Somatic and dendritic GABA<sub>B</sub> receptors regulate neuronal excitability via different mechanisms

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BRETON J-D, STUART GJ. Somatic and dendritic GABA<sub>B</sub> receptors regulate neuronal excitability via different mechanisms. J Neurophysiol 108: 2810–2818, 2012. First published September 5, 2012; doi:10.1152/jn.00524.2012.—GABA<sub>B</sub> receptors play a crucial role in regulating neuronal excitability in the brain. Whereas the impact of somatic GABA<sub>B</sub> receptors on neuronal excitability has been studied in some detail, much less is known about the role of dendritic GABA<sub>B</sub> receptors. Here, we investigate the impact of GABA<sub>B</sub> receptor activation on the somato-dendritic excitability of layer 5 pyramidal neurons in the rat barrel cortex. Activation of GABA<sub>B</sub> receptors led to hyperpolarization and a decrease in membrane resistance that was greatest at somatic and proximal dendritic locations. These effects were occluded by low concentrations of barium (100 μM), suggesting that they are mediated by potassium channels. In contrast, activation of dendritic GABA<sub>B</sub> receptors decreased the width of backpropagating action potential (APs) and abolished dendritic calcium electrogenesis, indicating that dendritic GABA<sub>B</sub> receptors regulate excitability, primarily via inhibition of voltage-dependent calcium channels. These distinct actions of somatic and dendritic GABA<sub>B</sub> receptors regulated neuronal output in different ways. Activation of somatic GABA<sub>B</sub> receptors led to a reduction in neuronal output, primarily by increasing the AP rheobase, whereas activation of dendritic GABA<sub>B</sub> receptors blocked burst firing, decreasing AP output in the absence of a significant change in somatic membrane properties. Taken together, our results show that GABA<sub>B</sub> receptors regulate somatic and dendritic excitability of cortical pyramidal neurons via different cellular mechanisms. Somatic GABA<sub>B</sub> receptors activate potassium channels, leading primarily to a subtractive or shunting form of inhibition, whereas dendritic GABA<sub>B</sub> receptors inhibit dendritic calcium electrogenesis, leading to a reduction in bursting firing.

GABA<sub>B</sub> receptor; excitability; GIRK channels; calcium channels; output gain

GABA is the primary inhibitory neurotransmitter in the brain. The release of GABA leads to fast postsynaptic inhibition mediated by the activation of GABA<sub>A</sub> receptors (Allen et al. 1977), whereas activation of GABA<sub>B</sub> receptors coupled to the G-protein G<sub>iso</sub> 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 (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 been shown recently that postsynaptic GABA<sub>B</sub> receptors can activate TREK-2 channels, a two pore-domain potassium channel (Deng et al. 2009). By increasing membrane permeability to potassium, GABA<sub>B</sub> 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<sub>B</sub> receptors can act presynaptically to inhibit voltage-dependent calcium channels and thereby modulate transmitter release. Whereas this action of GABA<sub>B</sub> receptors was initially thought only to be important in presynaptic terminals (Campbell et al. 1993; Mintz and Bean 1993; Scholz and Miller 1991), there is increasing evidence that GABA<sub>B</sub> 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-Garcia et al. 2006) as well as modulate N-methyl-D-aspartate (NMDA) receptor activation (Chalifoux and Carter 2010).

The role these different forms of GABA<sub>B</sub>-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). It therefore seems likely that somatic and dendritic GABA<sub>B</sub> 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<sub>B1</sub> subunit (GABA<sub>B1a</sub> and GABA<sub>B1b</sub>) is segregated, with the GABA<sub>B1a</sub> subunit mediating inhibition of voltage-dependent calcium channels, whereas the GABA<sub>B1b</sub> subunit is at least partly responsible for postsynaptic hyperpolarization (Perez-Garcia et al. 2006).

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

MATERIALS AND METHODS

Slice preparation. All procedures are performed in accordance with methods approved by the Animal Ethics Committee of the Australian National University. Wistar rats (4–9 wk old of either sex) were deeply anesthetized by isoflurane inhalation (3% in oxygen) and decapitated. The brain was removed quickly and coronal slices containing the 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<sub>3</sub>, 3 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 0.5 CaCl<sub>2</sub>, 6
MgCl₂, 25 glucose, 75 sucrose, pH 7.4, oxygenated with carbogen (95% O₂/5% CO₂). After cutting, slices were immersed in artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 NaHCO₃, 3 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, 1 MgCl₂, 25 glucose, pH 7.4, oxygenated with carbogen (95% O₂/5% CO₂), maintained at 35°C for 30 min, and 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% O₂/5% CO₂). Barrel cortex layer 5 was visualized under low magnification using an upright microscope (5× magnification; BX50WI, Olympus, Tokyo, Japan). Pyramidal neurons were observed at higher magnification (×60) using differential interference contrast 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 Apparatus, 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 with sucrose. Recordings were obtained at a temperature of 34 ± 1°C.

