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 electrogenesis, 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 though 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_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 cutting, 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 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 Na_2GTP, 10 Na_2-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, Mineapolis, 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 compare 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 $GABA_B$ 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).

Table 1 near here

Impact of dendritic $GABA_B$ receptor activation

To investigate the impact of $GABA_B$ receptors at somatic and dendritic sites, baclofen (50 μM) was focally applied (3 sec application) 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 somatic baclofen application was significantly larger than the dendritic response to dendritic baclofen application (Fig. 2B; dendritic baclofen applications 435 ± 23 μm from the soma; n= 12; $P < 0.01$, unpaired Student's *t* test).

Figure 2 near here

To further study the effect of dendritic $GABA_B$ receptor activation on dendritic membrane excitability we examined the impact of bath application of baclofen (20 μM) 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_{rest}= -73.46 ± 1.00 mV; baclofen V_{rest}= -74.25 ± 0.73 mV; distance from soma: 395 ± 16 μm; 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 ANONA: 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.

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.

Figure 3 near here

Impact of $GABA_B$ receptors activation on action potential backpropagation

To understand the role of $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ 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

283

284 **$GABA_B$ receptors inhibit dendritic calcium electrogenesis**

285 Given that baclofen reduced bAP duration by blocking dendritic calcium channels, we
286 next investigated the impact of baclofen on dendritic calcium electrogenesis elicited
287 by AP trains. Previous work indicates that trains of bAPs lead to generation of
288 dendritic calcium electrogenesis in a frequency dependent manner as observed
289 previously (Larkum et al. 1999a; Williams and Stuart 2000). The frequency of bAPs
290 required to evoke dendritic calcium electrogenesis is called the "critical frequency"
291 and can be used to determine the degree of dendritic excitability (Larkum et al.
292 1999a). To examine the impact of $GABA_B$ receptor activation on dendritic
293 excitability we therefore tested the effect of bath application of baclofen on the
294 critical frequency. The dendritic response to trains of five somatic APs evoked at
295 frequencies of 20 to 200 Hz (increment of 10 Hz) was recorded during dual dendritic
296 and somatic whole-cell recording (Fig. 5A). In control, the observed critical
297 frequency was 96.7 ± 3.1 Hz (n= 46), consistent with previous studies (Breton and
298 Stuart 2009; Larkum et al. 1999a). Bath application of baclofen (20 μM) abolished
299 dendritic calcium electrogenesis (Fig. 5B), leading to a significant decrease in the
300 integral of dendritic responses measured at supra-critical frequencies (Fig. 5C, left;
301 distance from soma: 518 ± 18 μm; n= 30, $P < 0.001$, paired Student's t test).
302 Furthermore, this effect of baclofen was blocked by CGP52432 (1 μM; n= 8; data not
303 shown) and by low concentrations (100 μM) of nickel (Fig. 5C, right; n= 8).

304

305 **Figure 5 near here**

306

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 electrogenesis, 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 electrogenesis 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_{rest}: $P < 0.01$, 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 $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ receptor activation leads primarily to a reduction in AP output through an increase in the rheobase, whereas dendritic $GABA_B$ 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

