Somatic and dendritic GABA$_B$ receptors regulate neuronal excitability via different mechanisms

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Abstract:
GABA_B receptors play a key role in regulating neuronal excitability in the brain. While the impact of somatic GABA_B receptors on neuronal excitability has been studied in some detail, much less is known about the role of dendritic GABA_B receptors. Here we investigate the impact of GABA_B receptor activation on the somato-dendritic excitability of layer 5 pyramidal neurons in rat barrel cortex. Activation of GABA_B receptors led to hyperpolarization and a decrease in membrane resistance, that was restricted largely to somatic or proximal dendritic locations. These effects were blocked by low concentrations of barium (100 µM), consistent with the idea that they are mediated by potassium channels. In contrast, activation of dendritic GABA_B receptors decreased the width of backpropagating action potential (APs) and abolished dendritic calcium electrogensis, indicating that dendritic GABA_B receptors regulate excitability primarily via inhibition of voltage-dependent calcium channels. These distinct actions of somatic and dendritic GABA_B receptors regulated neuronal output in different ways. Activation of somatic GABA_B receptors led to a reduction in neuronal output primarily by increasing AP rheobase, whereas dendritic GABA_B receptors blocking burst firing, decreasing the number of elicited APs in the absence of a significant change in somatic membrane properties. Taken together, our results show that GABA_B receptors regulate somatic and dendritic excitability of cortical pyramidal neurons via different cellular mechanisms, with somatic GABA_B receptors leading to a subtractive or shunting form of inhibition, whereas dendritic GABA_B receptors reduce neuronal output via an inhibition of bursting firing.

Key words: GABA_B receptor, excitability, GIRK channels, calcium channels, output gain.
Introduction:

GABA is the primary inhibitory neurotransmitter in the brain. The release of GABA leads to fast postsynaptic inhibition mediated by the activation of GABA_A receptors (Allen et al. 1977), whereas activation of GABA_B receptors coupled to the G-protein \( G_{i/o} \) provides a mechanism for slow inhibition (Lüscher et al. 1997; Tamas et al. 2003). This slow inhibition is thought to be mediated via activation of G-protein coupled inwardly rectifying potassium channels (GIRK channels) belonging to the Kir3 potassium channel family (Chen and Johnston 2005; Gähwiler and Brown 1985; Lüscher et al. 1997; Newberry and Nicoll 1985). In addition, it has recently been shown that postsynaptic GABA_B receptors can activate TREK-2 channels, a two pore-domain potassium channel (Deng et al. 2009). By increasing membrane permeability to potassium, GABA_B receptors play a crucial role in regulating neuronal excitability via hyperpolarizing the resting membrane potential and reducing the input resistance (Gähwiler and Brown 1985; Lüscher et al. 1997). In addition, GABA_B receptors can act presynaptically to inhibit voltage-dependent calcium channels and thereby modulate transmitter release. While this action of GABA_B receptors was initially thought to only be important in presynaptic terminals (Campbell et al. 1993; Mintz and Bean 1993; Scholz and Miller 1991), there is increasing evidence that GABA_B receptors can also act to modulate voltage-dependent calcium channels in dendrites and spines (Chalifoux and Carter 2011; Kavalali et al. 1997; Sabatini and Svoboda 2000), where they can influence dendritic excitability (Perez-Garci et al. 2006) as well as modulate NMDA receptor activation (Chalifoux and Carter 2010).

The role these different forms of GABA_B-mediated inhibition play in regulating neuronal output is less clear. Different classes of GABAergic neurons are known to
target different cellular compartments of cortical pyramidal neurons (Chu et al. 2003; Gonchar and Burkhalter 1999; 2003; Houser et al. 1983; Tamas et al. 2003; Zhu et al. 2004). As such, it seems possible that somatic and dendritic GABA_B receptors may regulate the excitability of cortical neurons in different ways. Further evidence for this idea comes from the observation that the function of the two varieties of the GABA_B1 subunit (GABA_B1a and GABA_B1b) are segregated, with the GABA_B1a subunit mediating inhibition of voltage-dependent calcium channels, whereas the GABA_B1b subunit being at least partly responsible for postsynaptic hyperpolarization (Perez-Garci et al. 2006).

Here we address the mechanisms by which somatic and dendritic GABA_B receptors regulate the excitability of layer 5 pyramidal neurons in rat barrel cortex. We find that somatic excitability is regulated primarily via GABA_B receptor activation of potassium channels, whereas dendritic excitability is regulated primarily via inhibition of voltage-dependent calcium channels, indicating that somatic and dendritic GABA_B receptors regulate neuronal excitability via different mechanisms.
Materials and methods:

Slice preparation:

All procedures are performed in accordance to methods approved by the Animal Ethics Committee of the Australian National University. Wistar rats (4 to 9 week old of either sex) were deeply anaesthetized by isoflurane inhalation (3% in oxygen) and decapitated. The brain was quickly removed and coronal slices containing barrel cortex prepared (300 µm thick). During this procedure, the brain was maintained in an ice-cold solution containing (in mM): 87 NaCl, 25 NaHCO$_3$, 3 KCl, 1.25 NaH$_2$PO$_4$, 0.5 CaCl$_2$, 6 MgCl$_2$, 25 Glucose, 75 Sucrose; pH=7.4; oxygenated with carbogen (95%O$_2$/5%CO$_2$). After cutting, slices were immersed in artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 NaHCO$_3$, 3 KCl, 1.25 NaH$_2$PO$_4$, 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 for electrophysiological recording.

