PROF. GREG J STUART (Orcid ID : 0000-0001-9395-2219)

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Title: $GABA_B$ receptors in neocortical and hippocampal pyramidal neurons are coupled to different potassium channels

Authors: Jean-Didier Breton* and Greg J. Stuart

Address: Eccles Institute of Neuroscience and Australian Research Council Centre of Excellence for Integrative Brain Function, John Curtin School of Medical Research, Australian National University, Canberra, Australia.

*Current address: Medical School, Australian National University, Canberra, Australia.

Corresponding author: Greg Stuart, Eccles Institute of Neuroscience, John Curtin School of Medical Research, Australian National University, Canberra ACT 2601, Australia. Phone: +61-2-6125-8927; E-mail: greg.stuart@anu.edu.au

Running title: Differential coupling of $GABA_B$ to GIRK and TASK

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Abstract:

Classically, $GABA_B$ receptors are thought to regulate neuronal excitability via G-protein coupled inwardly rectifying potassium (GIRK) channels. Recent data, however, indicates that $GABA_B$ receptors can also activate two-pore domain potassium channels. Here, we investigate which potassium channels are coupled to $GABA_B$ receptors in rat neocortical layer 5 and hippocampal CA1 pyramidal neurons. Bath application of the non-specific GIRK channel blocker barium (200 µM) abolished outward currents evoked by $GABA_B$ receptors in CA1 pyramidal, but only partially blocked $GABA_B$ responses in layer 5 neurons. Layer 5 and CA1 pyramidal neurons also showed differential sensitivity to tertiapin-Q, a specific GIRK channel blocker. Tertiapin-Q partially blocked $GABA_B$ responses in CA1 pyramidal neurons, but was ineffective in blocking $GABA_B$ responses in neocortical layer 5 neurons. Consistent with the idea that $GABA_B$ receptors are coupled to two-pore domain potassium channels, the non-specific blockers quinidine and bupivacaine partially blocked $GABA_B$ responses in both layer 5 and CA1 neurons. Finally, we show that lowering external pH, as occurs in hypoxia, blocks the component of $GABA_B$ responses mediated by two-pore domain potassium channels in neocortical layer 5 pyramidal neurons, while at the same time revealing a GIRK channel component. These data indicate that $GABA_B$ receptors in neocortical layer 5 and hippocampal CA1 pyramidal neurons are coupled to different channels, with this coupling pH dependent in neocortical layer 5 pyramidal neurons. This pH-dependency may act to maintain constant levels of $GABA_B$ inhibition during hypoxia by enhancing GIRK channel function following a reduction in two-pore domain potassium channel activity.

Abbreviations:

ACSF	Artificial cerebrospinal fluid
CA1	*Cornu Ammonis* area 1
GABA	*gamma*-Aminobutyric acid
GIRK	G-protein coupled inwardly rectifying potassium channel
K2P	Two-pore domain potassium channel
PKA	Protein kinase A
SEM	Standard Error of the Mean
TASK	TWIK-related acid-sensitive potassium channel

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TREK	TWIK-related potassium channel
TWIK	Tandem P-domain weak inward rectifying potassium channel
TTX	Tetrodotoxin

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Introduction:

The metabotropic $GABA_B$ receptor is widely distributed throughout the brain (Bowery *et al.*, 1987; Chu *et al.*, 1990), and plays an important role in physiology and disease (Couve *et al.*, 2000; Bowery *et al.*, 2002; Bettler *et al.*, 2004). Functionally, $GABA_B$ receptors consist of heterodimers of $GABA_{B1}$ and $GABA_{B2}$ subunits coupled to the G-protein $G_{i/o}$ (Menon-Johansson *et al.*, 1993; White *et al.*, 1998; Kuner *et al.*, 1999; Obrietan & van den Pol, 1999), with the main final effectors regulated by $GABA_B$ receptors identified so far being voltage-gated calcium channels and G-protein coupled inwardly rectifying potassium (GIRK) channels (Couve *et al.*, 2000; Bowery *et al.*, 2002; Bettler *et al.*, 2004).

Previous studies indicate that presynaptic $GABA_B$ receptors down-regulate voltage-activated calcium channels, restricting neurotransmitter release from synaptic terminals (Scholz & Miller, 1991; Campbell *et al.*, 1993; Mintz & Bean, 1993). Postsynaptic $GABA_B$ receptors can also modulate voltage-activated calcium channels in dendrites and spines (Kavalali *et al.*, 1997; Sabatini & Svoboda, 2000; Chalifoux & Carter, 2011), where they regulate dendritic excitability and neuronal output both *in vitro* and *in vivo* (Perez-Garci *et al.*, 2006; Breton & Stuart, 2012; Palmer *et al.*, 2012). $GABA_B$ receptors also regulate neuronal excitability via the activation of potassium channels leading to a slow membrane hyperpolarization, first identified in hippocampal neurons (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Luscher *et al.*, 1997; Chen & Johnston, 2005). Importantly, this slow inhibition is absent in hippocampal pyramidal neurons in animals lacking the GIRK2 gene, indicating a role of GIRK channels in this cell type (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Luscher *et al.*, 1997; Chen & Johnston, 2005). Co-localization of $GABA_B$ receptors and GIRK channel mRNA is observed in many brain regions, including the cerebral cortex and hippocampus (Karschin *et al.*, 1996; Liao *et al.*, 1996; Ponce *et al.*, 1996; Saenz del Burgo *et al.*, 2008). Furthermore, $GABA_B$ receptors and GIRK channels are co-localized in molecular complexes in the cerebellum (Ciruela *et al.*, 2010) and in dendritic spines in the hippocampus (Kulik *et al.*, 2006).

