated channel, HCN4, albeit not at clinically relevant drug concentrations (Tae et al., 2017). Conversely, gabapentin was augmented in hippocampal and inhibitory interneurons, cells that highly express HCN1 and HCN2 (Surges et al., 2003; Peng et al., 2011). Thus, indirect modes of action of gabapentin may occur in vivo, as reported for K<sub>v</sub> currents in rat DRG neurons (McClelland et al., 2004).

## Conclusions

Perhaps the two most important take-home points from this study are the following. First, that we have discovered a new chemical space for KCNQ2/3 activation by synthetic compounds. Future structure-activity relationship studies guided by what we now know regarding the difference between gabapentin versus pregabalin with respect to KCNQ opening, and our previous work identifying endogenous activators for KCNQ3 and KCNQ5, including GABA, GABOB (gamma-amino-beta-hydroxybutyrate), and β-hydroxybutyrate (Manville et al., 2018), can start to inform synthesis of a new class of KCNQ activators for potential therapeutic use. Second, the high potency but relatively low efficacy of gabapentin compared with, e.g., retigabine, suggests the possibility that gabapentin could act as a partial agonist and disrupt therapeutic actions of retigabine and related anticonvulsants. Furthermore, it is possible that gabapentin competes with the binding of endogenous GABA and its metabolites to neuronal KCNQ channels but shares similar or lower efficacy to them with respect to KCNQ activation, possibly explaining why KCNQ activation may not be an important determinant of gabapentin's beneficial effects. Thus, further exploration of gabapentinoids and related compounds with respect to KCNQ activation might uncover superior compounds, which either avoid KCNQ activation (and thus potentially disruptive partial agonism) or alternatively are more effective than gabapentin in activating neuronal KCNQs (and thus are clinically superior because of an additional, beneficial target site).

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## Authorship Contributions

*Participated in research design:* Manville, Abbott.

*Conducted experiments:* Manville.

*Performed data analysis:* Manville, Abbott.

*Wrote or contributed to the writing of the manuscript:* Manville, Abbott.

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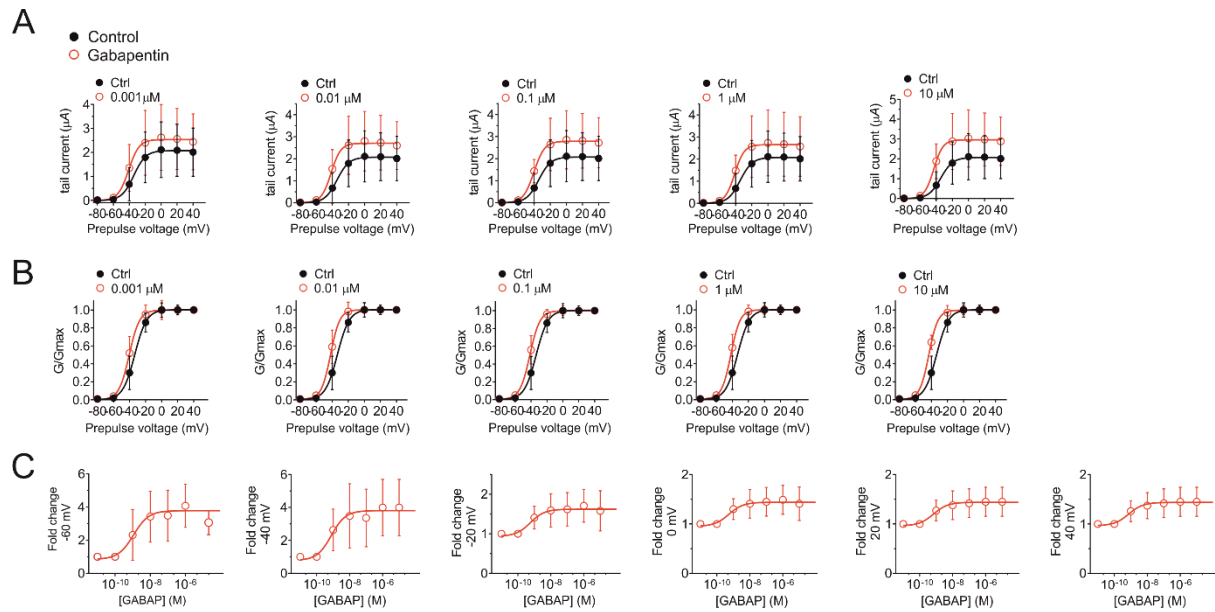
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# **Gabapentin is a potent activator of KCNQ3 and KCNQ5 potassium channels**