Two identical BVC-700A current-clamp amplifiers (Dagan, Minneapolis, MN) 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, HEKA Instruments, Bellmore, NY), analog filtered online at 10 kHz, and acquired using the data acquisition software AxoGraph X (AxoGraph Scientific, Bellmore, NY), and checked throughout the experiment. Recordings were obtained with a stable resting membrane potential more negative than −65 mV, respectively, have been discarded. Furthermore, only neurons where the somatic or dendritic access resistance exceeded 20 MΩ set to 280 mosmol/l with sucrose. Recordings were obtained at a temperature of 34 ± 1°C.

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. 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. 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 V/s, respectively. AP duration (half width) was determined at 50% of AP amplitude, measured between AP threshold and peak. The amplitude of the medium after-hyperpolarization (mAHF) was defined as the voltage difference between AP threshold and the membrane potential 40 ms after AP onset. Rhobase was defined as the amplitude of the minimum somatic current pulse that evoked APs. The propagation velocity of backpropagating APs (bAPs) was determined from the time difference between the peak of somatic and dendritic APs. The critical frequency for dendritic calcium electrogenesis was determined during trains of five APs evoked via somatic current injections 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 described previously by Breton and Stuart (2009). The output gain was determined by fitting a linear function to the integral of the input-output relationship (the over four current steps above rhobase; 100-pA increments). We defined an AP burst (see Figs. 5 and 6) as two or more APs at a frequency >200 Hz. Numerical values given in the text and figures represent mean ± SE, and the level of statistical significance was set.
RESULTS

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

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

**Impact of dendritic GABA<sub>B</sub> receptor activation.** To investigate the impact of GABA<sub>B</sub> receptor activation at somatic and dendritic sites, baclofen (50 μM) was focally applied (3-s applications) via a pipette placed in the vicinity of the somatic or dendritic recording pipette (within ~30 μm) 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 local somatic baclofen application was significantly larger than the dendritic response to local dendritic baclofen application (Fig. 2B; dendritic baclofen applications 435 ± 23 μm from the soma; n = 12; P < 0.01, unpaired Student’s t-test). This difference may indicate a lower density of GABA<sub>B</sub> receptors at dendritic locations or less-effective coupling of dendritic GABA<sub>B</sub> receptors to potassium channels. Alternatively, it may reflect differences in the surface area exposed to baclofen during local applications, with more GABA<sub>B</sub> receptors activated at the soma compared with dendritic sites.

To distinguish between these possibilities, we examined the impact of bath applications of baclofen (20 μM) on voltage responses to hyperpolarizing and depolarizing subthreshold current injections at different apical dendritic locations. As seen at the soma, baclofen application led to a decrease in the dendritic voltage response to hyperpolarizing dendritic current injections and a reduction in the slope of the dendritic current-voltage relationship, which was occluded by co-application of low concentrations of barium (Fig. 2, C and D). This reduction...
in dendritic input resistance occurred in the absence of a significant change in dendritic membrane potential (control = $-73.46 \pm 1.00$ mV; baclofen = $-74.25 \pm 0.73$ mV; distance from soma: $395 \pm 16 \mu m$; $n = 12$; $P > 0.05$, paired Student’s $t$-test). Furthermore, the impact of bath application of baclofen on dendritic input resistance was distance dependent and greatest at proximal dendritic locations (Fig. 2, $E$ and $F$; two-way ANOVA, $P < 0.0001$). 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 have their greatest impact on resting membrane properties at somatic and proximal dendritic locations.

