Outwardly Rectifying Tonically Active GABA_A Receptors in Pyramidal Cells Modulate Neuronal Offset, Not Gain

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Hippocampal pyramidal cell excitability is regulated both by fast synaptic inhibition and by tonically active high-affinity extrasynaptic GABA_A receptors. The impact of tonic inhibition on neuronal gain and offset, and thus on information processing, is unclear. Offset is altered by shunting inhibition, and the gain of a neuronal response to an excitatory input can be modified by changing the level of “background” synaptic noise. Therefore, tonic activation of GABA_A receptors would be expected to modulate offset and, in addition, to alter gain through a shunting effect on synaptic noise. Here we show that tonically active GABA_A receptors in CA1 pyramidal cells show marked outward rectification, while the peaks of IPSCs exhibit a linear current–voltage relationship. As a result, tonic GABA_A receptor-mediated currents have a minimal effect upon subthreshold membrane potential variation due to synaptic noise, but predominantly affect neurons at spiking threshold. Consistent with this, tonic GABA_A receptor-mediated currents in pyramidal cells exclusively affect offset and not gain. Modulation of tonically active GABA_A receptors by fluctuations in extracellular GABA concentrations or neuromodulators acting on high-affinity receptors potentially provides a powerful mechanism to alter neuronal offset independently of neuronal gain.

Introduction

In addition to fast synaptic GABA_A receptor (GABA_AR)-mediated signaling, there is a slower form of signaling resulting from the tonic activity of extrasynaptic, high-affinity GABA_ARs (Semyanov et al., 2004; Farrant and Nusser, 2005; Glykys and Mody, 2007). Tonic conductances in the hippocampus modulate cognitive function: genetic deletion or pharmacological inhibition of $\alpha 5$ subunit-containing GABA_ARs, which contribute to tonic inhibition in CA1 pyramidal cells (Caraiscos et al., 2004; Scimemi et al., 2005; Prenosil et al., 2006; Glykys et al., 2008), improves spatial learning and memory (Collinson et al., 2002; Atack et al., 2006; Dawson et al., 2006). A possible mechanism linking tonically active GABA_ARs to cognition is the modulation of long-term potentiation (Cheng et al., 2006). However, blocking $\alpha 5$ subunit-containing GABA_ARs also increases neuronal excitability (Bonin et al., 2007), implying that tonically active GABA_ARs also affect neuronal computations.

Elemental neuronal computation can be represented by the input–output (I–O) function, which relates action potential generation to synaptic excitation. The I–O relationship can be modified through a change in gain (slope) or in offset (threshold). These two operations have distinct effects on information processing (Abbott et al., 1997; Salinas and Thier, 2000; Schwartz and

Simoncelli, 2001; Prescott and De Koninck, 2003; Cavelier et al., 2005; Mehaffey et al., 2005). Increasing gain increases the sensitivity of the neuron to a change in excitatory input. However, because neurons have finite maximal firing rates, this is accompanied by a decrease in the range of inputs that can be discriminated. Changes in offset alter the input detection threshold without changing the neuron’s sensitivity to changes in input.

How inhibitory signaling affects neuronal computations has attracted considerable attention (Carvalho and Buonomano, 2009). Because the time-averaged voltage in a firing neuron remains approximately constant (close to threshold potential), introducing a shunting conductance results in constant-current offset. This has been confirmed theoretically (Gabbiani et al., 1994; Holt and Koch, 1997) and experimentally with excitation mimicked by constant-current injection (Brickley et al., 1996; Chance et al., 2002; Ulrich, 2003). However, *in vivo*, neurons are bombarded by a noisy, stochastic input (Paré et al., 1998; Destexhe and Paré, 1999; Destexhe et al., 2001), which influences I–O gain and increases the input range of a neuron (Hó and Destexhe, 2000; Doiron et al., 2001; Chance et al., 2002; Fellous et al., 2003; Wolfart et al., 2005; Higgs et al., 2006). Tonic inhibition would be expected to shunt membrane noise and so to influence both gain and offset. This would have significant implications for signal processing, because it would imply that the offset of the I–O function cannot be modified independently of its gain.