379 inwardly rectifying potassium or GIRK (Kir3) channels (Chen and Johnston 2005;
380 Lüscher et al. 1997; Takigawa and Alzheimer 1999). Consistent with this idea,
381 $GABA_B$-mediated slow inhibition is absent from hippocampal pyramidal neurons in
382 the GIRK2 knockout mouse (Lüscher et al. 1997), although Deng et al. (2009) have
383 recently described that $GABA_B$ receptors in entorhinal cortex can regulate neuronal
384 excitability through activation of a TREK-2 (a two-pore domain) potassium channel.
385 To investigate the contribution of GIRK channels to the effects observed in our study
386 we co-applied baclofen with low concentrations of barium (100 μM), a non-selective
387 blocker of GIRK channels (Chen and Johnston 2005; Coetzee et al. 1999; Takigawa
388 and Alzheimer 1999). Application of baclofen with barium antagonized the effects of
389 baclofen at the soma (Fig. 1), consistent with the idea that these effects are mediated
390 by GIRK channels. That said, barium is quite non-specific even at low concentrations,
391 hence we cannot rule out a role of TREK-2 or other potassium channels in mediating
392 these effects of baclofen.
393
394 Dendritic $GABA_B$-mediated effects on input resistance at proximal dendritic locations
395 were also barium-sensitive (Fig. 2), consistent with the idea that they are also
396 mediated by GIRK channels. Activation of dendritic GIRK channels by $GABA_B$
397 receptors has previously been described in both cortical layer 5 and hippocampal
398 pyramidal neurons (Chen and Johnston 2005; Takigawa and Alzheimer 1999). In our
399 experiments the impact of baclofen on dendritic input resistance was restricted to
400 proximal dendritic locations. This observation suggests that the baclofen-sensitive
401 GIRK channel responses in the earlier work by Takigawa and Alzheimer (1999) are
402 likely to be from proximal dendritic segments of cortical pyramidal neurons. In
403 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 $GABA_B$-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 balcofen 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).

$GABA_B$-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 electrogenesis 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 $GABA_B$ receptors located at distal dendritic sites as it was observed during local dendritic applications of baclofen (Fig. 5D). These data suggest that dendritic $GABA_B$ 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 $GABA_B$ 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 $GABA_B$ receptors on dendritic excitability in cortical pyramidal neurons is primarily via this mechanism. In addition, we find that the functional impact of $GABA_B$-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), $GABA_B$-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

454 dendritic depolarization associated with bAPs is key to removal of the voltage-
455 dependent magnesium block of NMDA receptors (Nowak et al. 1984). This action of
456 $GABA_B$ receptors on synaptic plasticity will be further enhanced by the impact of
457 $GABA_B$-receptor activation on bAP duration (Fig. 4E, F).
458
459 **Impact of dendritic and somatic $GABA_B$ receptors on neuronal output**
460 With respect to the impact of $GABA_B$ receptors on neuronal output we show that
461 distal dendritic baclofen applications decrease bursting firing, decreasing the number
462 of APs elicited for suprathreshold current injections, in the absence of an impact on
463 somatic membrane properties. This observation is consistent with the recent
464 observations of Palmer *et al.* (2012), who also showed that $GABA_B$ receptor-mediated
465 down-regulation of dendritic calcium channels leads to a reduction in AP firing in the
466 absence of a significant change in somatic properties.
467
468 In contrast, somatic baclofen applications increase AP rheobase, shifting the f/I
469 relationship to the right without influencing AP bursting firing. This leads to a
470 subtractive or shunting form of inhibition (Silver 2010). Interesting, activation of
471 somatic $GABA_B$ receptors also leads to an increase the slope of the f/I relationship.
472 This effect may be due to the impact of $GABA_B$ receptor activation on the medium
473 AHP (Fig 6H). Consistent with this idea, previous work indicates a role of the AHP in
474 regulation of output gain (Higgs et al. 2006).
475
476 In summary, we show that $GABA_B$ receptors in cortical layer 5 pyramidal neurons act
477 to decrease somatic and dendritic excitability via different mechanisms. Somatic
478 $GABA_B$ 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.