Ríán W. Manville and Geoffrey W. Abbott

## ***MOLECULAR PHARMACOLOGY***

## **SUPPLEMENTAL DATA**



**Supplementary Figure 1. Effects of Gabapentin on KCNQ2/3 channels**

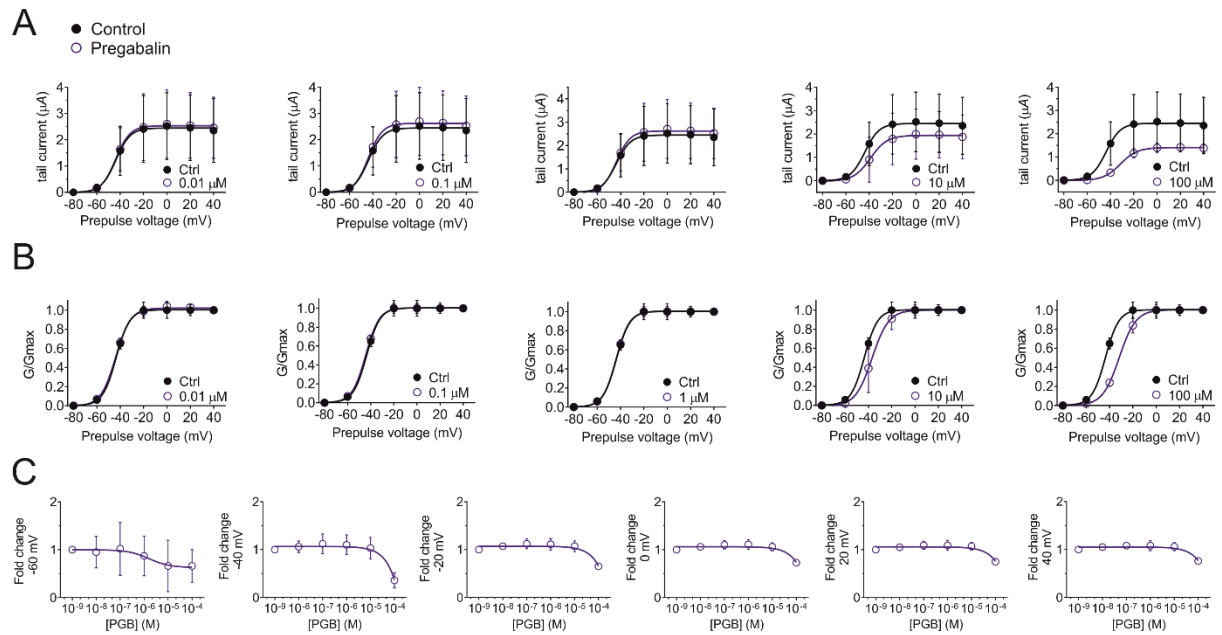
- A. Mean tail current versus prepulse voltage relationship for KCNQ2/3 channels in the absence (black) and presence (red) of Gabapentin,  $n = 7-8$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 7-8$ .
- C. Dose response of KCNQ2/3 channels between  $-40$  and  $+40$  mV,  $n = 7-8$ . Error bars indicate SD.

KCNQ2/3	$\tau$ act, $-40$ mV (ms)	$\tau$ deact, $-80$ mV (ms)	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$1141 \pm 369$ ( $n=8$ )	$115 \pm 35$ ( $n=8$ )	$-33.5 \pm 3.8$ ( $n=8$ )	$-33.4 \pm 15.8$ ( $n=8$ )	$7.4 \pm 2.9$ ( $n=8$ )
0.001 $\mu$ M Gabapentin	$853 \pm 379$ ( $n=7$ )	$146 \pm 49$ ( $n=7$ )	$-40.5 \pm 2.8$ ( $n=7$ ) **	$-40.9 \pm 12.8$ ( $n=7$ )	$6.4 \pm 4.3$ ( $n=7$ )
0.01 $\mu$ M Gabapentin	$797 \pm 379$ ( $n=7$ )	$153 \pm 17$ ( $n=7$ ) *	$-41.9 \pm 2.7$ ( $n=7$ ) ***	$-41.6 \pm 11.6$ ( $n=7$ )	$5.7 \pm 4.2$ ( $n=7$ )
0.1 $\mu$ M Gabapentin	$835 \pm 350$ ( $n=7$ )	$156 \pm 26$ ( $n=7$ ) *	$-41.4 \pm 2.1$ ( $n=7$ ) ***	$-41.3 \pm 10.2$ ( $n=7$ )	$6.3 \pm 3.0$ ( $n=7$ )
1 $\mu$ M Gabapentin	$800 \pm 290$ ( $n=7$ )	$154 \pm 36$ ( $n=7$ )	$-42.8 \pm 2.3$ ( $n=7$ ) ***	$-42.2 \pm 12.3$ ( $n=7$ )	$6.0 \pm 3.4$ ( $n=7$ )
10 $\mu$ M Gabapentin	$820 \pm 186$ ( $n=8$ )	$160 \pm 46$ ( $n=8$ )	$-43.0 \pm 1.2$ ( $n=8$ ) ***	$-43.2 \pm 13.5$ ( $n=8$ )	$5.6 \pm 1.8$ ( $n=8$ )

**Supplementary Table 1. Summary of Effects of Gabapentin on KCNQ2/3 channels.**

Statistics versus same channel in absence of Gabapentin: \*\*\* $p=0.0006$ , \*\* $p=0.002$ . Values indicate mean  $\pm$  SD.