Here we show that the current–voltage (*I–V*) relationship of tonically active GABA_ARs exhibits marked outward rectification, a feature also shared by the late phases of IPSCs. This outward rectification implies that tonic GABA_AR-mediated conductances would have a minimal effect upon subthreshold membrane variations. Consequently (and contrary to expectation), tonic inhi-

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bition in hippocampal pyramidal cells acts predominantly as an offset and has minimal effect on neuronal gain.

Materials and Methods

Hippocampal slice preparation. Transverse hippocampal slices (350 μm thick) were used for *in vitro* electrophysiological recordings. Slices were prepared from 3- to 4-week-old male Sprague Dawley rats.

Animals were killed according to the United Kingdom Animals (Scientific Procedures) Act of 1986. After decapitation, brains were rapidly removed and dissected, and hippocampi were cut with a Leica VT1000S vibratome in ice-cold sucrose-based solution containing the following (in mM): 70 sucrose, 80 NaCl, 2.5 KCl, 7 MgCl_2 , 0.5 CaCl_2 , 25 NaHCO_3 , 1.25 NaH_2PO_4 , 22 glucose, equilibrated with 95% O_2 plus 5% CO_2 to yield a pH of 7.4. The slices were then allowed to recover in sucrose-free solution in an interface chamber for at least 1 h at room temperature before being transferred into recording chamber. Recordings were performed at 32°C. The storage and perfusion solution contained the following (in mM): 119 NaCl, 2.5 KCl, 1.3 MgSO_4 , 2.5 CaCl_2 , 26.2 NaHCO_3 , 1 NaH_2PO_4 , 22 glucose and was gassed with 95% O_2 and 5% CO_2 .

Electrophysiological recordings. Visualized patch-clamp recordings from the CA1 pyramidal cells were performed using infrared differential interference contrast imaging system. Tonic GABA_AR-mediated currents were measured in a voltage-clamp mode ($V_{\text{hold}} = -70$ mV) with AMPA, NMDA, and GABA_B receptors blocked. We used a Cs-based internal solution containing the following (in mM): 120 CsCl, 10 HEPES, 2 EGTA, 8 NaCl, 0.2 MgCl_2 , 2 Mg-ATP, 0.3 Na-GTP, 5 QX-314 Br^- salt, pH 7.2, 290 mOsm. The tonic GABA_AR-mediated current was measured as a reduction in holding current following application of picrotoxin (100 μM). Another GABA_AR antagonist, bicuculline methiodide (10 μM), was used in some experiments. The GABA uptake inhibitor SKF-89976A (30 μM) was used to block GAT1.

To estimate the I - V relationship of tonic GABA_AR-mediated inhibition the holding current was recorded during slow (5-s-long) voltage ramps from -70 mV to $+40$ mV in the presence of Cd^{2+} (100 μM) to prevent activation of voltage-dependent Ca^{2+} channels. Ramps were performed before and after the application of picrotoxin and then current values were subtracted to obtain the picrotoxin-sensitive component. The I - V curve with low (8 mM)-Cl $^-$ internal solution (calculated $E_{\text{GABA}} \sim -74$ mV) for Figure 4 was obtained by using a step protocol. A liquid junction potential of 14.2 mV was corrected off-line. The pipette solution contained the following (in mM): 140 K-gluconate, 8 NaCl, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, 5 QX-314 Br^- salt, pH and osmolarity adjusted to 7.2, 290 mOsm. Recordings for supplemental Fig. S2A, available at www.jneurosci.org as supplemental material, were done in bicarbonate-free HEPES-buffered extracellular solution containing the following (in mM): 125 NaCl, 2.5 KCl, 2 CaCl_2 , 2 MgCl_2 , 30 glucose, 25 HEPES, pH 7.4 adjusted with NaOH, and gassed by 100% O_2 . The solution also included membrane-permeable inhibitor of carbonic anhydrase, ethoxzolamide (50 μM). The rectification index (RI) represented the ratio of conductances underlying the GABA_AR-mediated current at -60 and $+30$ mV and was calculated as follows:

$$\text{RI} = [I_{+30}/(30 - E_{\text{rev}})]/[I_{-60}/(-60 - E_{\text{rev}})].$$

To record evoked GABA_A receptor-mediated currents, IPSCs were evoked by Schaffer collateral stimulation (200 μs , 50–500 μA) using a bipolar stainless steel electrode positioned close to the recording site. Action potential independent miniature IPSCs (mIPSCs) were recorded with the Na^+ -channel blocker tetrodotoxin (1 μM) in the bathing solution.