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622

623

Figure captions

Figure 1: $GABA_B$-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 $GABA_B$-mediated change in the slope of the I/V curve is occluded by a prior application of the $GABA_B$ 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 suptrathreshold 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 $GABA_B$ 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 balcofen 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

674 distance dependence of bAP amplitude (**B**), velocity (**C**), rate-of-rise (**D**) and half-
675 width (**E**) in control (black) and during bath application of baclofen (grey, 20 μM). **F,**
676 Left: The duration of bAPs is significantly decreased during bath application of
677 baclofen (20 μM) at distal dendritic recording sites (dendritic recordings 520 ± 18 μm
678 from the soma, n= 31). Right: In the presence of nickel (100 μM) baclofen no longer
679 has an impact on bAP duration (dendritic recording 522 ± 30 μm from the soma,
680 n= 8). **G,** The impact of baclofen (20 μM) on bAP width is blocked by the $GABA_B$
681 antagonist CGP52432 (1 μM). *: $P < 0.05$; ***: $P < 0.001$; n.s.: non significant.
682
683 **Figure 5: $GABA_B$ receptors decrease dendritic calcium electrogenesis.**
684 **A,** Dendritic responses (620 μm from the soma) to trains of five APs evoked by
685 somatic current injection (bottom) at the indicated frequencies in control (top) and
686 following bath application of balcofen (20 μM, middle). **B,** Graph of dendritic voltage
687 integral versus AP frequency for the data illustrated in **A**. Note the non-linear increase
688 in dendritic integral in control (black), indicative of dendritic calcium electrogenesis,
689 is abolished by baclofen (grey). **C,** The average dendritic voltage integral obtained at
690 supra-critical frequencies (200 Hz) is significantly decreased in baclofen (left). This
691 effect is occluded by a prior bath application of nickel (right; 100 μM). **D,** Dendritic
692 responses (420 μm from the soma) during trains of somatic APs (5 spikes at 200Hz)
693 at the times indicated in the graph (bottom) during local application of baclofen to the
694 dendritic recording site (grey bar). **: $P < 0.01$; ***: $P < 0.001$; n.s.: non significant.
695
696 **Figure 6: Impact of somatic and dendritic $GABA_B$ receptors on somatic**
697 **membrane properties and neuronal output.**

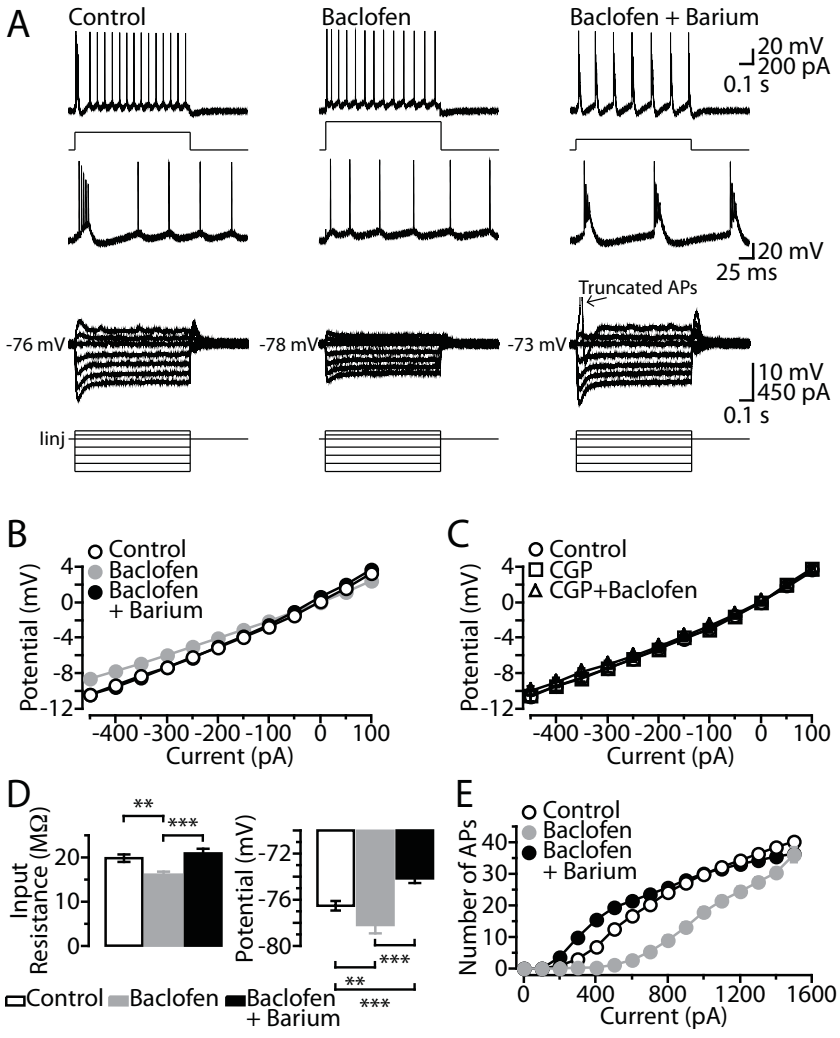
A, Impact of somatic and dendritic baclofen application (50 μM) on somatic input resistance (left) and resting membrane potential (right). Note that dendritic application of baclofen does not affect somatic resting membrane properties (distance from soma 568 ± 12 μm; n= 7). **B,** Examples of APs evoked 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.