Electrophysiological procedures:

Barrel cortex slices were transferred to an immersed recording chamber continuously perfused with oxygenated ACSF (95%O$_2$/5%CO$_2$). Barrel cortex layer 5 was visualized under low magnification using an upright microscope (5x magnification; BX50WI, Olympus, Tokyo, Japan). Pyramidal neurons were observed at higher magnification (x60) using differential interference contrast (DIC) optics combined with infrared illumination (Stuart et al. 1993) allowing somatic and dendritic recordings under visual control. Somatic and dendritic patch pipettes were made from borosilicate glass (Harvard Appparatus, Edenbridge, Kent, UK) and pulled to obtain a
resistance of 5 and 10 MΩ, respectively. Whole-cell current-clamp recordings were obtained using glass pipettes filled with a potassium gluconate-based solution consisting of the following (in mM): 130 potassium gluconate, 10 KCl, 10 Hepes, 4 MgATP, 0.3 Na₂GTP, 10 Na₂-phosphocreatine; pH= 7.3 with KOH and osmolarity set to 280 mosmol.l⁻¹ with sucrose. Recordings were obtained at a temperature set at 34 ± 1°C.

Two identical BVC-700A current-clamp amplifiers (Dagan, Minneapolis, MN, USA) were used to record somatic and dendritic signals in the whole-cell configuration. These signals were digitized with an ITC-18 computer interface at 50 KHz (Instrutech, Port Washington, NY, USA), analogue filtered on-line at 10 KHz and acquired using the data acquisition software Axograph X (Axograph Scientific, Australia). The pipette bridge balance and capacitance were compensated on-line and checked throughout the experiment. Recordings where the somatic or dendritic access resistance exceeded 20 MΩ and 30 MΩ, respectively, have been discarded. Furthermore, only neurons with a stable resting membrane potential more negative than -65 mV were used. All membrane potentials have been corrected for the experimentally determined liquid junction potential of ~12 mV.

**Data analysis:**
The distance of dendritic recordings from the soma was estimated *in situ* using the linear distance between the somatic and the dendritic pipettes. The amplitude and the width of somatic and dendritic action potentials (APs) were measured from threshold, defined as a dV/dt of 50 and 25 mV/s, respectively. AP duration was measured at 50% of peak amplitude. The amplitude of the medium after-hyperpolarization (AHP)
was defined as the voltage difference between AP threshold and the membrane potential 40 ms after AP onset. The rheobase was defined as the amplitude of the minimum somatic current required to elicit APs. The propagation velocity of backpropagated APs was determined from the time difference between the peak of somatic and dendritic APs. Somatic and dendritic input resistances were 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, respectively. The critical frequency for dendritic calcium electrogenesis was determined during trains of five APs evoked via somatic current injection at frequencies ranging from 20 to 200 Hz (Larkum et al. 1999a). The integral of the dendritic response was plotted against the somatic AP frequency, and the critical frequency defined as previously described by Breton and Stuart (2009). The output gain of recorded neurons was analysed by fitting a linear function to the input-output relationship between the first four data points above the rheobase. Numerical values given in the text and figures represent mean ± S.E.M, and the level of statistical significance was set to $P < 0.05$. Statistical tests used in this study are indicated in the Results section.
Results:

Recordings were obtained from rats aged 4 to 9 weeks of either sex (n= 80 neurons). As no significant difference was observed between male and female rats the data have been combined.

Impact of GABA_B receptor activation at the soma

We first investigated the impact of GABA_B receptor activation on the somatic resting membrane properties of layer 5 pyramidal neurons during bath application of baclofen (20 µM; a GABA_B receptor agonist). Bath application of baclofen decreased the steady-state voltage response to hyperpolarizing somatic current injections compared to control (Fig. 1A, bottom). This led to a change in slope of the current-voltage relationship (Fig. 1B), indicating a reduction in input resistance (Rn) (Fig. 1D left; n= 42; P < 0.01, Tukey’s post hoc test). In parallel, GABA_B receptor activation induced a significant hyperpolarization of the resting membrane potential (V_rest) (Fig. 1D, right; n= 42; P < 0.01, Tukey’s post hoc test). These effects of baclofen on somatic membrane properties were blocked by co-application of barium (Fig. 1D; 100 µM), a wide spectrum blocker of potassium channels including GIRK channels, and by CGP52432 (1 µM), a potent GABA_B receptor antagonist (n= 8; Fig. 1C). As the impact of baclofen was sensitive to low concentrations of barium these data suggest that GABA_B receptors reduce somatic excitability via activation of a potassium conductance.