**Impact of GABA$_{B}$ receptor activation on bAPs.** To understand the role of GABA$_{B}$ receptors in regulating active dendritic properties, we first assessed their impact on bAPs during dual somatic and dendritic whole-cell recordings (Fig. 3A). The amplitude of bAPs decreased as they propagated into the apical dendrite and in some cases, failed to propagate distally (Fig. 3B) (Breton and Stuart 2009; Larkum et al. 2001; Stuart and Hausser 2001). Bath application of baclofen (20 $\mu M$) did not significantly affect the amplitude of bAPs at distal dendritic locations (Fig. 3B; control = $38.75 \pm 3.44$ mV; baclofen = $37.78 \pm 3.56$ mV; distance from soma: $520 \pm 18 \mu m$; $n = 31$; $P > 0.05$, paired Student’s $t$-test). Furthermore, no significant impact of baclofen was observed on the bAP rate of rise (Fig. 3C; control = $78.9 \pm 8.8$ V/s; baclofen = $79.8 \pm 9.8$ V/s; $n = 31$; distance from soma: $520 \pm 18 \mu m$; $n = 31$; $P > 0.05$, paired Student’s $t$-test). Bath application of baclofen (20 $\mu M$), however, caused a distance-dependent decrease in bAP duration (Fig. 3, $E$ and $F$). At distal dendritic locations ($520 \pm 18 \mu m$ from the soma), baclofen (20 $\mu M$) significantly reduced bAP duration from $2.34 \pm 0.14$ ms (in control) to $1.67 \pm 0.08$ ms ($n = 31$; $P < 0.001$, paired Student’s $t$-test). This effect was independent of whether bAPs propagated efficiently into distal dendrites and absent when baclofen was co-applied with the GABA$_{B}$ antagonist CGPS2432 (1 $\mu M$; $n = 8$; data not shown) or low concentrations of nickel (100 $\mu M$) to block T- and R-type voltage-gated calcium channels (Fig. 3, $G$ and $H$; $n = 8$; $P < 0.05$, Tukey’s post hoc test). Bath applications of nickel (100 $\mu M$) did not have an impact on somatic and dendritic resting membrane properties ($n = 8$; data not shown), indicating that nickel did not influence potassium leak channels. Nickel also did not block the impact of baclofen on somatic or dendritic input resistance. These data suggest that GABA$_{B}$ receptor activation causes a decrease in the duration of bAPs at distal locations by inhibiting dendritic voltage-gated calcium channels.

**GABA$_{B}$ receptors inhibit dendritic calcium electrogenesis.** Given that the data above suggest 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 (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$_{B}$ receptor activation on dendritic excitability, we 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 20–200 Hz (increment of 10 Hz), was recorded during dual dendritic and somatic whole-cell recordings (Fig. 4A). In control, the observed critical frequency was $96.7 \pm 3.1$ Hz ($n = 46$), consistent with previous studies (Breton and Stuart 2009; Larkum et al. 1999a). Bath application of baclofen (20 $\mu M$) abolished dendritic calcium electrogenesis (Fig. 4B), leading to a significant decrease in the integral of dendritic responses measured at supracritical frequencies (Fig. 4C; distance from soma: $518 \pm 18 \mu m$; $n = 30$; $P < 0.001$, paired Student’s $t$-test). Furthermore, this effect of baclofen was blocked by CGPS2432 (1 $\mu M$; $n = 8$; data not shown) and by low concentrations (100 $\mu M$) of nickel (Fig. 4, $D$–$F$; distance from soma: $522 \pm 30 \mu m$; $n = 8$).

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 supracritical frequency of 200 Hz, and baclofen (50 μM) was applied locally in the vicinity of the dendritic recording pipette (within ~30 μm). Local application of baclofen to the dendritic recording location led to a transient decrease in dendritic calcium electrogenesis, consistent with the idea that this decrease is due to activation of dendritic rather than somatic GABA<sub>B</sub> receptors (Fig. 4G; 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<sub>B</sub> receptors decreases dendritic excitability by reducing dendritic calcium electrogenesis in layer 5 pyramidal neurons, presumably following downregulation of dendritic T- and/or R-type voltage-gated calcium channels.

Impact of somatic and dendritic GABA<sub>B</sub> receptors on neuronal output. We next investigated the impact of GABA<sub>B</sub> receptor activation on the input-output properties of layer 5 pyramidal neurons. Suprathreshold somatic current injections evoked regular and intrinsic-bursting firing patterns similar to those observed in earlier 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 APs, shifting the input-output relationship (f/I curve) to the right (Fig. 5, A and B). This led to a significant decrease in the number of APs generated by suprathreshold current injections (control: 31.0 ± 0.9 APs; baclofen: 17.1 ± 1.3 APs; n = 42; 1 nA current pulse of 900 ms duration; P < 0.001, paired Student’s t-test). Furthermore, bath application of baclofen abolished burst firing and converted all intrinsic-bursting neurons to regular firing (Fig. 5C; n = 29). 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 (Table 1). These effects of baclofen were reversible after washing for at least 30 min (Fig. 5A; n = 23) and were blocked by CGP52432 (1 μM; n = 8; data not shown).