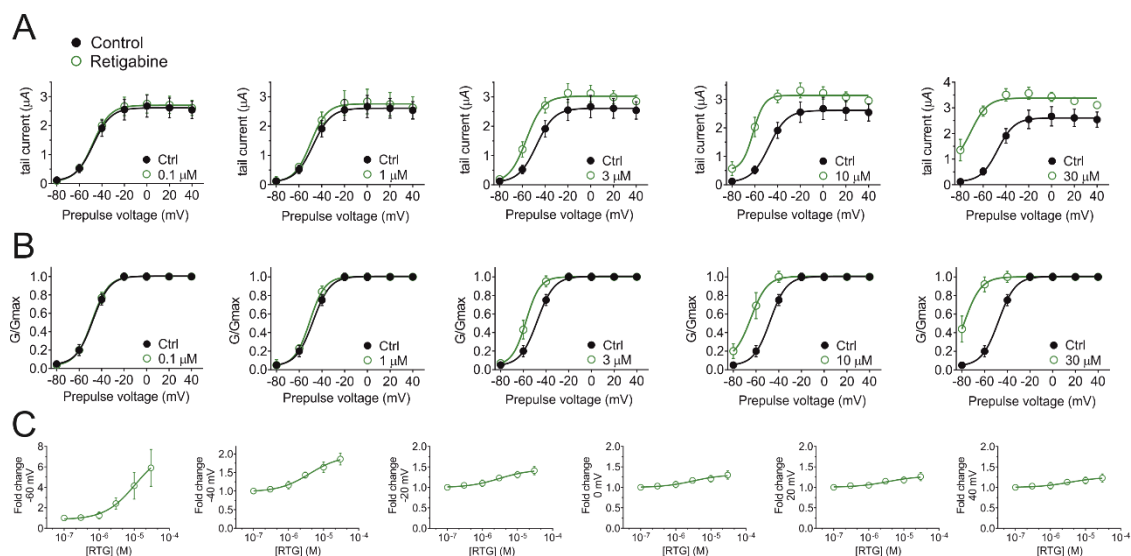
**Supplementary Figure 2. Effects of Pregabalin on KCNQ2/3 channels**

- A. Mean tail current versus prepulse voltage relationship for KCNQ2/3 channels in the absence (black) and presence (red) of Pregabalin,  $n = 4-8$ .  
 B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 4-8$ .  
 C. Dose response of KCNQ2/3 channels between  $-40$  and  $+40$  mV,  $n = 4-8$ . Error bars indicate SD.

KCNQ2/3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-43.5 \pm 4.5$ ( $n = 8$ )	$-43.7 \pm 13.8$ ( $n = 8$ )	$5.7 \pm 4.9$ ( $n = 8$ )
0.01 $\mu$ M Pregabalin	$-43.8 \pm 1.1$ ( $n = 4$ )	$-43.8 \pm 14.8$ ( $n = 4$ )	$6.4 \pm 1.2$ ( $n = 4$ )
0.1 $\mu$ M Pregabalin	$-44.4 \pm 1.2$ ( $n = 4$ )	$-43.9 \pm 14.4$ ( $n = 4$ )	$5.8 \pm 1.1$ ( $n = 4$ )
1 $\mu$ M Pregabalin	$-43.8 \pm 1.4$ ( $n = 4$ )	$-43.7 \pm 13.9$ ( $n = 4$ )	$5.7 \pm 1.4$ ( $n = 4$ )
10 $\mu$ M Pregabalin	$-36.8 \pm 3.7$ ( $n = 8$ ) **	$-39.0 \pm 14.1$ ( $n = 8$ )	$7.1 \pm 3.9$ ( $n = 8$ )
100 $\mu$ M Pregabalin	$-31.9 \pm 0.8$ ( $n = 4$ ) ***	$-31.5 \pm 1.9$ ( $n = 4$ )	$7.1 \pm 0.5$ ( $n = 4$ )

**Supplementary Table 2. Summary of Effects of Pregabalin on KCNQ2/3 channels.**

Statistics versus same channel in absence of Pregabalin: \*\*\* $p=0.0001$ , \*\* $p=0.006$ . Values indicate mean  $\pm$  SD.



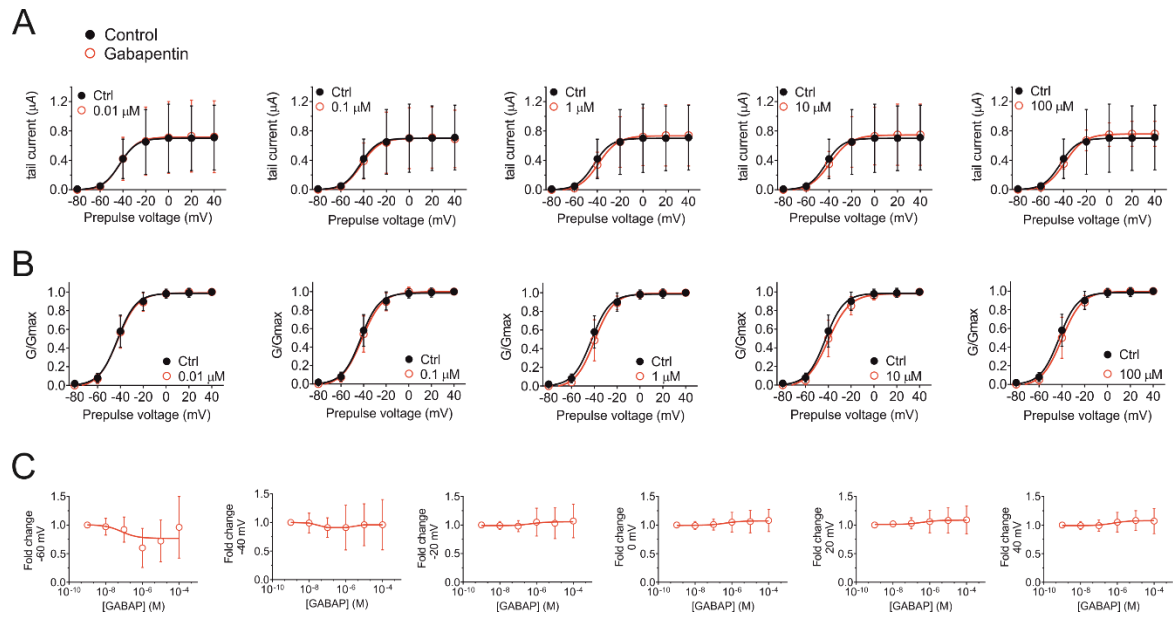
**Supplementary Figure 3. Effects of retigabine on KCNQ2/3 channels**

- A. Mean tail current versus prepulse voltage relationship for Q2/Q3 channels in the absence (black) and presence (green) of retigabine,  $n = 4$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 4$ .
- C. Dose response of Q2/Q3 channels between -40 and +40 mV,  $n = 4$ . Error bars indicate SD.