Intracellular solution containing the following (in mM): 140 K-gluconate, 8 NaCl, 10 HEPES, 0.2 EGTA, 2 Mg-ATP, 0.3 Na-GTP, 10 Na-phosphocreatine, pH and osmolarity adjusted to 7.2, 290 mOsm, was used for the majority of the current-clamp recordings. Dynamic clamp (Robinson and Kawai, 1993; Sharp et al., 1993) was used to inject simulated synaptic inputs into cells. Background synaptic noise was simulated by injecting noisy conductance template (g_{noise}) containing Poisson-

distributed excitatory synaptic conductances of constant amplitude (times the unitary conductance g_e of 0.7 nS) and a reversal potential of 0 mV. The time course of synaptic conductances ($\tau_{\text{rise}} = 0.5$ ms, $\tau_{\text{decay}} = 5$ ms) was used to fit evoked AMPAR-mediated EPSCs. The amplitude range and mean interevent interval of simulated background synaptic conductances (60 Hz) were chosen to mimic membrane potential variations observed *in vivo* in cortical and hippocampal pyramidal cells (Destexhe and Paré, 1999; Hahn et al., 2007). Variations of the membrane potential were measured as root mean square (RMS) noise. RMS values generated by g_{noise} injections in dynamic current-clamp experiments (see Fig. 6) were of a similar magnitude to those in other studies (Destexhe et al., 2001; Wolfart et al., 2005) and were an order of magnitude larger than those due to spontaneous GABA_AR channel activity. Signal EPSPs were simulated by injecting synaptic conductances of varying amplitudes (g_{SEPS}) at 500 ms intervals, and the probability of spiking (P_i) in response to varying peak excitatory postsynaptic conductance amplitudes was calculated from the results of 20 trials at each g_{SEPS} to obtain I-O curves. Spike probability functions were fitted by the following sigmoid function:

$$F = A_2 + (A_1 - A_2)/(1 + \exp[(x - x_0)/dx]).$$

The slope of spike probability curves was calculated at 0.5 probability as in the study by Wolfart et al. (2005). Such a measure, however, does not always give an accurate index of changes in the I-O function, especially when it is poorly fitted by a sigmoid function. Therefore, in addition, we estimated the area under the curve (AUC), a parameter which allowed us to assess the effect of the tonic conductance on the shape of the I-O relationship without making any assumptions about the distribution of the action potential probability function. AUC was calculated by integrating I-O curves using a trapezoidal rule over the interval of inputs in which P_i changed from 0 to 1. This parameter is sensitive both to changes in the dynamic range of the neuronal input and to the shape of the distribution of the probability function.

Dynamic clamp was implemented with G-clamp software (Kullmann et al., 2004) running under LabView RealTime with an iteration frequency of 20 kHz. Experiments were done in the presence of ionotropic glutamate receptor blockers DL-AP5 (50 μM) and NBQX (20 μM) and GABA_B receptor blocker CGP52432 (5 μM), as well as GABA_A receptor blocker picrotoxin (100 μM).

Series resistance was monitored throughout the experiment using a -5 mV step command. Cells showing a $>20\%$ change in series resistance, a resistance of >20 M Ω , or an unstable holding current were rejected. Recordings were obtained using a MultiClamp 700B amplifier (Molecular Devices), filtered at 2 kHz, digitized at 5 kHz, and stored on a personal computer. LabView (National Instruments) software was used for data acquisition and off-line analysis. Two-tailed Student's t test was used for statistical analysis. Differences were considered significant when $p < 0.05$. Data are shown as mean $\pm$ SEM.

Drugs and reagents. Receptor antagonists, GABA uptake inhibitor, and GABA were purchased from Tocris Bioscience and Ascent Scientific. Other chemicals were from Sigma-Aldrich.