Figure 1 near here
We also investigated the impact of GABA<sub>B</sub> receptor activation on active membrane properties at the soma. Supra-threshold current injections evoked regular and intrinsic bursting firing patterns similar to those observed in other studies (Breton and Stuart 2009; Williams and Stuart 1999). Bath application of baclofen (20 µM) reduced the ability of layer 5 pyramidal neurons to generate action potentials (APs), shifting the input-output relationship (f/I relationship) to the right (Fig. 1A, E; table 1). This led to a significant decrease in the number of APs generated by supra-threshold current injections (1 nA; control: 31.0 ± 0.9 APs; baclofen: 17.1 ± 1.3 APs, n= 42; P < 0.001; Tukey’s post hoc test). Furthermore, bath application of baclofen converted intrinsic bursting neurons to regular firing neurons (n= 29 bursting neurons; Fig. 1A, top). Bath application of baclofen had a small but significant impact on somatic AP properties, leading to a slight reduction in AP amplitude, rate of rise and half-width without changing AP threshold (see table 1). These effects of baclofen were reversible after washing for at least 30 minutes (n= 23, data not shown) and were blocked by CGP52432 (1 µM; n= 8; data not shown). Co-application of baclofen (20 µM) with barium (100 µM) antagonized these effects of baclofen on the input-output properties of neurons (Fig. 1A, E; n= 42), suggesting it is mediated by activation of a potassium conductance. Furthermore, co-application of barium profoundly changed the firing properties of neurons, converting regular AP firing observed in baclofen to strong AP bursts (Fig. 1A).

| Impact of dendritic GABA<sub>B</sub> receptor activation |
To investigate the impact of GABA\textsubscript{B} receptors at somatic and dendritic sites, baclofen (50 \mu M) was focally applied (3 sec application) via a pipette placed in the vicinity of the somatic or dendritic recording pipette (within ~30 \mu m) during simultaneous somatic and dendritic recording. As seen during bath application, somatic application of baclofen evoked a hyperpolarization of the somatic membrane potential, which attenuated as it propagated to the dendritic recording site. Similarly, dendritic application of baclofen evoked a hyperpolarization of the dendritic membrane potential, which attenuated as it propagated to the somatic recording site (Fig. 2A). On average, however, the somatic response to somatic baclofen application was significantly larger than the dendritic response to dendritic baclofen application (Fig. 2B; dendritic baclofen applications 435 ± 23 \mu m from the soma; n= 12; \( P < 0.01 \), unpaired Student’s \( t \) test).

To further study the effect of dendritic GABA\textsubscript{B} receptor activation on dendritic membrane excitability we examined the impact of bath application of baclofen (20 \mu M) on voltage responses to hyperpolarizing and depolarizing sub-threshold current injections at different apical dendritic locations. As seen at the soma, baclofen application lead to a decrease in the voltage response to hyperpolarizing dendritic current injections and a reduction in the slope of the I/V relationship, which was blocked by co-application of barium (Fig. 2C, D). This reduction in dendritic input resistance occurred in the absence of a significant change in dendritic membrane potential (control \( V_{\text{rest}}= -73.46 \pm 1.00 \) mV; baclofen \( V_{\text{rest}}= -74.25 \pm 0.73 \) mV; distance from soma: 395 ± 16 \mu m; n= 12; \( P > 0.05 \), paired Student’s \( t \) test). Furthermore, the
impact of bath application of baclofen on dendritic input resistance was distance
dependent and only observed at proximal dendritic locations (Fig. 2E, F; two-way
ANOVA: effect of distance from soma: $P= 0.0004$ and effect of treatment:
$P < 0.0001$; 100-300 µm: n= 9, 300-500 µm: n= 12; 500-800 µm: n= 9). Together,
these results show that in layer 5 pyramidal neurons of the somatosensory cortex
coupling of dendritic GABA$_B$ receptors to barium-sensitive potassium channels is
restricted to proximal dendritic locations.

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Impact of GABA$_B$ receptor activation on steady-state voltage attenuation

We next determined the impact of GABA$_B$ receptor activation on steady-state voltage
attenuation during hyperpolarizing current injections (-450 pA, duration: 900 ms) at
the soma or apical dendrite (Fig. 3A). Voltage attenuation was calculated as the ratio
of the steady-state voltage response at the “receiving” location divided by that
recorded at the site of current injection. Voltage attenuation from the soma to the
dendritic recording site, and vice versa, was greater the larger the distance between
the current injecting and receiving pipette due to the filtering properties of the apical
dendrite (Fig. 3B, C). Importantly, however, we failed to observe an impact of bath
application of baclofen (20 µM) on voltage attenuation irrespective of the direction of
voltage spread (Fig. 3D; one-way ANOVA; n= 54; $P= 0.4146$ and $P= 0.3494$ for
somatic and dendritic current injection, respectively). These data indicate that
activation of dendritic GABA$_B$ receptors has minimal impact on steady-state voltage
attenuation in layer 5 pyramidal neurons.