KCNQ2/3	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-47.8 \pm 1.7$ ( $n = 4$ )	$-47.6 \pm 4.9$ ( $n = 4$ )	$7.4 \pm 1.2$ ( $n = 4$ )
0.1 $\mu$ M Retigabine	$-48.6 \pm 1.6$ ( $n = 4$ )	$-47.9 \pm 4.1$ ( $n = 4$ )	$7.3 \pm 1.0$ ( $n = 4$ )
1 $\mu$ M Retigabine	$-50.7 \pm 1.6$ ( $n = 4$ ) *	$-49.7 \pm 5.2$ ( $n = 4$ )	$6.7 \pm 1.0$ ( $n = 4$ )
3 $\mu$ M Retigabine	$-57.6 \pm 1.6$ ( $n = 4$ ) ***	$-56.5 \pm 3.2$ ( $n = 4$ ) *	$6.1 \pm 2.2$ ( $n = 4$ )
10 $\mu$ M Retigabine	$-64.6 \pm 2.8$ ( $n = 4$ ) ***	$-61.6 \pm 5.0$ ( $n = 4$ ) **	$7.2 \pm 1.4$ ( $n = 4$ )
30 $\mu$ M Retigabine	$-78.1 \pm 2.5$ ( $n = 4$ ) ****	$-70.8 \pm 8.1$ ( $n = 4$ ) **	$7.4 \pm 1.6$ ( $n = 4$ )

**Supplementary Table 3. Summary of Effects of retigabine on KCNQ2/3 channels.**

Statistics versus same channel in absence of retigabine: \*\*\*\* $p < 0.0001$ , \*\*\* $p = 0.0002$ , \* $p = 0.04$ . Values indicate mean  $\pm$  SD.



#### Supplementary Figure 4. Effects of Gabapentin on KCNQ2 channels

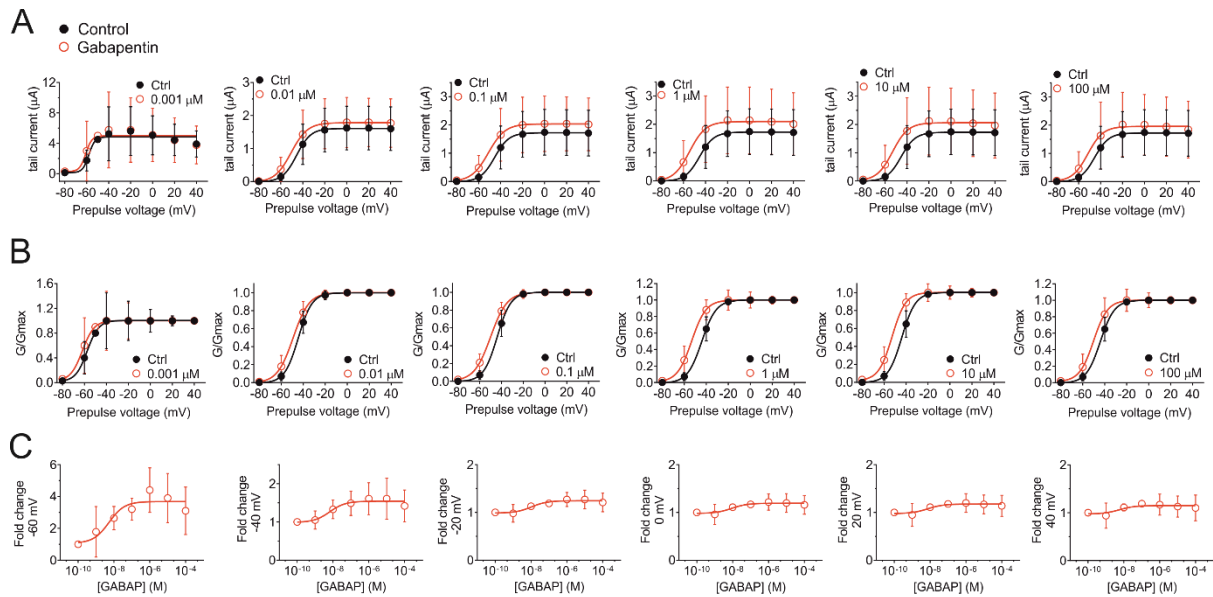
- A. Mean tail current versus prepulse voltage relationship for KCNQ2 channels in the absence (black) and presence (red) of Gabapentin,  $n = 6$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 6$ .
- C. Dose response of KCNQ2 channels between -40 and +40 mV,  $n = 6$ . Error bars indicate SD.