Modeling. All simulations were conducted with NEURON 6.0 (Hines and Carnevale, 1997), using a variable time step dt and a simulated temperature of 34°C. We used two different models: one based on the realistic morphology of a CA1 hippocampal pyramidal cell [used for supplemental Fig. S7, available at www.jneurosci.org as supplemental material, modified from the study by Migliore et al. (1999)], and the other with a simplified morphology. The latter contained a cylindrical soma of diameter and length of 20 μm , four dendrites (one 800 μm long and three others 200 μm long, with a diameter of 2 μm), and an axon of 100 μm and diameter of 1 μm . Both models had Na^+ , K^+ , and mixed conductances with the following peak values: $g_{\text{Na}} = 32$ mS/cm 2 , $g_{\text{KDR}} = 10$ mS/cm 2 , $g_{\text{KA}} = 8$ mS/cm 2 , $g_{\text{ih}} = 0.05$ mS/cm 2 . In the realistic neuron model, g_{KA} and g_{ih} increased linearly in apical dendrites ($d/100$ and $3d/100$, respectively), where d is the distance from the soma. The model obeyed the following current balance equation:

$$C_m \frac{dV}{dt} = -I_{\text{Na}} - I_{\text{KDR}} - I_{\text{KA}} - I_{\text{ih}} - I_{\text{GABA}} - I_{\text{syn}},$$

where $C_m = 1 \mu\text{F}/\text{cm}^2$, I_{GABA} is the shunting tonic GABA_A receptor-mediated current ($E_{\text{GABA}} = -65 \text{ mV}$), and I_{syn} is a synaptic current. We compared the effects of either a constant or voltage-dependent conductance on the I - O function of the neuron. The current mediated by a constant tonic conductance $I_{\text{GABA}} = g_{\text{GABA}} \times (V - E_{\text{GABA}})$ was calculated using g_{GABA} in the range of 0–3 mS/cm². The current carried by rectifying receptors was calculated as $I_{\text{GABA}} = g_{\text{GABA}} \times O \times (V - E_{\text{GABA}})$, where the state O is a proportion of channels in the open state. The transition from open to closed state was described by the following simple kinetic scheme:

where $a_{OC} = 50x/(1 - e^{-x})$ and $x = 0.1(V + 20)$;

$b_{CO} = 20x/(1 - e^{-x})$ and $x = -0.08(V - 10)$.

The I - V relationship obtained with such a kinetic scheme fits our experimental data (see Fig. 4A). The resting membrane potential of simulated neurons was -65 mV . Excitatory synaptic conductance waveforms used in simulations were similar to those used in dynamic clamp experiments and were represented by a double exponential function with τ_{rise} of 0.5 ms, τ_{decay} of 5 ms, and reversal potential of 0 mV. Inhibitory synaptic conductance waveforms used in simulations presented in supplemental Fig. S6, available at www.jneurosci.org as supplemental material, had the following kinetics: τ_{rise} of 0.8 ms, τ_{decay} of 8 ms, and reversal potential of -65 mV . Individual events were Poisson distributed, occurred at the same rate as excitatory noise (60 Hz), and had the same unitary conductance as noisy excitatory synapses in the same simulation rounds.

Results

Tonic inhibition exhibits strong outward rectification

There is growing evidence from single channel recordings from neurons that GABA_A receptors can under certain circumstances show outward rectification (Birnir et al., 1994), which is dependent upon the presence of low GABA concentrations (Pytel et al., 2006). How does this affect the I - V relationship of phasic and tonic GABA_A-mediated signals? At the later phases of IPSCs, the GABA concentrations within the synaptic cleft are low and so this phenomenon would be expected to affect predominantly the shape of IPSC tails. To test this, we recorded from CA1 pyramidal cells in whole-cell patch-clamp mode in acute rat hippocampal slices at near-physiological temperature. AMPA, NMDA, and GABA_B receptors were blocked with NBQX (25 μM), D,L-2-amino-5-phosphonovalerate (APV, 50 μM), and CGP52432 (5 μM), respectively. We used a high internal Cl^- concentration to avoid Goldman-Hodgkin-Katz rectification. Under these conditions, the decay of evoked and miniature GABA_A-mediated synaptic currents displayed outward rectification that was more marked at later phases (Fig. 1; supplemental Fig. S1, available at www.jneurosci.org as supplemental material), consistent with other studies (Ashwood et al., 1987; Mellor and Randall, 1998; Pytel and Mozrzymas, 2006). This is strikingly different from the peaks of evoked and miniature IPSCs which displayed a linear I - V relationship, suggesting that rectification plays a limited role in phasic inhibition during synaptic transmission but may contribute to IPSP summation.