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Figure 3 near here
Impact of GABA\textsubscript{B} receptors activation on action potential backpropagation

To understand the role of GABA\textsubscript{B} receptors in regulating active dendritic properties we first assessed their impact on backpropagating APs (bAPs) during dual somatic and dendritic whole-cell recordings (Fig. 4A). The amplitude of bAPs decreased as they invaded the apical dendrite and in some cases failed to propagate distally (Fig. 4B, see Breton and Stuart 2009; Larkum et al. 2001; Stuart and Hausser 2001). Bath application of baclofen (20 µM) did not significantly affect the amplitude of bAPs at distal dendritic locations (Fig. 4B; control= 38.75 ± 3.44 mV, baclofen= 37.78 ± 3.56 mV; distance from soma 520 ± 18 µm; n= 31; \( P > 0.05 \), paired Student’s \( t \) test). Furthermore, no significant impact of baclofen was observed on bAP velocity (Fig. 4C; control= 0.41 ± 0.02 mV, baclofen= 0.43 ± 0.03 mV; n= 31; \( P > 0.05 \), paired Student’s \( t \) test) or rate of rise (Fig. 4D; control= 78.9 ± 8.8 mV.s\(^{-1}\), baclofen= 79.8 ± 9.8 mV.s\(^{-1}\); n= 31; \( P > 0.05 \), paired Student’s \( t \) test). Bath application of baclofen (20 µM) did, however, induce a distance-dependent decrease in bAPs duration (Fig. 4E, F). At distal dendritic location (520 ± 18 µm from the soma), baclofen (20 µM) significantly reduced bAP duration from 2.34 ± 0.14 ms (in control condition) to 1.67 ± 0.08 ms (n= 31; \( P < 0.001 \), paired Student’s \( t \) test). This effect was absent when baclofen was co-applied with the GABA\textsubscript{B} antagonist CGP52432 (1 µM, n= 8; Fig. 4G) or low concentrations of nickel (100 µM), to block T- and R-type voltage-gated calcium channels (Fig. 4F, right; n= 8; \( P < 0.05 \), Tukey’s post hoc test). These data suggest that GABA\textsubscript{B} receptor activation causes a decrease in the duration of bAPs at distal locations by inhibiting dendritic T- and/or R-type voltage-gated calcium channels.

Figure 4 near here
GABA<sub>B</sub> receptors inhibit dendritic calcium electrogensis

Given that baclofen reduced bAP duration by blocking dendritic calcium channels, we next investigated the impact of baclofen on dendritic calcium electrogenesis elicited by AP trains. Previous work indicates that trains of bAPs lead to generation of dendritic calcium electrogenesis in a frequency dependent manner as observed previously (Larkum et al. 1999a; Williams and Stuart 2000). The frequency of bAPs required to evoke dendritic calcium electrogenesis is called the “critical frequency” and can be used to determine the degree of dendritic excitability (Larkum et al. 1999a). To examine the impact of GABA<sub>B</sub> receptor activation on dendritic excitability we therefore tested the effect of bath application of baclofen on the critical frequency. The dendritic response to trains of five somatic APs evoked at frequencies of 20 to 200 Hz (increment of 10 Hz) was recorded during dual dendritic and somatic whole-cell recording (Fig. 5A). In control, the observed critical frequency was 96.7 ± 3.1 Hz (n= 46), consistent with previous studies (Breton and Stuart 2009; Larkum et al. 1999a). Bath application of baclofen (20 µM) abolished dendritic calcium electrogenesis (Fig. 5B), leading to a significant decrease in the integral of dendritic responses measured at supra-critical frequencies (Fig. 5C, left; distance from soma: 518 ± 18 µm; n= 30, P < 0.001, paired Student’s t test). Furthermore, this effect of baclofen was blocked by CGP52432 (1 µM; n= 8; data not shown) and by low concentrations (100 µM) of nickel (Fig. 5C, right; n= 8).

Figure 5 near here
Because bath application of baclofen activates both somatic and dendritic GABA$_B$ receptors, we tested the effect of local dendritic application of baclofen on the critical frequency. In these experiments a train of five somatic APs was generated at a supra-critical frequency of 200 Hz and baclofen (50 µM) was applied locally in the vicinity of the dendritic recording pipette (with ~30 µm). Local application of baclofen to the dendritic recording location lead to a transient decrease in dendritic calcium electrogensis, consistent with the idea that it is due to activation of dendritic GABA$_B$ receptors (Fig. 5D; control integral= 1.31 ± 0.07 mV.s; baclofen integral= 1.03 ± 0.08 mV.s; n= 9; $P < 0.001$, paired Student’s $t$ test). Together, these data show that activation of dendritic GABA$_B$ receptors decrease dendritic excitability by reducing dendritic calcium electrogensis in layer 5 pyramidal neurons, presumably following down-regulation of dendritic T- and/or R-type voltage-gated calcium channels.