KCNQ2	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-42.5 \pm 3.0$ ( $n = 6$ )	$-42.8 \pm 19$ ( $n = 6$ )	$8.1 \pm 3.4$ ( $n = 6$ )
0.01 $\mu$ M Gabapentin	$-42.5 \pm 3.3$ ( $n = 6$ )	$-42.6 \pm 19.8$ ( $n = 6$ )	$8.6 \pm 3.3$ ( $n = 6$ )
0.1 $\mu$ M Gabapentin	$-41.4 \pm 3.8$ ( $n = 6$ )	$-41.8 \pm 17.4$ ( $n = 6$ )	$8.9 \pm 3.8$ ( $n = 6$ )
1 $\mu$ M Gabapentin	$-39.8 \pm 3.4$ ( $n = 6$ )	$-38.8 \pm 15.4$ ( $n = 6$ )	$8.4 \pm 3.7$ ( $n = 6$ )
10 $\mu$ M Gabapentin	$-40.3 \pm 3.9$ ( $n = 6$ )	$-38.9 \pm 16.4$ ( $n = 6$ )	$9.5 \pm 3.9$ ( $n = 6$ )
100 $\mu$ M Gabapentin	$-40.1 \pm 3.5$ ( $n = 6$ )	$-38.8 \pm 15.3$ ( $n = 6$ )	$8.9 \pm 3.6$ ( $n = 6$ )

Supplementary Table 4. Summary of Effects of Gabapentin on KCNQ2 channels.

Values indicate mean  $\pm$  SD.





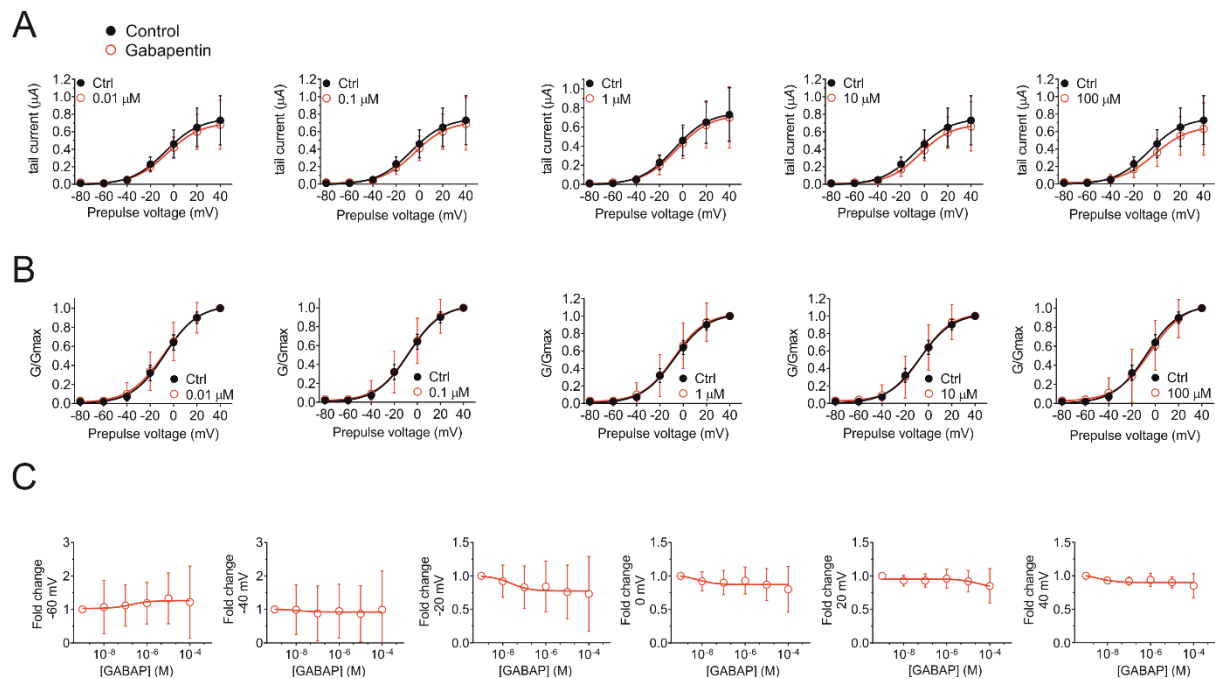
### Supplementary Figure 5. Effects of Gabapentin on KCNQ3\* channels

- A. Mean tail current versus prepulse voltage relationship for KCNQ3\* channels in the absence (black) and presence (red) of Gabapentin,  $n = 6$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 6$ .
- C. Dose response of KCNQ3\* channels between -40 and +40 mV,  $n = 6$ . Error bars indicate SD.

KCNQ3*	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-44.5 \pm 2.0$ ( $n = 6$ )	$-45.5 \pm 13.9$ ( $n = 6$ )	$6.2 \pm 1.8$ ( $n = 6$ )
0.001 $\mu$ M Gabapentin	$-44.9 \pm 2.1$ ( $n = 6$ )	$-60.1 \pm 17.0$ ( $n = 7$ )	n.d.
0.01 $\mu$ M Gabapentin	$-49.2 \pm 2.4$ ( $n = 6$ ) **	$-50.5 \pm 17.7$ ( $n = 6$ )	$7.2 \pm 1.6$ ( $n = 6$ )
0.1 $\mu$ M Gabapentin	$-49.6 \pm 3.3$ ( $n = 6$ ) *	$-51.1 \pm 17.7$ ( $n = 6$ )	$7.5 \pm 2.1$ ( $n = 6$ )
1 $\mu$ M Gabapentin	$-53.3 \pm 3.2$ ( $n = 6$ ) ***	$-54.5 \pm 19.2$ ( $n = 6$ )	n.d.
10 $\mu$ M Gabapentin	$-52.7 \pm 3.5$ ( $n = 6$ ) **	$-53.2 \pm 19.9$ ( $n = 6$ )	n.d.
100 $\mu$ M Gabapentin	$-50.2 \pm 3.7$ ( $n = 6$ ) *	$51.7 \pm 19.7$ ( $n = 6$ )	n.d.