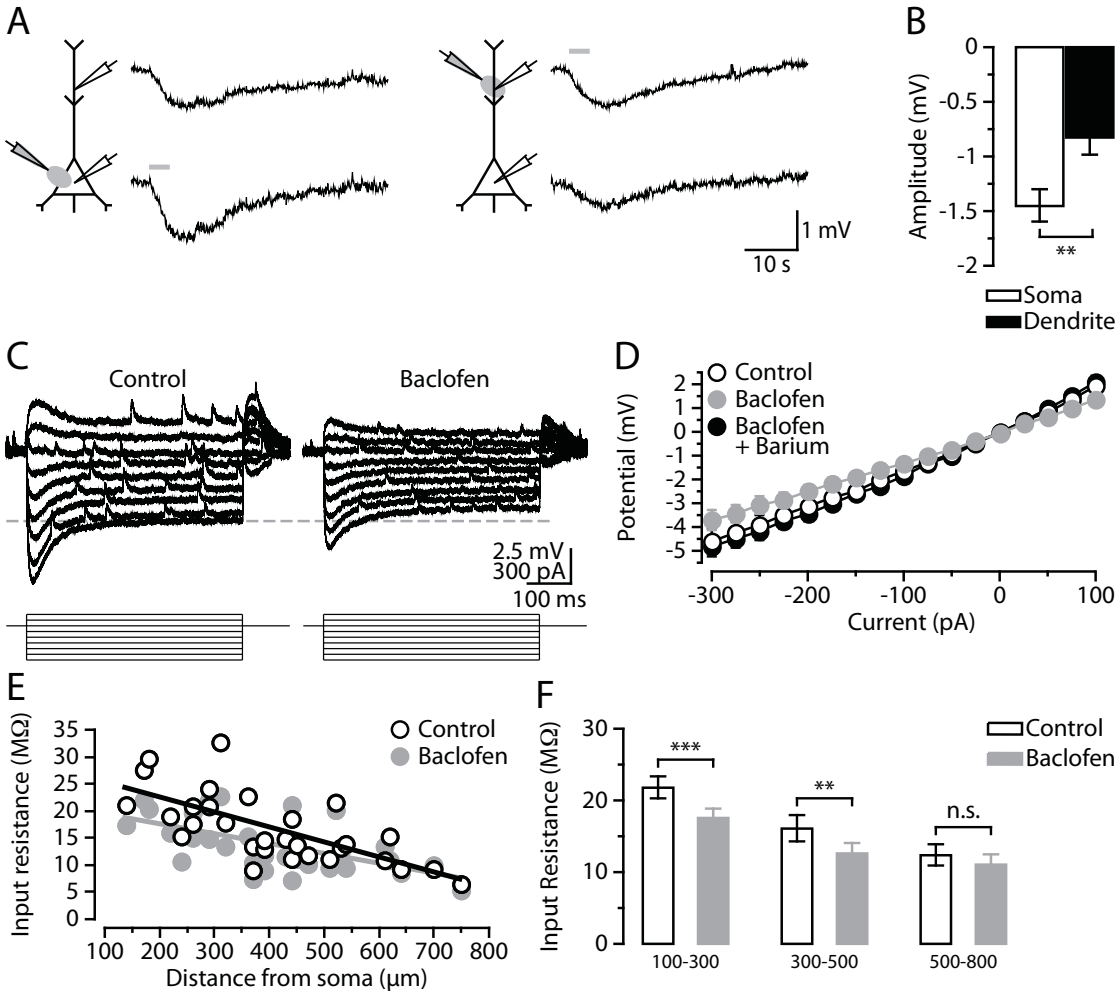
719 Table:
720
721 **Table 1: Effects of $GABA_B$ receptor activation on somatic AP properties.**