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While these studies provide strong support for the idea that activation of GIRK channels underlies $GABA_B$ receptor-mediated slow inhibition, recent data in entorhinal cortex indicates that $GABA_B$ receptors also activate TREK-2 channels, a two-pore domain potassium channel of the KCNK channel family, via a G_i/protein kinase A (PKA)-dependent pathway (Deng *et al.*, 2009). Here we re-evaluate which G-protein coupled potassium channels underlie $GABA_B$-mediated responses in both neocortical and hippocampal pyramidal neurons. In addition, as some two-pore domain potassium channels are pH sensitive we also investigate the pH sensitivity of $GABA_B$-mediated responses.

Materials and Methods:

Slice preparation:

All procedures were performed in accordance to methods approved by the Animal Ethics Committee of the Australian National University. Wistar rats (4 to 8 week old of either sex) were obtained from the Australian Phenomics Facility at the Australian National University and were deeply anaesthetized by isoflurane inhalation (3% in oxygen), decapitated, and the brain quickly removed. A total of 111 animals were used in this study. Coronal brain slices (300 µm thick) containing barrel cortex and hippocampus were prepared. During the slicing procedure the brain was maintained in an ice-cold solution containing (in mM): 87 NaCl, 25 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 6 $MgCl_2$, 25 Glucose, 75 sucrose; pH=7.4; oxygenated with carbogen (95% O_2/5% CO_2). After slicing slices were immersed in artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 $NaHCO_3$, 3 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 25 glucose; pH=7.4; oxygenated with carbogen (95%O_2/5%CO_2) and maintained at 35°C for 30 minutes, then stored at room temperature. The same ACSF solution was used during electrophysiological recording.

Electrophysiology:

Brain slices were transferred to an immersed recording chamber continuously perfused with oxygenated ACSF (95% O_2/5% CO_2) at a constant temperature set at 34 ± 1°C. The layer 5 region of barrel cortex and the CA1 region of the hippocampus were visualized under low magnification using an upright microscope (5x magnification; BX50WI, Olympus, Tokyo, Japan). Somatic whole-cell patch-clamp recordings from neocortical layer 5 or hippocampal CA1 pyramidal neurons were performed under visual control at high magnification (x60) using differential interference contrast (DIC) optics combined with infrared illumination

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(Stuart *et al.*, 1993). Patch pipettes were made from borosilicate glass (Harvard Apparatus, Edenbridge, Kent, UK) and filled with a potassium gluconate-based solution consisting of (in mM): 130 potassium gluconate, 10 KCl, 10 HEPES, 4 MgATP, 0.3 Na_2GTP, 10 Na_2-phosphocreatine (pH=7.3 with KOH and osmolarity set to 280 mosmol/l with sucrose). Somatic whole-cell voltage-clamp recordings (open tip pipette resistance of 5 MΩ) were made at a holding potential of -72 mV using an Axopatch 200A amplifier (Molecular Devices, Sunnyvale, CA, USA). Only recordings with access resistances less than 15 MΩ were considered for analysis. Dendritic whole-cell current-clamp recordings (open tip pipette resistance of 10 MΩ) were made at the resting membrane potential using a BVC-700A current-clamp amplifier (Dagan, Mineapolis, MN, USA). Pipette bridge balance and capacitance were compensated on-line and checked routinely. Only dendritic recordings with access resistance less than 30 MΩ were considered for analysis.

All data were acquired on a Machintosh computer running Axograph X software (Axograph Scientific, Australia) using an ITC-18 A/D board (Instrutech, USA). Responses to baclofen were analogue filtered at 2 kHz and digitally sampled at 10 kHz, except during voltage ramps where data were analogue filtered at 10 kHz and digitally sampled at 50 kHz. Data were corrected for the experimentally determined liquid junction potential of 12 mV.

Solutions:

Unless otherwise stated $GABA_B$ receptors were activated by local applications of the $GABA_B$ agonist baclofen (50 µM in ACSF) applied close to the soma of neocortical layer 5 or hippocampal CA1 pyramidal neurons (within ~30 µm). Local applications of baclofen were made using 3-second pressure ejections from a patch pipette. Recordings were typically 90 minutes in duration with baclofen application every 3 minutes. Under these conditions we saw that baclofen responses changed less than 10% over the course of the experiment (n=4). The timing of pressure ejections was controlled by a picospritzer. A high concentration of baclofen (50 µM) was used during local applications to account for the lower effective concentration of baclofen under these conditions. Bath applications of baclofen (20 µM) were used to determine the impact of $GABA_B$ receptor activation on input resistance, and also for measurement of the reversal potential of $GABA_B$ responses during voltage ramps. Bath applications were made via the gravity feed line (flow rate 2 to 4 ml/minute).

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The specific GIRK channel antagonist tertiapin-Q (Tocris, UK; IC50=13.3 nM) (Jin & Lu, 1999), a derivative and more stable form of the toxin tertiapin found in bee venom, was dissolved in ACSF on the day of the experiment from a stock solution (x1000 final concentration in water kept at -20 °C) and bath applied at the indicated concentrations. The TASK channel blockers bupivacaine (Sigma-Aldrich, Germany) and quinidine (Tocris, UK) were also dissolved in ACSF on the day of the experiment from stock solutions (x1000 final concentration in water kept at -20 °C) and bath applied at the indicated concentrations. Low pH experiments were performed using a modified ACSF with 10 mM MES (2-(*N*-Morpholino)ethanesulfonic acid hydrate) with the pH set to 6.3 (34ºC) using 1M HCl. No significant change of the solution osmolarity was observed compared to normal ACSF solution.