### Supplementary Table 5. Summary of Effects of Gabapentin on KCNQ3\* channels.

Statistics versus same channel in absence of Gabapentin: \*\*\* $p=0.0004$ , \*\* $p=0.003$ , \* $p=0.01$ . Values indicate mean  $\pm$  SD.



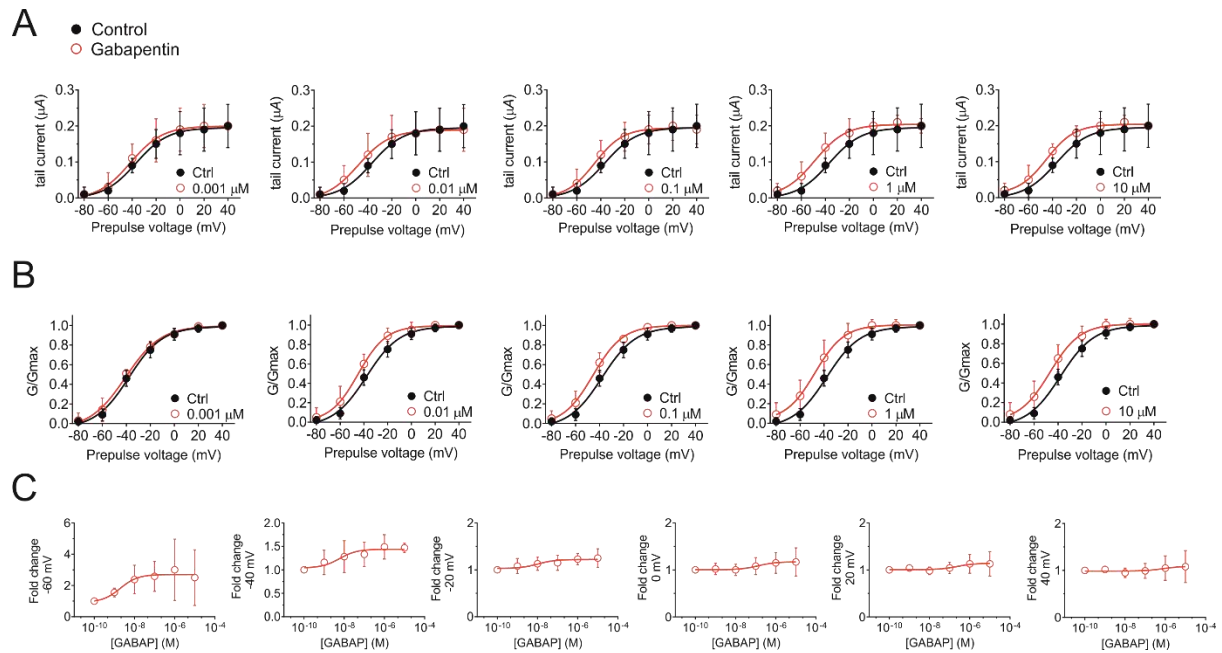
**Supplementary Figure 6. Effects of Gabapentin on KCNQ4 channels**

- A. Mean tail current versus prepulse voltage relationship for KCNQ4 channels in the absence (black) and presence (red) of Gabapentin,  $n = 4$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 4$ .
- C. Dose response of KCNQ4 channels between  $-40$  and  $+40$  mV,  $n = 4$ . Error bars indicate SD.

KCNQ4	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-42.5 \pm 3.3$ ( $n = 4$ )	$-42.8 \pm 13.2$ ( $n = 4$ )	$8.6 \pm 3.1$ ( $n = 4$ )
0.01 $\mu$ M Gabapentin	$-42.5 \pm 2.1$ ( $n = 4$ )	$-42.6 \pm 12.9$ ( $n = 4$ )	$8.7 \pm 1.9$ ( $n = 4$ )
0.1 $\mu$ M Gabapentin	$-41.4 \pm 2.0$ ( $n = 4$ )	$-41.8 \pm 14.6$ ( $n = 4$ )	$8.6 \pm 1.9$ ( $n = 4$ )
1 $\mu$ M Gabapentin	$-39.8 \pm 2.8$ ( $n = 4$ )	$-38.8 \pm 15.7$ ( $n = 4$ )	$8.4 \pm 2.6$ ( $n = 4$ )
10 $\mu$ M Gabapentin	$-40.3 \pm 1.8$ ( $n = 4$ )	$-38.9 \pm 13.0$ ( $n = 4$ )	$9.5 \pm 1.6$ ( $n = 4$ )
100 $\mu$ M Gabapentin	$-40.1 \pm 2.6$ ( $n = 4$ )	$-38.8 \pm 18.0$ ( $n = 4$ )	$8.9 \pm 2.4$ ( $n = 4$ )

**Supplementary Table 6. Summary of Effects of Gabapentin on KCNQ4 channels.**

Values indicate mean  $\pm$  SD.