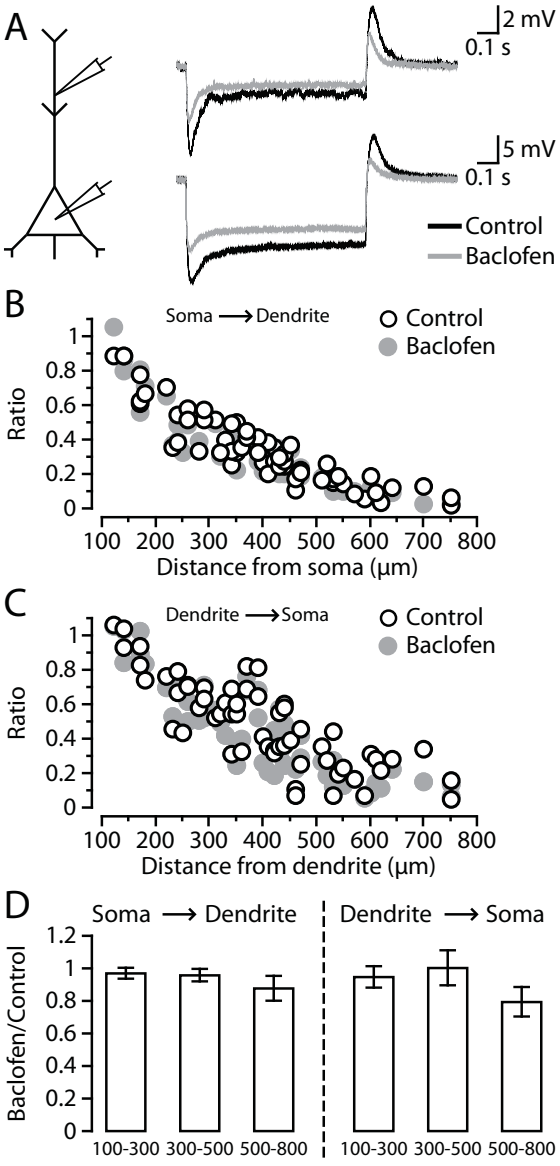
	Threshold (mV)	Amplitude (mV)	dV/dt ($V.s^{-1}$)	Half-width (ms)	Rheobase (pA)	n
Control	-59.4 ± 0.4	96.3 ± 0.5	634 ± 9	0.56 ± 0.01	275 ± 13	73
Baclofen	-59.5 ± 0.4	95.5 ± 0.5	619 ± 8	0.53 ± 0.01	522 ± 20	73
Probability	n.s.	**	*	**	***	