Impact of somatic and dendritic GABA$_B$ receptors on neuronal output

We next investigated how activation of GABA$_B$ receptors at different locations regulates neuronal output. In these experiments we recorded AP output in response to somatic current injections during local application of baclofen (50 µM) to the distal apical dendrite or to the soma. Local application of baclofen to distal dendritic locations (distance from soma 568 ± 12 µm; n= 7) failed to influence somatic resting membrane potential or input resistance (Fig. 6A; n= 7, $P > 0.05$, paired Student’s $t$ test). These observations are in good agreement with the absence of a significant effect of bath application of baclofen on distal dendritic membrane properties (> 500 µm from the soma, see Fig. 2). Conversely, local application of baclofen to the soma lead to hyperpolarization of the somatic resting membrane potential and a
decrease in input resistance, similar to that seen during bath application of baclofen (Fig 6A; n= 7, V<sub>rest</sub>: P < 0.01, Rn: P < 0.001, paired Student’s t test).

**Figure 6 near here**

Despite having no detectable impact on somatic resting membrane properties, activation of dendritic GABA<sub>B</sub> receptors reduced AP output during somatic current injections (Fig. 6B, G; distance from soma 568 ± 12 µm; n= 7). This was associated with a reduction in both AP burst firing (Fig. 6B, C; n= 7; P < 0.001, paired Student’s t test) and the number of APs elicited for a given current injection (Fig. 6G; current: +1000 pA; n=7; P < 0.05, paired Student’s t test), but no change in the slope of the input-output (f/I) relationship (Fig. 6D, F) and the rheobase (Fig. 6E) was observed. In contrast, activation of somatic GABA<sub>B</sub> receptors lead to an increase in both rheobase (Fig. 6E; n= 7; P < 0.001, paired Student’s t test) and the slope of the f/I relationship (Fig. 6F; n= 7; P < 0.01, paired Student’s t test), but no significant change on burst firing (Figure 6B,C). Somatic GABA<sub>B</sub> receptor activation was also associated with a significant decrease in the amplitude of the medium after-hyperpolarization (AHP) following AP generation (Fig. 6H; n= 7; P < 0.01, paired Student’s t test). These results indicate that somatic GABA<sub>B</sub> receptor activation leads primarily to a reduction in AP output through an increase in the rheobase, whereas dendritic GABA<sub>B</sub> receptor activation reduces burst firing.
Discussion:

In this study we provide evidence that GABA$_B$ receptors regulate the somatic and dendritic excitability of layer 5 pyramidal neurons in the barrel cortex via different mechanisms. At the soma activation of GABA$_B$ receptors leads to a decrease in AP firing by hyperpolarizing the somatic resting membrane potential and decreasing the somatic membrane resistance via activation of putative GIRK channels. In contrast, at dendritic locations GABA$_B$ receptors reduce dendritic excitability primarily by down regulating dendritic calcium channels, leading to an increase in the threshold for generation of dendritic calcium electrogenesis and a decrease in burst firing. The consequence on neuronal output is a subtractive form of inhibition following activation of somatic and proximal dendritic GABA$_B$ receptors, whereas activation of distal dendritic GABA$_B$ receptors leads primarily to a divisive form of inhibition.

Cellular mechanisms underlying GABA$_B$-mediated slow inhibition

Our results show that both bath and local somatic application of baclofen lead to hyperpolarization and a decrease in resistance at the soma of layer 5 pyramidal neurons. This effect is similar to that observed previously at the soma of neurons in other cortical areas and brain regions (Benardo 1994; Deng et al. 2009; Gähwiler and Brown 1985; Lüscher et al. 1997; Newberry and Nicoll 1985). Furthermore, we show that bath application of baclofen decreases neuronal excitability, as observed by a rightward shift of the input-output relationship (Fig. 1E). Similar results have been reported in neurons from the entorhinal cortex (Deng et al. 2009). These effects of GABA$_B$ receptors are likely to be due to activation of a potassium conductance. Previous studies indicate that GABA$_B$ receptors can activate G-protein coupled
inwardly rectifying potassium or GIRK (Kir3) channels (Chen and Johnston 2005; Lüscher et al. 1997; Takigawa and Alzheimer 1999). Consistent with this idea, GABA$_B$-mediated slow inhibition is absent from hippocampal pyramidal neurons in the GIRK2 knockout mouse (Lüscher et al. 1997), although Deng et al. (2009) have recently described that GABA$_B$ receptors in entorhinal cortex can regulate neuronal excitability through activation of a TREK-2 (a two-pore domain) potassium channel.

To investigate the contribution of GIRK channels to the effects observed in our study we co-applied baclofen with low concentrations of barium (100 µM), a non-selective blocker of GIRK channels (Chen and Johnston 2005; Coetzee et al. 1999; Takigawa and Alzheimer 1999). Application of baclofen with barium antagonized the effects of baclofen at the soma (Fig. 1), consistent with the idea that these effects are mediated by GIRK channels. That said, barium is quite non-specific even at low concentrations, hence we cannot rule out a role of TREK-2 or other potassium channels in mediating these effects of baclofen.