**Supplementary Figure 7. Effects of Gabapentin on KCNQ5 channels**

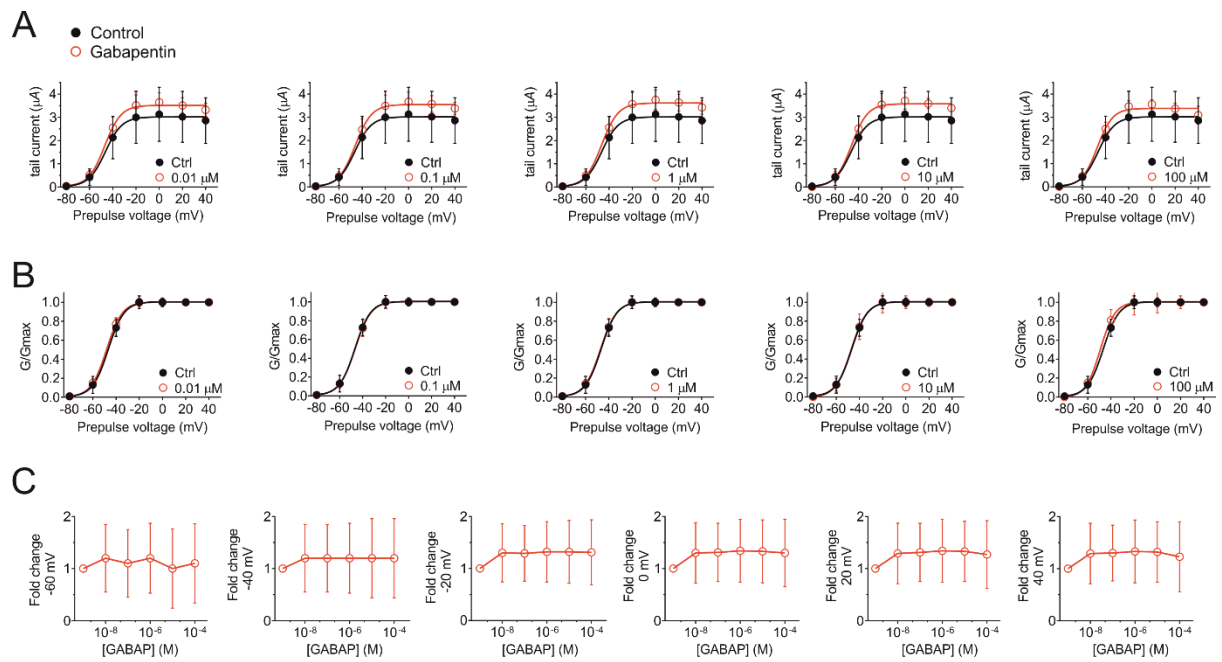
- A. Mean tail current versus prepulse voltage relationship for KCNQ5 channels in the absence (black) and presence (red) of Gabapentin,  $n = 4$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 4$ .
- C. Dose response of KCNQ5 channels between -40 and +40 mV,  $n = 4$ . Error bars indicate SD.

KCNQ5	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-37.9 \pm 3.8$ ( $n = 4$ )	$-36.8 \pm 12.8$ ( $n = 4$ )	$13.9 \pm 4.4$ ( $n = 4$ )
0.001 $\mu$ M Gabapentin	$-40.7 \pm 4.9$ ( $n = 4$ )	$-41.5 \pm 12.9$ ( $n = 4$ )	$14.7 \pm 4.9$ ( $n = 4$ )
0.01 $\mu$ M Gabapentin	$-44.8 \pm 5.7$ ( $n = 4$ )	$-44.9 \pm 12.3$ ( $n = 4$ )	$11.7 \pm 5.7$ ( $n = 4$ )
0.1 $\mu$ M Gabapentin	$-45.7 \pm 4.8$ ( $n = 4$ ) *	$-45.0 \pm 12.6$ ( $n = 4$ )	$12.5 \pm 4.8$ ( $n = 4$ )
1 $\mu$ M Gabapentin	$-47.6 \pm 4.2$ ( $n = 4$ ) *	$-48.9 \pm 8.2$ ( $n = 4$ )	$12.2 \pm 8.2$ ( $n = 4$ )
10 $\mu$ M Gabapentin	$-46.8 \pm 7.5$ ( $n = 4$ )	$-45.0 \pm 7.5$ ( $n = 4$ )	$12.6 \pm 7.5$ ( $n = 4$ )

**Supplementary Table 7. Summary of Effects of Gabapentin on KCNQ5 channels.**

Statistics versus same channel in absence of Gabapentin: \* $p=0.02$ . Values indicate mean  $\pm$  SD.