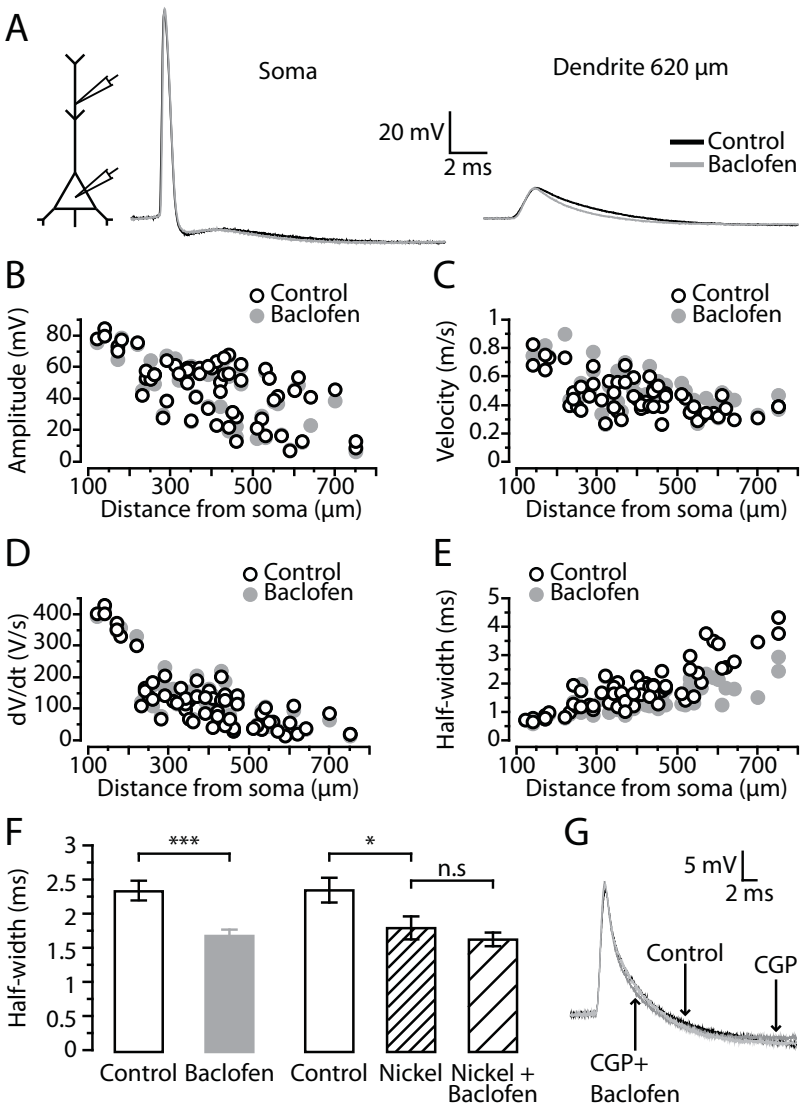
722 Data are shown as mean ± S.E.M. n.s: non significant, *: $P < 0.05$; **: $P < 0.01$; ***:
723 $P < 0.001$; paired Student's *t* test.
724