Data analysis:

The reversal potential of $GABA_B$ responses was determined during voltage ramps from –132 mV to –72 mV (slope: +20 mV/s) by subtraction of the response in baclofen from that in control. It was defined as the membrane potential where the response passed through zero current, established by fitting a linear function to the baclofen-sensitive response in this region. Somatic input resistance in control and baclofen was determined from the slope of the control current-voltage relationship during ramps. Based on the external and internal potassium concentrations (including the amount of KOH added to the intracellular solution to adjust the pH) the predicted potassium equilibrium potential estimated from the Nernst equation was -103 mV. Dendritic input resistance was calculated from the fit to the linear region of the current-voltage relationship measured at steady state during subthreshold current injections at somatic and dendritic recording sites

In each cell baclofen applications were repeated three times for each experimental condition with a period of at least 3 minutes between applications to ensure full recovery. The data were average and the peak amplitude of the baclofen response determined. Recordings were not used if baclofen responses differed by more than 10% on different trials. Average data in the text and figures are presented as mean ± SEM (Standard Error of the Mean). Statistical significance was assessed using the paired t-test and one or two-way ANOVA with repeated measures followed by either a Bonferroni's or Tukey's *post hoc* multiple comparison test. Log transformations were performed on the data prior to statistical tests to normalise distributions and to equalize variance in control and drug conditions. In the figures a single

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asterisk (*) indicates $P < 0.05$, double asterisks (**) indicates $P < 0.01$, triple asterisks (***) indicates $P < 0.001$, quadruple asterisks (****) indicates $P<0.0001$ and "n.s." indicates not statistically significant ($P > 0.05$).

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Results:

$GABA_B$ responses in layer 5 and CA1 pyramidal neurons have different sensitivity to barium

$GABA_B$ receptors were activated by local applications of the $GABA_B$ receptor agonist baclofen applied to the soma of neocortical layer 5 and CA1 pyramidal neurons during somatic whole-cell voltage clamp. Local (3 second) applications of baclofen induced a transient outward current in both layer 5 and CA1 pyramidal neurons that lasted for tens of seconds (Fig 1A,B), consistent with previous studies (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Luscher *et al.*, 1997). All effects of baclofen were postsynaptic as they were also observed in the presence of TTX (1 µM; n=4).

To determine which G-protein coupled channels underlie baclofen-mediated responses in layer 5 and CA1 pyramidal neurons we first tested the impact of low concentrations of barium (200 µM), a non-specific blocker of GIRK potassium (Dascal *et al.*, 1993; Kubo *et al.*, 1993). This concentration of barium, which should completely block GIRK channels (Dascal *et al.*, 1993; Kubo *et al.*, 1993), only partially blocked baclofen responses in layer 5 pyramidal neurons (Fig. 1A,B; n=10; $P=7.2x10^{-4}$). In contrast, this concentration of barium essentially abolished baclofen-evoked outward currents in CA1 pyramidal neurons (Fig. 1C,D; n=5; $P= 6.0x10^{-8}$). The difference in barium sensitivity of baclofen responses in layer 5 and CA1 pyramidal neurons was highly significant ($P=4.8x10^{-4}$).

To further characterise the channels underlying baclofen responses in CA1 and layer 5 pyramidal neurons we next determined their reversal potential using voltage ramps during bath applications of baclofen (20 µM; see Materials & Methods). The average reversal potential of baclofen-mediated currents was found to be –99.6 ± 1.2 mV (n=6) in layer 5 pyramidal neurons and –101.4 ± 1.5 (n=6) in CA1 pyramidal neurons; close to the estimated

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potassium equilibrium potential of -103 mV in both cell types. These data suggest that while $GABA_B$-mediated responses in layer 5 and CA1 pyramidal neurons are both generated solely by potassium channels, the specific potassium channels coupled to $GABA_B$ receptors in these two cell types have different sensitivity to barium.

$GABA_B$ receptors activate GIRK channels in CA1 but not layer 5 pyramidal neurons

$GABA_B$ receptors are classically thought to activate GIRK channels. To investigate the coupling of $GABA_B$-receptors to GIRK channels we tested the sensitivity of $GABA_B$-mediated responses in layer 5 and CA1 pyramidal neurons to tertiapin-Q, a specific GIRK channel blocker (Jin & Lu, 1999). Tertiapin-Q at 100 nM and 500 nM (~10 and 50 times the IC_{50}) had no significant impact on $GABA_B$ responses evoked by local applications of baclofen (50 µM) to the soma of layer 5 pyramidal neurons (Fig. 2A,B; n=5; P=0.74 and P=0.31, respectively). In contrast, 100 nM tertiapin-Q significantly decreased baclofen-evoked responses in CA1 pyramidal neurons (Fig. 2C,D; n=8; $P=3.0\text{x}10^{-6}$). Increasing the tertiapin-Q concentration to 500 nM did not significantly increase the block of baclofen responses in CA1 pyramidal neurons (P=0.10), indicating that under our recording conditions tertiapin-Q at 100 nM is saturating in CA1 pyramidal neurons (Fig. 2C,D; n=8). The difference in tertiapin-Q sensitivity of baclofen responses in layer 5 and CA1 pyramidal neurons was highly significant ($P=6.0\text{x}10^{-4}$). Similar to the experiments using low concentrations of barium, these data show that under our experimental conditions somatic $GABA_B$-mediated responses in layer 5 and CA1 pyramidal neurons also show differential sensitivity to the GIRK channel blocker tertiapin-Q.