Dendritic GABA$_B$-mediated effects on input resistance at proximal dendritic locations were also barium-sensitive (Fig. 2), consistent with the idea that they are also mediated by GIRK channels. Activation of dendritic GIRK channels by GABA$_B$ receptors has previously been described in both cortical layer 5 and hippocampal pyramidal neurons (Chen and Johnston 2005; Takigawa and Alzheimer 1999). In our experiments the impact of baclofen on dendritic input resistance was restricted to proximal dendritic locations. This observation suggests that the baclofen-sensitive GIRK channel responses in the earlier work by Takigawa and Alzheimer (1999) are likely to be from proximal dendritic segments of cortical pyramidal neurons. In contrast, observations in hippocampal pyramidal neurons suggest that GABA$_B$
receptors can activate GIRK channels also at distal dendritic locations (Chen and Johnston, 2005), where they may play a role in synaptic plasticity (Chen and Johnston 2005; Chung et al. 2009). Consistent with this observation, an interplay between GABA<sub>B</sub>-activated GIRK channels and HCN channels has been observed in hippocampal pyramidal neurons (Takigawa and Alzheimer 2003), which are known to express HCN channels at high density in the distal apical dendrite (Magee 1999).

Despite the capacity of baclofen to activate GIRK channels at proximal dendritic locations and at the soma, steady-state voltage attenuation was unaffected by bath application of baclofen irrespective of the direction of voltage spread (Fig. 3). While one might expect that steady-state voltage attenuation is relatively insensitive to changes in membrane resistance when voltage spreads away from the site of current injection, this should not be the case when voltage spreads towards the location of a decrease in membrane resistance. Yet no change in steady-state voltage attenuation was observed in our experiments. We predict this was the case as the change in voltage attenuation during baclofen applications was too small to be detected given the sensitivity of our experiments (unpublished simulations). Consistent with this idea, the density of GIRK channels at the soma and at proximal dendrite locations of CA1 hippocampal pyramidal neurons is low (Chen and Johnston 2005).

**GABA<sub>B</sub>-mediated modulation of dendritic excitability and calcium channels**

Action potentials attenuate and broaden as they propagate along the apical dendrite of cortical layer 5 pyramidal neurons (Stuart et al. 1997). Broadening of bAPs is in part due to activation of voltage-gated calcium channels, which also play a key role in generation of dendritic calcium electrogensis that can feedback to the soma.
triggering AP burst firing (Breton and Stuart 2009; Larkum et al. 1999a; Williams and Stuart 1999). Our data show that bath application of baclofen reduces the half-width of bAPs and abolishes dendritic calcium electrogenesis evoked by high frequency AP bursts (Fig. 4 & 5). This effect on dendritic excitability was mediated by GABA\textsubscript{B} receptors located at distal dendritic sites as it was observed during local dendritic applications of baclofen (Fig. 5D). These data suggest that dendritic GABA\textsubscript{B} receptors reduced dendritic excitability primarily through down regulation of dendritic voltage-gated calcium channels. Consistent with this idea, the impact of baclofen on bAP half-width and calcium electrogenesis during high-frequency AP firing was blocked in the presence of low concentrations of nickel (100 µM), which blocks T- and R-type voltage-gated calcium channels (Fig. 4F, 5C).

Previous work indicates that activation of GABAergic input to somatosensory cortex can selectively block the initiation of dendritic calcium spikes (Larkum et al. 1999b). This effect involves, at least in part, the activation of dendritic GABA\textsubscript{B} receptors, through down regulation of dendritic calcium channels in layer 5 pyramidal neurons (Perez-Garci et al. 2006). Consistent with this earlier study, we also find that the impact of GABA\textsubscript{B} receptors on dendritic excitability in cortical pyramidal neurons is primarily via this mechanism. In addition, we find that the functional impact of GABA\textsubscript{B}-mediated inhibition of dendritic calcium channels and associated dendritic calcium electrogenesis is a reduction in burst firing at the soma (Fig. 1A, 6C). Given that AP burst firing is required for the induction of spike-timing-dependent plasticity (STDP) in layer 5 pyramidal neurons (Kampa et al. 2006; Letzkus et al. 2006), GABA\textsubscript{B}-receptor activation is likely to have a significant impact on STDP and other forms of NMDA receptor-dependent synaptic plasticity where the magnitude of the
dendritic depolarization associated with bAPs is key to removal of the voltage-dependent magnesium block of NMDA receptors (Nowak et al. 1984). This action of GABA_B receptors on synaptic plasticity will be further enhanced by the impact of GABA_B-receptor activation on bAP duration (Fig. 4E, F).

Impact of dendritic and somatic GABA_B receptors on neuronal output

With respect to the impact of GABA_B receptors on neuronal output we show that distal dendritic baclofen applications decrease bursting firing, decreasing the number of APs elicited for suprathreshold current injections, in the absence of an impact on somatic membrane properties. This observation is consistent with the recent observations of Palmer et al. (2012), who also showed that GABA_B receptor-mediated down-regulation of dendritic calcium channels leads to a reduction in AP firing in the absence of a significant change in somatic properties.