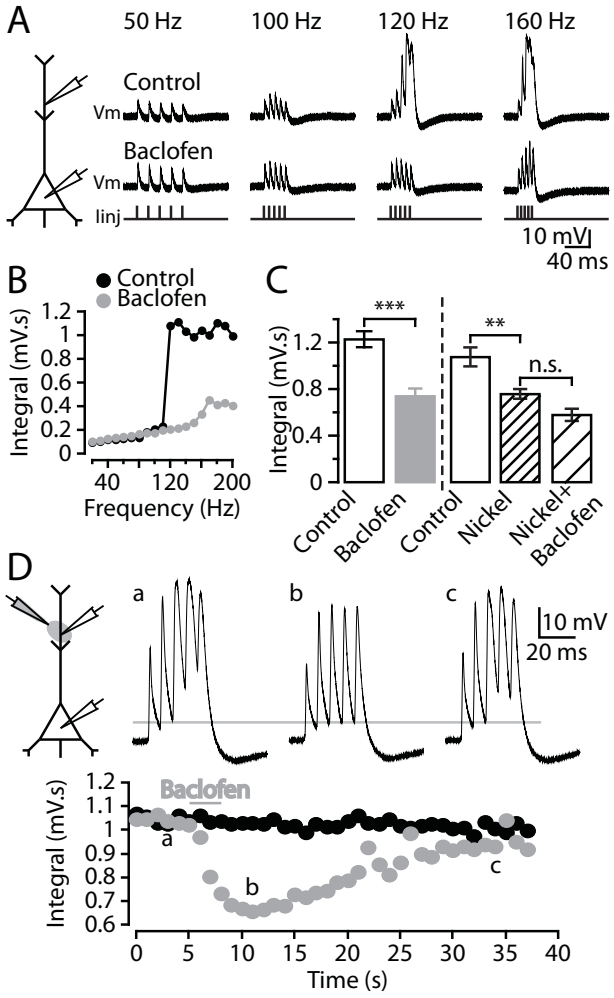
725

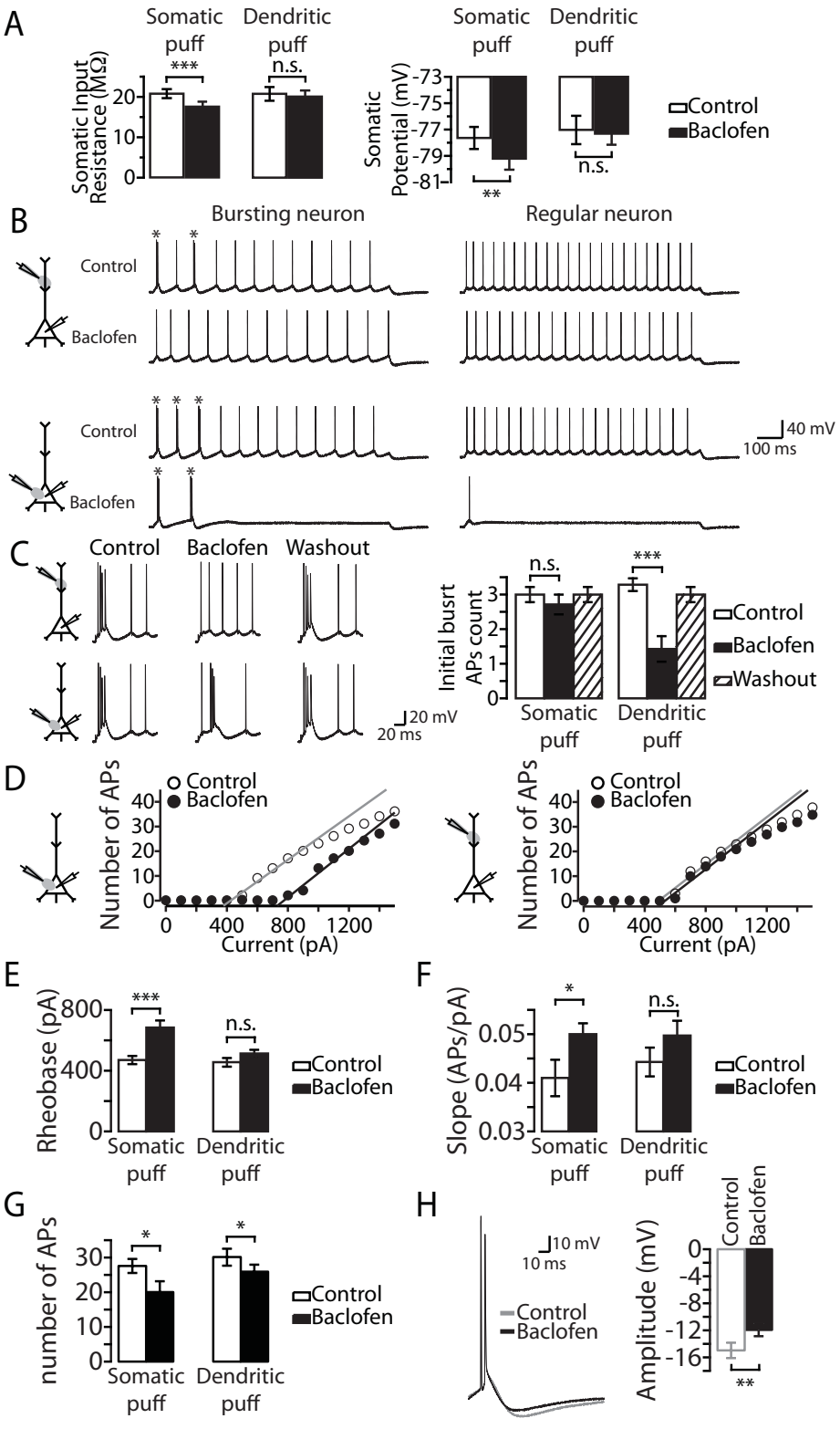

A
Control
Baclofen
Baclofen + Barium
20 mV
200 pA
0.1 s
20 mV
25 ms
Truncated APs
-76 mV
-78 mV
-73 mV
10 mV
450 pA
0.1 s
Iinj
B
Control
Baclofen
Baclofen
+ Barium
Potential (mV)
Current (pA)
C
Control
CGP
CGP+Baclofen
Potential (mV)
Current (pA)
D
Input Resistance (MΩ)
Potential (mV)
Control
Baclofen
Baclofen
+ Barium
E
Control
Baclofen
Baclofen
+ Barium
Number of APs
Current (pA)


A
1 mV
10 s
B
Amplitude (mV)
0
-0.5
-1
-1.5
-2
**
Soma
Dendrite
C
Control
Baclofen
2.5 mV
300 pA
100 ms
D
Control
Baclofen
Baclofen
+ Barium
Potential (mV)
2
1
0
-1
-2
-3
-4
-5
-300
-200
-100
0
100
Current (pA)
E
Input resistance (MΩ)
35
30
25
20
15
10
5
0
Control
Baclofen
100
200
300
400
500
600
700
800
Distance from soma (µm)
F
Input Resistance (MΩ)
30
20
10
0
***
**
n.s.
Control
Baclofen
100-300
300-500
500-800


A
2 mV
0.1 s
5 mV
0.1 s
Control
Baclofen
B
Soma ⟶ Dendrite
Control
Baclofen
Ratio
Distance from soma (μm)
C
Dendrite ⟶ Soma
Control
Baclofen
Ratio