To investigate the possibility that somatic and dendritic $GABA_B$ receptors have different sensitivity to tertiapin-Q, we also tested the impact of tertiapin-Q on dendritic $GABA_B$ responses in layer 5 pyramidal neurons during bath applications of baclofen (20 µM). These experiments showed that dendritic input resistance (R_n) measured between 300 and 500 µm from the soma (n=5; average: 436 ± 13 µm) decreased significantly from 26.4 ± 1.5 MΩ in control to 19.8 ± 1.5 MΩ in the presence of baclofen (n=5; P<0.05), as previously described (Breton & Stuart, 2012). Concomitant application of tertiapin-Q (100 nM) with baclofen failed to block the impact of baclofen on dendritic input resistance (R_n baclofen + tertiapin-Q: 19.4 ± 1.9 MΩ; n=5, P>0.05). These data suggest that both somatic and dendritic $GABA_B$ receptors in layer 5 pyramidal neurons are insensitive to tertiapin-Q.

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$GABA_B$ receptors activate TASK-like potassium channels in both layer 5 and CA1 pyramidal neurons

Previous work indicates that $GABA_B$ receptors can activate two-pore domain potassium channels such as TASK and TREK (Fearon *et al.*, 2003; Deng *et al.*, 2009). To investigate the potential coupling of $GABA_B$ receptors to these channels in layer 5 and CA1 pyramidal neurons we focused our attention on TASK channels, as previously work indicates that the mRNAs coding for TASK-1 and TASK-3 channels are highly expressed in layer 5 of the neocortex, whereas the expression of TREK channel mRNAs is low (Talley *et al.*, 2001). To block TASK channels we bath applied bupivacaine (40 µM) and quinidine (50 µM), two relatively specific TASK channel blockers at these concentrations (Lotshaw, 2007). Bupivacaine partially blocked responses to local somatic applications of baclofen (50 µM) in both layer 5 and CA1 pyramidal neurons (Fig. 3A,B; Layer 5: P=0.04, n=5; CA1: P=4.5x10^{-5}, n=8). Similarly, local somatic applications of baclofen (50 µM) were also partially blocked by quinidine in both layer 5 and CA1 pyramidal neurons (Fig. 3C; Layer 5: P=3.0x10^{-3}, n=5; CA1: P=2.7x10^{-4}, n=8). The effects of bupivacaine and quinidine were similar in layer 5 and CA1 pyramidal neurons (P=0.05 and 0.37, respectively). These data suggesting that $GABA_B$ responses in both layer 5 and CA1 pyramidal neurons are in part be mediated by TASK channels.

We next verified that the TASK blockers quinidine and bupivacaine were acting specifically, and were not blocking GIRK channels. To test this we applied the GIRK channel blocker tertiapin-Q in the presence of quinidine or bupivacaine. Consistent with the idea that the TASK channel blockers were acting specifically, addition of tertiapin-Q (100 nM) in the presence of quinidine (50 µM) lead to a further reduction in baclofen-evoked responses in CA1 neurons (Fig 4A,B; $P < 0.01$, n=5). Similarly, while application of tertiapin-Q had no impact on baclofen responses in layer 5 pyramidal neurons, as shown in Figure 2, addition of quinidine (50 µM) in the presence of tertiapin-Q (100 nM) significantly reduced baclofen-evoked responses (Fig 4A,C; $P < 0.01$, n=5). Similar results were observed in layer 5 neurons when the TASK blocker bupivacaine (40 µM) was applied in the presence of tertiapin-Q (500 nM; Fig 4D; $P < 0.0001$, n=9). These results suggest that tertiapin-Q and the TASK channel blockers quinidine and bupivacaine are acting independently and blocking different populations of potassium channels (GIRK and TASK channels, respectively).

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Impact of external pH on the coupling of $GABA_B$ receptors to GIRK and TASK channels

TASK channels are sensitive to changes in pH, with a decrease in external pH leading to a reduction in TASK channel activity via a decrease in the channel opening probability (Reyes *et al.*, 1998; Kang & Kim, 2004). Given that our data suggest an important role of TASK channels in mediating $GABA_B$ responses in neocortical layer 5 pyramidal neurons we tested the impact of lowering external pH on these responses. In these experiments we recorded baclofen-mediated responses in layer 5 pyramidal neurons in physiological pH (control) and in a buffered acidic extracellular solution (pH=6.3; see Materials and Methods). In acidic conditions, when TASK channel activity is reduced, application of the TASK channel antagonist bupivacaine (40 µM) had no significant impact on baclofen evoked responses in layer 5 pyramidal neurons (Fig 5A; P=0.08, n=4). These data are consistent with the idea that TASK channels are involved in mediating $GABA_B$ receptor-mediated responses in these neurons under control conditions.

Surprisingly, lowering external pH revealed a GIRK component to $GABA_B$ responses in layer 5 pyramidal neurons. In acidic conditions application of the GIRK channel blocker tertiapin-Q (100 nM) significantly decreased baclofen responses in layer 5 pyramidal neurons (Fig 5; P=1.2x10^{-4}; n=5), consistent with earlier work showing that decreasing external pH enhances GIRK channel activity (Mao *et al.*, 2002; Mao *et al.*, 2003). Finally, in layer 5 pyramidal neurons we found that the amplitude of $GABA_B$ when external pH was lower to 6.3 was similar to control (n=10, P > 0.34), indicating that during a reduction in external pH up-regulation of presumably GIRK channels compensates for the reduction in TASK channel activity. Together, these data further support the idea that TASK channels contribute to $GABA_B$ responses in layer 5 pyramidal neurons, and show that coupling of GIRK channels to $GABA_B$ receptors in these cells is pH dependent, and only functionally relevant under conditions of low external pH.