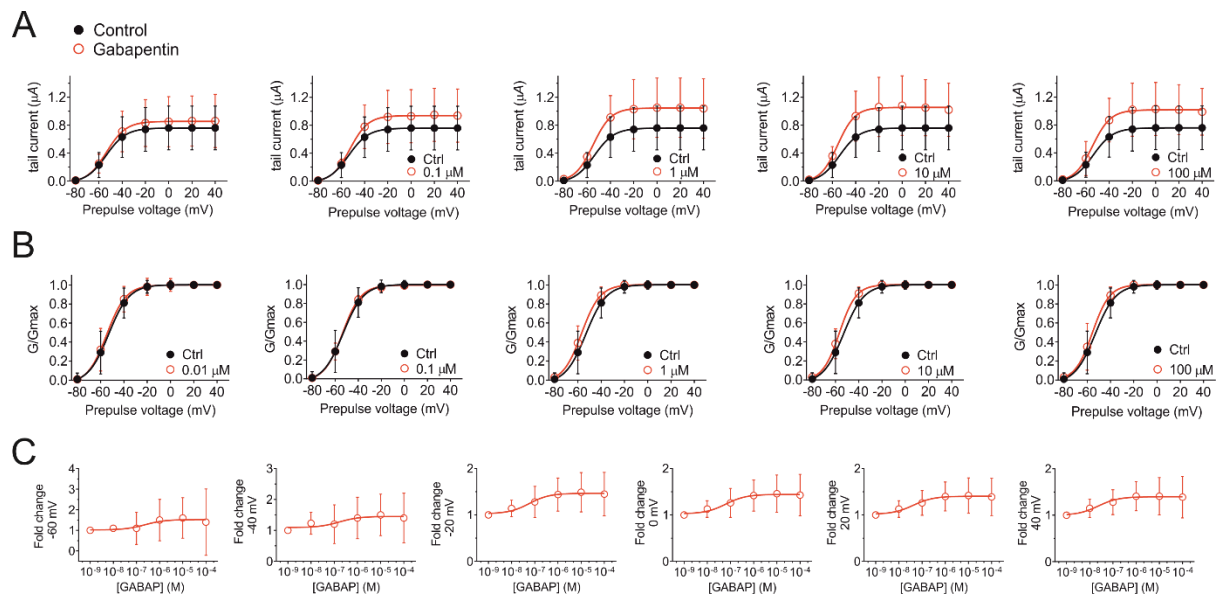
**Supplementary Figure 8. Effects of Gabapentin on KCNQ2/KCNQ3-W265L channels**

- A. Mean tail current versus prepulse voltage relationship for KCNQ2/KCNQ3-W265L channels in the absence (black) and presence (red) of Gabapentin,  $n = 5$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 5$ .
- C. Dose response of KCNQ2/KCNQ3-W265L channels between -40 and +40 mV,  $n = 5$ . Error bars indicate SD.

Q2/Q3-W265L	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-46.7 \pm 2.2$ ( $n = 5$ )	$-46.4 \pm 9.6$ ( $n = 5$ )	$6.7 \pm 2.2$ ( $n = 5$ )
0.01 $\mu$ M Gabapentin	$-48.1 \pm 1.7$ ( $n = 5$ )	$-46.9 \pm 4.1$ ( $n = 5$ )	$6.7 \pm 1.1$ ( $n = 5$ )
0.1 $\mu$ M Gabapentin	$-46.4 \pm 1.8$ ( $n = 5$ )	$-46.0 \pm 4.1$ ( $n = 5$ )	$6.9 \pm 1.8$ ( $n = 5$ )
1 $\mu$ M Gabapentin	$-47.2 \pm 2.1$ ( $n = 5$ )	$-46.4 \pm 4.2$ ( $n = 5$ )	$6.9 \pm 2.1$ ( $n = 5$ )
10 $\mu$ M Gabapentin	$-47.2 \pm 3.3$ ( $n = 5$ )	$-46.1 \pm 3.7$ ( $n = 5$ )	$6.8 \pm 3.3$ ( $n = 5$ )
100 $\mu$ M Gabapentin	$-49.2 \pm 3.3$ ( $n = 5$ )	$-47.2 \pm 2.7$ ( $n = 5$ )	$6.4 \pm 3.3$ ( $n = 5$ )

**Supplementary Table 8. Summary of Effects of Gabapentin on KCNQ2/KCNQ3-W265L channels.**

Values indicate mean  $\pm$  SD.



**Supplementary Figure 9. Effects of Gabapentin on KCNQ2-W236L/KCNQ3-W265L channels**

- A. Mean tail current versus prepulse voltage relationship for KCNQ2-W236L/KCNQ3-W265L channels in the absence (black) and presence (red) of Gabapentin,  $n = 5$ .
- B. Normalized tail current versus prepulse voltage relationships as in panel A,  $n = 5$ .
- C. Dose response of KCNQ2-W236L/KCNQ3-W265L channels between -40 and +40 mV,  $n = 5$ . Error bars indicate SD.

Q2-W236L/ Q3-W265L	Normalized tail current $V_{0.5}$ (mV)	Non-normalized tail current $V_{0.5}$ (mV)	Slope (mV)
Ctrl	$-53.1 \pm 5.2$ ( $n = 5$ )	$-53.8 \pm 15.9$ ( $n = 5$ )	$8.8 \pm 4.0$ ( $n = 5$ )
0.01 $\mu$ M Gabapentin	$-54.7 \pm 4.7$ ( $n = 5$ )	$-53.5 \pm 15.6$ ( $n = 5$ )	$8.3 \pm 3.8$ ( $n = 5$ )
0.1 $\mu$ M Gabapentin	$-53.6 \pm 1.9$ ( $n = 5$ )	$-52.6 \pm 13.9$ ( $n = 5$ )	$7.9 \pm 1.4$ ( $n = 5$ )
1 $\mu$ M Gabapentin	$-56.9 \pm 3.2$ ( $n = 5$ )	$-54.8 \pm 13.7$ ( $n = 5$ )	$7.9 \pm 3.1$ ( $n = 5$ )
10 $\mu$ M Gabapentin	$-56.6 \pm 2.7$ ( $n = 5$ )	$-55.4 \pm 12.7$ ( $n = 5$ )	$7.1 \pm 2.7$ ( $n = 5$ )
100 $\mu$ M Gabapentin	$-55.5 \pm 4.0$ ( $n = 5$ )	$-54.3 \pm 13.0$ ( $n = 5$ )	$7.4 \pm 3.5$ ( $n = 5$ )

**Supplementary Table 9. Summary of Effects of Gabapentin on KCNQ2-W236L/KCNQ3-W265L channels.**

Values indicate mean  $\pm$  SD.