To investigate how activation of GABA<sub>B</sub> receptors at different locations regulates neuronal output, 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 the soma led 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; P < 0.01; paired Student’s t-test). In contrast, local application of baclofen to distal dendritic locations (distance from soma: 568 ± 12 μm;
Bursting; increase in both rheobase (Fig. 6; 0.05, paired Student’s t-test), during bath application of baclofen (20 μM) in control (left), and following washout of baclofen (right). Top: responses at rheobase. Middle: responses to a 550-pA current pulse. Bottom: responses to 550-pA current pulses on an expanded time scale. Asterisks above traces indicate AP bursts. A: impact of baclofen on the input-output relationship (f/I curve; average ± SE; n = 42). Baclofen causes a rightward shift in the f/I curve. C: graph shows the distribution of regular and intrinsically bursting firing neurons (n = 42) in the different experimental conditions. Bath application of baclofen (20 μM) abolishes burst firing.

Despite having no detectable impact on the somatic resting membrane potential or input resistance (Fig. 6A; n = 7; P > 0.05, paired Student’s t-test), in agreement with the absence of a significant effect of bath applications of baclofen on distal dendritic membrane properties (see Fig. 2).

DISCUSSION

In this study, we provide evidence that GABA<sub>B</sub> receptors regulate the somatic and dendritic excitability of layer 5 pyramidal neurons in the somatosensory barrel cortex via different mechanisms. At the soma, activation of GABA<sub>B</sub> receptors leads to a decrease in AP firing by hyperpolarizing the somatic membrane potential and decreasing the somatic membrane resistance following activation of putative GIRK channels. In contrast, at dendritic locations, GABA<sub>B</sub> receptors reduce dendritic excitability, primarily by downregulating dendritic calcium channels, leading to an increase in the threshold for generation of dendritic calcium electrogensis 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<sub>B</sub> receptors, whereas activation of distal dendritic GABA<sub>B</sub> receptors leads to a decrease in burst firing.

Cellular mechanisms underlying GABA<sub>B</sub>-mediated slow inhibition. Our results show that both bath and local somatic application of baclofen lead to hyperpolarization and a decrease in somatic input resistance in 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. 5). Similar results have been reported in neurons from the entorhinal cortex (Deng et al. 2009). These effects of GABA<sub>B</sub> receptors are likely to be due to activation of a potassium conductance, as they were occluded by low concentrations of barium. Previous studies indicate that GABA<sub>B</sub> receptors can activate GIRK (Kir3) channels (Chen and Johnston 2005; Lüscher et al. 1997; Takigawa and Alzheimer 1999). Consistent with this idea, GABA<sub>B</sub>-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<sub>B</sub> receptors in the entorhinal cortex can also regulate neuronal excitability through...
activation of a TREK-2 (a two-pore domain) potassium channel. As barium is nonspecific, even at low concentrations, our experiments cannot distinguish among activation of GIRK, TREK-2, or other leak potassium channels in mediating these effects of baclofen.

Dendritic GABA_B-mediated effects on input resistance were also barium sensitive (Fig. 2), suggesting that they may also be mediated by GIRK channels. Activation of dendritic GIRK channels by GABA_B receptors has been described previously in both cortical layer 5 and hippocampal pyramidal neurons (Chen and Johnston 2005; Takigawa and Alzheimer 1999). At a functional level, we observed that the impact of GABA_B receptor activation on dendritic input resistance was greatest at proximal dendritic locations (Fig. 2). While it is tempting to

Table 1.  Effects of GABA_B receptor activation on somatic AP properties

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<th>Threshold (mV)</th>
<th>Amplitude (mV)</th>
<th>dV/dt (V/s)</th>
<th>Half Width (ms)</th>
<th>Rheobase (pA)</th>
<th>n</th>
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<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>
</tr>
<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>
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Data are shown as mean ± SE. AP, action potential; n.s., nonsignificant. *P < 0.01; **P < 0.05; ***P < 0.001, paired Student’s t-test.