Discussion:

In this study, we show that $GABA_B$ receptors are coupled to different potassium channels in neocortical layer 5 and hippocampal CA1 pyramidal neurons. We find that while $GABA_B$ receptors are coupled to TASK channels in both populations of pyramidal neurons, coupling of $GABA_B$ receptors to GIRK channels is functionally absent in layer 5 pyramidal neurons

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under physiological conditions. In contrast to these observations made under control conditions, lowering external pH blocks TASK channels in layer 5 pyramidal neurons and reveals a GIRK channel component to $GABA_B$ responses. In summary, we find that the coupling of $GABA_B$ receptors to potassium channels in pyramidal neurons from the cortex and hippocampus is cell-specific and regulated by external pH.

Ionic mechanisms underlying $GABA_B$ responses

Our results show that the $GABA_B$ agonist baclofen evokes an outward current in both neocortical layer 5 and CA1 pyramidal neurons with a reversal potential close to the equilibrium potential for potassium ions. Furthermore, this outward current was reduced by bath application of barium (Fig 1), a non-specific potassium channel blocker. These observations indicate that in both neocortical layer 5 and CA1 pyramidal neurons $GABA_B$ receptors activate a barium-sensitive potassium conductance, consistent with previous studies (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Luscher *et al.*, 1997; Chen & Johnston, 2005).

Surprisingly, however, we found that the sensitivity of $GABA_B$ responses to barium was cell specific (Fig 1), suggesting that the downstream targets of $GABA_B$ receptors in neocortical layer 5 and CA1 pyramidal neurons are different. Consistent with this idea $GABA_B$ responses in these two cells types showed differential sensitivity to the GIRK channel antagonist tertiapin-Q (Fig 2), which has not effect in layer 5 pyramidal neurons. In contrast, $GABA_B$ responses in both cell types were partially blocked by the TASK channel antagonists quinidine and bupivacaine (Fig 3 & 4). These data suggest that $GABA_B$ receptors are coupled to two-pore domain potassium (K2P) channels in both cell types, but that at physiological external pH $GABA_B$ receptors are only coupled to GIRK channels in CA1 pyramidal neurons. This idea is supported by the different sensitivity of $GABA_B$ responses in layer 5 and CA1 pyramidal neurons to external barium (Fig 1), as in comparison to GIRK channels TASK channels are less sensitivity to block by external barium (Coetzee *et al.*, 1999).

Our observation that $GABA_B$ receptors in neocortical and hippocampal pyramidal neurons are coupled to TASK channels is consistent with previous work indicating that $GABA_B$ receptors are coupled to TREK-2 channels (also of the K2P family) in the entorhinal cortex (Deng *et al.*, 2009). Furthermore, TASK channels are coupled to $GABA_B$ receptors in the carotid body (Fearon *et al.*, 2003), and TREK-1 channels have been shown to contribute to

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$GABA_B$ responses in cultured hippocampal CA1 pyramidal neurons (Sandoz *et al.*, 2012). This latter observation suggests that the response remaining in the presence of GIRK and TASK channel blockers in both neocortical layer 5 and hippocampal CA1 pyramidal neurons may be mediated by TREK-1 channels. While we cannot rule out that the concentration of the blockers used was too low to block all TASK and GIRK channels, it seems likely that $GABA_B$ receptors activate additional potassium conductances, such as TREK channels, particularly in layer 5 pyramidal neurons.

The $GABA_B$-mediated outward current in hippocampal CA1 pyramidal neurons was partially blocked by tertiapin-Q, a specific GIRK channel blocker (Jin & Lu, 1999), consistent with the idea that this response is mediated by GIRK channels (Gahwiler & Brown, 1985; Newberry & Nicoll, 1985; Luscher *et al.*, 1997; Chen & Johnston, 2005). However, tertiapin-Q, even at high (saturating) concentrations (500 nM), did not fully block $GABA_B$ responses in CA1 neurons, and had no impact on $GABA_B$ responses in layer 5 neurons under control conditions (Fig 2). Furthermore, the TASK channel blockers quinidine and bupivacaine were effective in blocking $GABA_B$ responses in both cell types in the presence of tertiapin-Q (Fig 4), suggesting that both GIRK and TASK channel antagonists were acting specifically. These results are surprising given that knockout of the GIRK2 gene largely abolishes $GABA_B$ responses in CA1 neurons (Luscher *et al.*, 1997). One explanation for this apparent discrepancy is that knocking down the GIRK2 gene disrupts TASK and TREK channel coupling to $GABA_B$ receptors in CA1 neurons, perhaps due to tight co-localisation of these channels with GIRK channels.

Surprisingly, tertiapin-Q did not affect $GABA_B$-mediated responses in neocortical layer 5 pyramidal neurons (Fig 2). These data suggest that GIRK channel coupling to $GABA_B$ receptors is not functional in neocortical layer 5 pyramidal neurons under physiological conditions, despite the dense expression of mRNA coding for different GIRK channel subunits throughout the neocortex, as well as GIRK channel protein expression in layer 5 on putative pyramidal neuron dendrites (Karschin *et al.*, 1996; Liao *et al.*, 1996; Ponce *et al.*, 1996; Saenz del Burgo *et al.*, 2008). Functional coupling of GIRK channels to $GABA_B$ receptors was observed, however, under conditions of low external pH, as can occur during hypoxia.

$GABA_B$ receptor signalling and K2P channels

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The K2P channels blockers bupivacaine and quinidine, used at low concentration, are relatively specific to TASK channels (Lotshaw, 2007), and were found to partially block baclofen-evoked responses in both neocortical layer 5 and hippocampal CA1 pyramidal neurons (Fig 3). This observation, and the barium sensitivity of the $GABA_B$ receptor-mediated outward current (Fig 1), are consistent with the TASK current described by Taverna *et al*. (2005) in CA1 pyramidal neurons. Furthermore, we found that $GABA_B$ responses in neocortical layer 5 pyramidal neurons were no longer sensitive to bupivacaine when external pH was decreased to 6.3 (Fig 5). This observation is also similar to that of Taverna *et al.* (2005), who showed that the impact of bupivacaine on TASK channels is occluded in low external pH conditions. Together, these observations support the idea that TASK channels are involved in $GABA_B$-mediated signalling in both neocortical and hippocampal pyramidal neurons.