In contrast, somatic baclofen applications increase AP rheobase, shifting the f/I relationship to the right without influencing AP bursting firing. This leads to a subtractive or shunting form of inhibition (Silver 2010). Interesting, activation of somatic GABA_B receptors also leads to an increase the slope of the f/I relationship. This effect may be due to the impact of GABA_B receptor activation on the medium AHP (Fig 6H). Consistent with this idea, previous work indicates a role of the AHP in regulation of output gain (Higgs et al. 2006).

In summary, we show that GABA_B receptors in cortical layer 5 pyramidal neurons act to decrease somatic and dendritic excitability via different mechanisms. Somatic GABA_B receptors are coupled to barium-sensitive, putative GIRK potassium
channels, whereas dendritic GABA_B receptors act primarily by down regulating dendritic calcium electrogenesis. As a result, activation of somatic GABA_B receptors leads to a shift to the right of the f/I relationship and an increase in neuronal output gain, whereas activation of dendritic GABA_B receptors causes a switch from burst to tonic firing, and a reduction in neuronal output. This location-dependent specificity of GABA_B receptor activation on neuronal excitability would be expected to further enhance the diversity with which different GABAergic interneuronal cell types orchestrate network activity in the cortex.
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Author contributions:

JDB and GJS conceived and designed the project. JDB performed experiments and analysed the data. Both authors discussed the results and wrote the manuscript. Both authors approved the final version of the manuscript.
References:


Figure captions

Figure 1: GABA<sub>B</sub>-mediated inhibition decreases somatic excitability.

A, Responses of an intrinsically bursting layer 5 pyramidal neuron to subthreshold (bottom) and suprathreshold (top, lower traces shown responses on an expanded time scale) somatic current injection (top, middle traces; bottom, lower traces) in control (left), during bath application of baclofen (20 µM, middle), and following application of baclofen (20 µM) plus barium (100 µM, right). B, Impact of baclofen on the subthreshold I/V relationship (n= 42). Baclofen reduces the slope of the I/V curve compare to control, which is antagonized by barium. C, The GABA<sub>B</sub>-mediated change in the slope of the I/V curve is occluded by a prior application of the GABA<sub>B</sub> antagonist CGP52432 (1µM). D, Effect of baclofen or baclofen plus barium on somatic input resistance (left) and resting membrane potential (right). E, Impact of baclofen on the suprathreshold input-output relationship (f/I curve). Baclofen causes a rightward shift in the f/I curve (n= 42), which is antagonized by barium. **: \( P < 0.01 \); ***: \( P < 0.001 \).

Figure 2: The impact of GABA<sub>B</sub> receptors on resting membrane properties is restricted to somatic and proximal dendritic locations.

A, Impact of local somatic (left) or dendritic (right) application of baclofen (50 µM, grey bar indicates application duration) on the somatic and dendritic membrane potential during dual somatic and dendritic recording (dendritic recording 410 µm from the soma). B, Average membrane potential hyperpolarization at the site of baclofen application. Dendritic recordings 435 ± 23 µm from the soma (n= 12). C, Dendritic voltage responses to dendritic current injection (bottom) in control and
following bath application of baclofen (20 µM). Bath application of baclofen (20 µM) decreased the amplitude of responses (C) and the slope of the I/V curve (D). These effects were antagonized by barium (100 µM). E, Distance-dependence of the decrease in dendritic input resistance during bath application of baclofen. Black and grey lines represent the linear fits to the data. F, Impact of bath application of balcofen (20 µM) on the dendritic input resistance at different dendritic locations. Balcofen significantly reduces the input resistance only at distances < 500 µm from the soma. **: P < 0.01; ***: P < 0.001; n.s.: non significant.

Figure 3: GABA_B receptor activation fails to influence steady-state attenuation.

A, Somatic and dendritic (570 µm from the soma) responses recorded simultaneously during somatic current injection (-450 pA, 900 ms) in control (black) and bath application of baclofen (20 µM, grey). B, C, Steady-state attenuation measured at different locations along the apical dendrite during somatic (B) and dendritic (C) current injection. Steady-state attenuation calculated as the ratio of the steady-state voltage response recorded at the "receiving" pipette divided by the response recorded at the site of current injection. D, Average steady-state attenuation in baclofen divided by that in control at different dendritic locations. No statistically significant impact of baclofen on steady-state voltage attenuation was observed irrespective of the site of dendritic recording or direction of steady-state voltage propagation.

Figure 4: Impact of GABA_B receptors on backpropagating APs.