Fig. 6. Impact of somatic compared with dendritic GABA_B receptors on AP 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. B: left: examples of the impact of dendritic (top; 540 μM from the soma) and somatic (bottom) baclofen application on burst firing. Right: average number of APs/burst in the different experimental conditions. C: average (±SE) f/I curves during somatic (left) and dendritic (right) application of baclofen. D–F: graphs of the average number of APs generated by 1 nA current injections (D), rheobase (E), and slope of the initial region of the f/I curve (F) in the different experimental conditions. G: impact of somatic baclofen application on the medium after-hyperpolarization following an AP burst. *P < 0.05; **P < 0.01; ***P < 0.001. Dendritic baclofen applications 568 ± 12 μM from the soma in all panels (n = 7).

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conclude from this data that the density or coupling of GABA\textsubscript{B} receptors to GIRK or other potassium channels is highest at somatic and proximal dendritic locations in cortical pyramidal neurons, it can be shown that a uniform increase in resting conductance leads to a greater decrease in somatic compared with dendritic input resistance (unpublished simulations). Our data therefore suggest that the density and coupling of GABA\textsubscript{B} receptors to potassium channels are uniform across the somato-dendritic axis of cortical layer 5 pyramidal neurons. Consistent with this idea, in hippocampal pyramidal neurons, GABA\textsubscript{B} receptors can activate GIRK channels at both proximal and distal dendritic locations (Chen and Johnston 2005), where they may play a role in regulating synaptic plasticity (Chen and Johnston 2005; Chung et al. 2009).

**GABA\textsubscript{B}-mediated modulation of dendritic excitability.** APs 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 plays a key role in generation of dendritic calcium electrogenesis that can feed back 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 (Figs. 3 and 4). 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. 4G). These data suggest that dendritic GABA\textsubscript{B} receptors reduced dendritic excitability, primarily through downregulation of dendritic voltage-gated calcium channels. Consistent with this idea, the impact of baclofen on bAP half width and calcium electrogenesis was occluded by the presence of low concentrations of nickel (100 \(\mu\)M), which blocks T- and R-type voltage-gated calcium channels (Figs. 3 and 4).

Previous work indicates that activation of GABAergic input to layer 5 pyramidal neurons in the 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 downregulation of dendritic calcium channels (Perez-Garcia et al. 2006). Consistent with this earlier study, we 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 (Figs. 5 and 6). 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\textsubscript{B} receptors on synaptic plasticity will be enhanced further by the impact of GABA\textsubscript{B} receptor activation on bAP duration (Fig. 3).

**Impact of dendritic and somatic GABA\textsubscript{B} Receptors on neuronal output.** With respect to the impact of GABA\textsubscript{B} receptors on neuronal output, we show that distal dendritic baclofen applications decrease bursting firing, reducing the number of APs elicited by suprathreshold current injections in the absence of an impact on somatic membrane properties (Fig. 6). This observation is consistent with the recent observations of Palmer et al. (2012), who also showed that GABA\textsubscript{B} receptor-mediated downregulation 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 (Figs. 5 and 6). This leads to a subtractive or shunting form of inhibition (Silver 2010). It is interesting that activation of somatic GABA\textsubscript{B} receptors also leads to an increase in the slope of the f/I relationship. This effect may be mediated by the impact of somatic GABA\textsubscript{B} receptors on the mAHP. A reduction in the mAHP would be expected to limit membrane hyperpolarization following the AP, decreasing the interspike interval and thereby increasing neuronal output gain. Consistent with this idea, previous work indicates a role of the AHP in regulation of output gain (Higgs et al. 2006), with block of the mAHP causing an increase in the slope of the f/I relationship (Schwindt et al. 1988).

In summary, we show that GABA\textsubscript{B} receptors in cortical layer 5 pyramidal neurons act to decrease somatic and dendritic excitability via different mechanisms. Somatic GABA\textsubscript{B} receptors are coupled to barium-sensitive, putative GIRK potassium channels, whereas dendritic GABA\textsubscript{B} receptors act primarily by downregulating dendritic calcium electrogenesis. As a result, activation of somatic GABA\textsubscript{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\textsubscript{B} receptors causes a switch from burst to tonic firing and a reduction in neuronal output. This location-dependent specificity of GABA\textsubscript{B} receptor activation on neuronal excitability would be expected to further enhance the diversity with which different GABAergic interneuron cell types orchestrate network activity in the cortex.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: J-D.B. and G.J.S. conception and design of research; J-D.B. performed experiments; J-D.B. analyzed data; J-D.B. and G.J.S. interpreted results of experiments; J-D.B. prepared figures; J-D.B. drafted manuscript; J-D.B. and G.J.S. edited and revised manuscript; J-D.B. and G.J.S. approved final version of manuscript.

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