Among the K2P channels members, both TASK and TREK channels are pH sensitive. However, while TREK channels are sensitive to changes in internal pH and their activity very low at physiological external pH, the external acid-sensing TASK channels are modulated by external pH and are open at physiological external pH (Reyes *et al.*, 1998; Maingret *et al.*, 1999; Honore *et al.*, 2002; Kang & Kim, 2004). Hence, it is perhaps not surprising to the find that TASK channels are involved in mediating $GABA_B$ responses. Furthermore, TASK channels are widely express in the cortex as well as in the hippocampus (Talley *et al.*, 2001), and mRNA expression has been observed in single CA1 pyramidal neurons and interneurons (Taverna *et al.*, 2005; Torborg *et al.*, 2006).

In conclusion, this study provides evidence that TASK channels are involved in $GABA_B$ receptors-mediated signalling at the soma of both neocortical and hippocampal pyramidal neurons. Furthermore, in the cortex, the classical coupling between GIRK channels and $GABA_B$ receptor is functional only under conditions of low external pH, as might occur during hypoxia. While the reasons for these cell-specific differences are unclear, the opposite external pH-dependence of TASK and GIRK-mediated responses in neocortical layer 5 pyramidal neurons provides a mechanism to maintain $GABA_B$ responses during changes in external pH (Fig 5D), which may provide an important protective mechanism during hypoxia-induced acidosis. Overall, the results show that the activation of downstream potassium channels by $GABA_B$ receptors is far more complex than previously thought, and points to an

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important role of $GABA_B$ receptors in regulating neuronal excitability under different physiological/pathological conditions.

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Author contribution:

JDB and GJS designed study. JDB acquired the data and performed all data analysis. JDB and GJS wrote the paper.

Data statement:

Primary data is stored at the Australian National University and can be obtained on request.

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Figures legends:

Fig 1: $GABA_B$ responses in layer 5 and CA1 pyramidal neurons have different sensitive to barium.

(A,C) Baclofen-evoked responses (50 µM, red) recorded at the soma of layer 5 (**A**) and CA1 pyramidal neurons (**C**) in control (black) and barium (200 µM; green). (**B,D)** Average amplitude of baclofen responses in control (open symbols; ± SEM) and after addition of barium (200 µM; green) in layer 5 (**B**; 10 cells from 10 animals) and CA1 (**D**; 5 cells from 5 animals) pyramidal neurons. Grey symbols and dashed lines show data from individual neurons.

Fig 2: GIRK channels are not activated by $GABA_B$ receptors in layer 5 pyramidal neurons.

(A,C) Baclofen-evoked responses (50 µM, red) in layer 5 (**A**) and CA1 pyramidal neurons (**C**) in control (black) and tertiapin-Q (100 nM, light blue; 500 nM, dark blue). (**B,D**) Average amplitude of baclofen responses in control (open symbols; ± SEM) and after addition of tertiapin-Q (100 nM, light blue; 500 nM, dark blue) in layer 5 (**B**; 5 cells from 5 animals) and CA1 pyramidal neurons (**D**; 8 cells from 8 animals). Grey circles and dashed lines show data from individual neurons**.**

Fig 3: $GABA_B$ receptors are coupled to TASK channels in both layer 5 and CA1 pyramidal neurons.

(A) Baclofen-evoked responses (50 µM, red) in layer 5 (left) and CA1 pyramidal neurons (right) in control (black) and bupivacaine (40 µM; orange). (**B**) Average amplitude of baclofen responses in control (open symbols; ± SEM) and after addition of bupivacine (**B**; 40 µM; orange) in layer 5 (left; 5 cells from 5 animals) and CA1 pyramidal neurons (right; 8 cells from 8 animals). Grey circles and dashed lines show data from individual neurons. (**D**) Average amplitude of baclofen responses in control (open symbols; ± SEM) and after addition of quinidine (50 µM; pink) in layer 5 (left; 5 cells from 5 animals) and CA1

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pyramidal neurons (right; 6 cells from 6 animals). Grey circles and dashed lines show data from individual neurons.

Fig 4: TASK and GIRK antagonists block different populations of channels.

(A) Baclofen-evoked responses (50 µM, red) in CA1 pyramidal (top) and layer 5 pyramidal neurons (bottom) in control (black), quinidine (50 µM; pink) or tertiapin-Q (100 nM; blue) alone, and quinidine plus tertiapin-Q (mauve). (**B**) Average amplitude of baclofen responses in control (open symbols; ± SEM), quinidine alone (50 µM, pink) and quinidine plus tertiapin-Q (100 nM; mauve) in CA1 pyramidal neurons (5 cells from 5 animals). Grey circles and dashed lines show data from different neurons. (**C)** Average amplitude of baclofen responses in control (open symbols; ± SEM), tertiapin-Q alone (100 nM, light blue) and tertiapin-Q plus quinidine (50 µM; mauve) in layer 5 pyramidal neurons (5 cells from 5 animals). Grey circles and dashed lines show data from different neurons. (**D**) Average amplitude of baclofen responses in control (open symbols; ± SEM), tertiapin-Q alone (500 nM, dark blue) and tertiapin-Q plus bupivacaine (40 µM; olive) in layer 5 pyramidal neurons (9 cells from 9 animals). Grey circles and dashed lines show data from different neurons.

Fig 5: Lowing external pH reveals a GIRK channel component to $GABA_B$ responses in layer 5 pyramidal neurons.