A, Example of somatic and backpropagating dendritic APs (bAPs; recorded 620 µm from the soma) in control (black) and following bath application of baclofen (grey; 20 µM). Note the slight reduction in bAP width in baclofen. B-E, Graphs of the
distance dependence of bAP amplitude (B), velocity (C), rate-of-rise (D) and half-width (E) in control (black) and during bath application of baclofen (grey, 20 µM). F, Left: The duration of bAPs is significantly decreased during bath application of baclofen (20 µM) at distal dendritic recording sites (dendritic recordings 520 ± 18 µm from the soma, n= 31). Right: In the presence of nickel (100 µM) baclofen no longer has an impact on bAP duration (dendritic recording 522 ± 30 µm from the soma, n= 8). G, The impact of baclofen (20 µM) on bAP width is blocked by the GABA_B antagonist CGP52432 (1 µM). *: P < 0.05; ***: P < 0.001; n.s.: non significant.

**Figure 5: GABA_B receptors decrease dendritic calcium electrogenesis.**

A, Dendritic responses (620 µm from the soma) to trains of five APs evoked by somatic current injection (bottom) at the indicated frequencies in control (top) and following bath application of balcofen (20 µM, middle). B, Graph of dendritic voltage integral versus AP frequency for the data illustrated in A. Note the non-linear increase in dendritic integral in control (black), indicative of dendritic calcium electrogenesis, is abolished by baclofen (grey). C, The average dendritic voltage integral obtained at supra-critical frequencies (200 Hz) is significantly decreased in baclofen (left). This effect is occluded by a prior bath application of nickel (right; 100 µM). D, Dendritic responses (420 µm from the soma) during trains of somatic APs (5 spikes at 200Hz) at the times indicated in the graph (bottom) during local application of baclofen to the dendritic recording site (grey bar). **: P < 0.01; ***: P < 0.001; n.s.: non significant.

**Figure 6: Impact of somatic and dendritic GABA_B receptors on somatic membrane properties and neuronal output.**
A, Impact of somatic and dendritic baclofen application (50 µM) on somatic input resistance (left) and resting membrane potential (right). Note that dendritic application of baclofen does not affect somatic resting membrane properties (distance from soma 568 ± 12 µm; n= 7). B, Examples of APs evoked by somatic current injection in a bursting (left) and a regular firing neuron (right). Dendritic application of baclofen (top traces) abolished AP burst firing in the bursting neuron (distance from soma 560 µm) and reduced AP output in the regular firing neuron (distance from soma 600 µm). Somatic application of baclofen (bottom traces) reduced AP output in both bursting and regular firing neurons, but did not block burst firing. The asterisks above the traces indicate APs bursts. C, Left, Examples of the impact of dendritic (top; distance from soma 540) and somatic (bottom) baclofen application on bursting firing neuron. Right, Average number of APs per burst in different experimental conditions (dendritic baclofen application: 568 ± 12 µm from the soma; n= 7). D, Input-output (f/I) curves from a typical neuron during somatic (left) and dendritic (right; distance from soma 630 µm) application of baclofen. E-G, Graphs of the average rheobase, slope of the f/I relationship and number of APs generated during a 1 nA current injection in different experimental conditions (dendritic baclofen application: 568 ± 12 µm from the soma; n= 7). H, Impact of somatic application of baclofen on the medium AHP following an AP burst. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; n.s.: non significant.
Table 1: Effects of GABA<sub>B</sub> receptor activation on somatic AP properties.

<table>
<thead>
<tr>
<th></th>
<th>Threshold (mV)</th>
<th>Amplitude (mV)</th>
<th>dV/dt (V.s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Half-width (ms)</th>
<th>Rheobase (pA)</th>
<th>n</th>
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<tbody>
<tr>
<td>Control</td>
<td>-59.4 ± 0.4</td>
<td>96.3 ± 0.5</td>
<td>634 ± 9</td>
<td>0.56 ± 0.01</td>
<td>275 ± 13</td>
<td>73</td>
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<tr>
<td>Baclofen</td>
<td>-59.5 ± 0.4</td>
<td>95.5 ± 0.5</td>
<td>619 ± 8</td>
<td>0.53 ± 0.01</td>
<td>522 ± 20</td>
<td>73</td>
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<tr>
<td>Probability</td>
<td>n.s.</td>
<td>**</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td></td>
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Data are shown as mean ± S.E.M. n.s: non significant, *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; paired Student's t test.
A. Diagram showing dendrites with amplitude and time annotations.

B. Graph showing amplitude (mV) vs. current (pA) with control and Baclofen conditions.

C. Graph showing potential (mV) vs. current (pA) with control, Baclofen, and Baclofen + Barium conditions.

D. Graph showing input resistance (MΩ) vs. distance from soma (μm) with control and Baclofen conditions.

E. Graph showing input resistance (MΩ) vs. distance from soma (μm) with control and Baclofen conditions.

F. Graph showing input resistance (MΩ) with control and Baclofen conditions for different distance ranges.
A

B

C

D

Control
Baclofen

Soma Dendrite

Ratio

Distance from soma (μm)

Ratio

Distance from dendrite (μm)

Baclofen/Control

Ratio

Distance from soma (μm)

Baclofen/Control

Ratio

Distance from dendrite (μm)