Distance from dendrite (μm)
D
Soma ⟶ Dendrite
Dendrite ⟶ Soma
Baclofen/Control
100-300
300-500
500-800
100-300
300-500
500-800


A
Soma
Dendrite 620 µm
20 mV
2 ms
Control
Baclofen
B
Amplitude (mV)
Distance from soma (µm)
C
Velocity (m/s)
Distance from soma (µm)
D
dV/dt (V/s)
Distance from soma (µm)
E
Half-width (ms)
Distance from soma (µm)
F
Half-width (ms)
***
*
n.s
Control
Baclofen
Control
Nickel
Nickel + Baclofen
G
5 mV
2 ms
Control
CGP
CGP+ Baclofen


A
50 Hz
100 Hz
120 Hz
160 Hz
Control
Vm
Baclofen
Vm
Iinj
10 mV
40 ms
B
Control
Baclofen
Integral (mV.s)
Frequency (Hz)
C
Integral (mV.s)
***
**
n.s.
Control
Baclofen
Control
Nickel
Nickel+ Baclofen
D
a
b
c
10 mV
20 ms
Baclofen
Integral (mV.s)
Time (s)


A
Somatic puff
Dendritic puff
Somatic Input Resistance (MΩ)
***
n.s.
Somatic Potential (mV)
**
Control
Baclofen
B
Bursting neuron
Regular neuron
Control
Baclofen
40 mV
100 ms
C
Control
Baclofen
Washout
20 mV
20 ms
Initial busrt APs count
D
Number of APs
Current (pA)
E
Rheobase (pA)
F
Slope (APs/pA)
G
number of APs
H
10 mV
10 ms
Amplitude (mV)