(A) Left, Baclofen-evoked responses (50 µM, red) in layer 5 pyramidal neurons at low external pH (pH 6.3; black) and bupivacaine (40 µM, orange). Right, Average amplitude of baclofen responses in control (open symbols; ± SEM) and in the presence of bupivacaine (orange) in layer 5 pyramidal neurons at low external pH (pH 6.3; 4 cells from 4 animals). Grey circles and dashed lines show data from different neurons. (**B**) Left, Baclofen-evoked responses (50 µM, duration: 3s) in layer 5 pyramidal neurons at low external pH (pH 6.3; black) and tertiapin-Q (100 nM, blue). Right, Average amplitude of baclofen responses in control (open symbols; ± SEM) and tertiapin-Q (blue) in layer 5 pyramidal neurons at low external pH (pH 6.3; 5 cells from 5 animals). Grey circles and dashed lines show data from different neurons.

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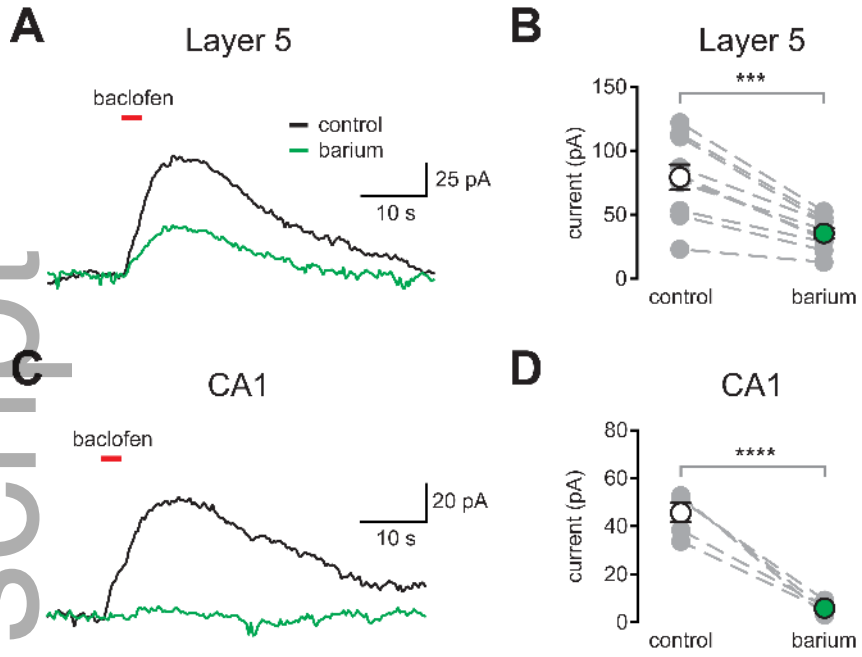

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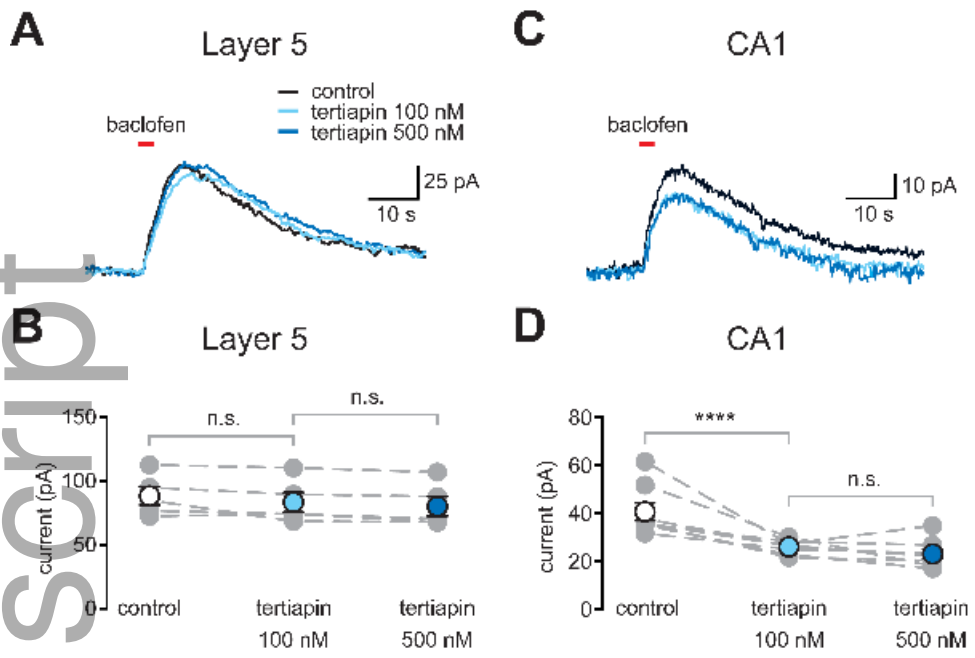

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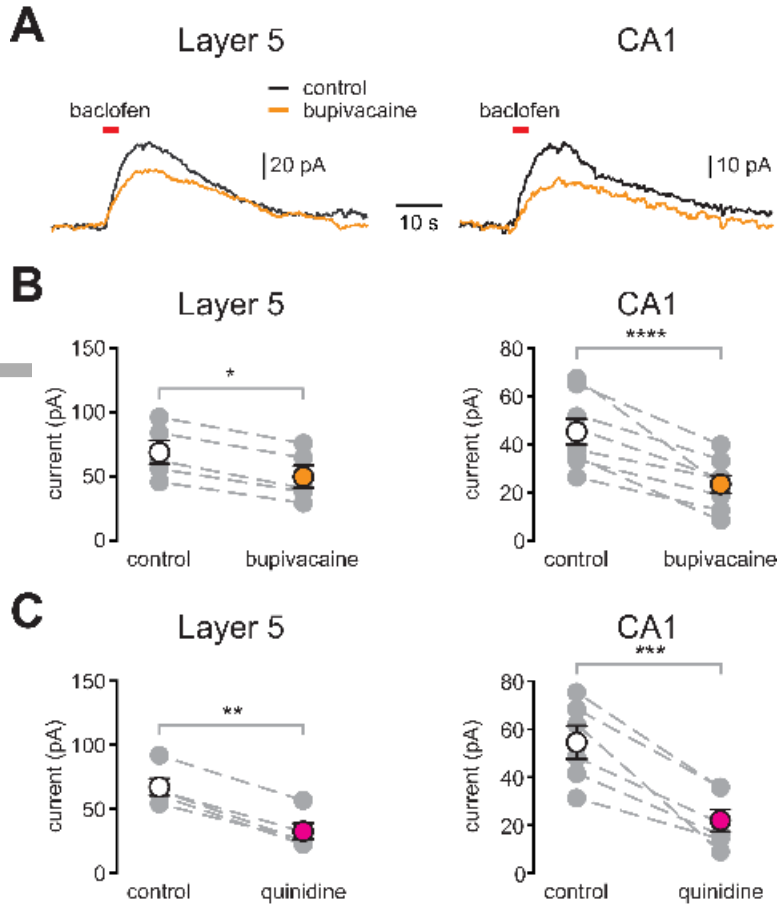

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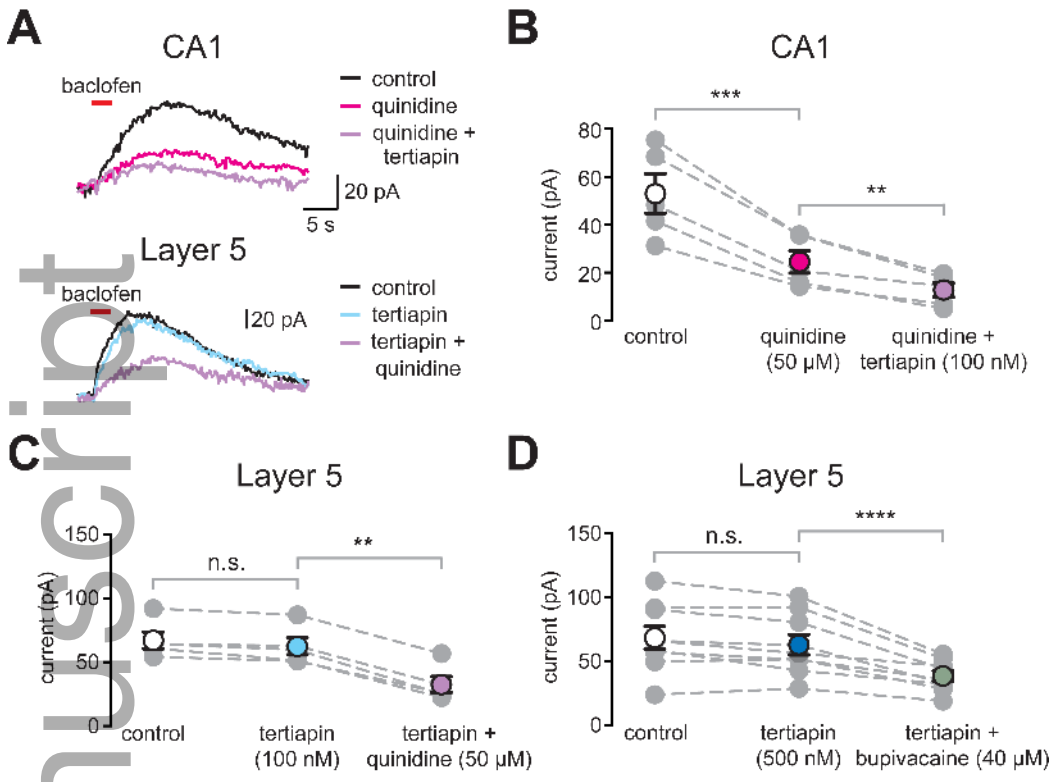

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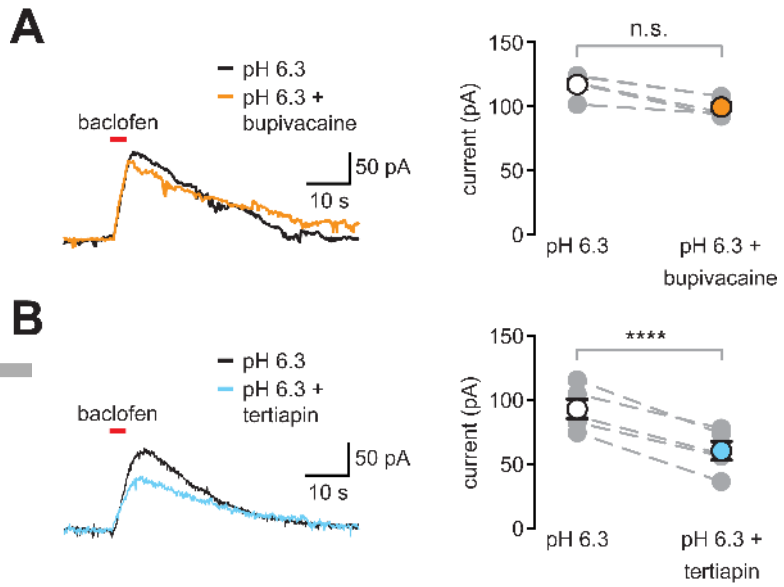

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