An important but previously untested prediction from these results is that tonic currents mediated by extrasynaptic GABA_ARs, which detect low GABA concentrations (in the nanomolar to micromolar range), would also show outward rectification. Using the same conditions in which we recorded evoked IPSCs, addition of the GABA_A receptor antagonist picrotoxin (100 μM) to neurons at -70 mV produced a small but detectable positive shift in holding current

Figure 1. The tails, but not peaks, of evoked IPSCs (eIPSCs) in hippocampal pyramidal neurons show outward rectification. **A**, Family of eIPSCs traces at different holding potentials between -90 and $+45 \text{ mV}$ from one neuron demonstrating slower decay at positive potentials. **B**, Superimposed normalized I - V relationships of peak amplitudes (closed circles) and amplitudes of the eIPSC tails measured at two different time points (20 ms, gray circles; 40 ms, open circles) as indicated in **A** showing outward rectification of IPSC tails with greater rectification further from the peak ($n = 7$; data points are fitted either with linear or second-order polynomial function). Norm. ampl., Normalized amplitude. **C**, The RI of the GABA_A-mediated current during the IPSC decay phase increases with time after stimulation (stim.). The bar on the graph shows the RI of the peak currents, indicating that they do not rectify ($n = 7$).

(Fig. 2A), consistent with the presence of a tonic GABA_A-mediated current in CA1 pyramidal cells.

We determined the I - V relationship of the signal carried by tonically active GABA_ARs by measuring the whole-cell current during a slow voltage ramp from -70 mV to $+40 \text{ mV}$ over 5 s (to minimize capacitive errors), in the presence of 100 μM Cd^{2+} (to block Ca^{2+} channels). We repeated the ramps in the absence and presence of picrotoxin (100 μM) to block GABA_ARs. Subtraction of the two curves revealed marked outward rectification (Fig. 2B). In a separate series of cells, rectification was assessed by using a voltage-step protocol, measuring the steady-state current at each voltage. This also revealed marked outward rectification (Fig. 2C).

Although outward rectification is most likely a property of tonically active GABA_ARs, we considered several alternative explanations. First, the rectification is unlikely to reflect trans-membrane Cl^- shifts secondary to the applied voltage because it persists when the ramp protocol is reversed (Fig. 3A). Second, the rectification

persists when HCO_3^- in the external solution is replaced with HEPES, arguing against the possibility that it reflects a transmembrane HCO_3^- gradient (supplemental Fig. S2A, available at www.jneurosci.org as supplemental material). Third, outward rectification is still present after compensating for voltage-clamp error (supplemental Fig. S2B, available at www.jneurosci.org as supplemental material). Rectification is also unlikely to reflect a hitherto unknown voltage dependence of blockade of GABA_A Rs by picrotoxin because spontaneous IPSCs were absent at all potentials in 100 μM picrotoxin indicating complete block of GABA_A Rs. Further, we confirmed that the competitive GABA_A R antagonist, bicuculline (10 μM) had an identical effect (supplemental Fig. S2C, available at www.jneurosci.org as supplemental material).

The experiments described thus far have determined the I - V relationship using slowly varying voltages. This is very different from many physiological processes in which voltage changes on a millisecond timescale. We therefore applied voltage steps from -60 mV to $+40$ mV before and after washing in picrotoxin and compared the subtracted currents 10 and 100 ms after depolarization. There was no significant difference in the magnitude of tonic current at 10 ms (62 ± 13 pA) and 100 ms (63 ± 9 pA) after the voltage step ($n = 4$) (Fig. 3B), implying that rectification is quasi-instantaneous.

To explore the possible physiological role of this rectification, we recorded with pipettes containing a lower Cl^- concentration (8 mM) that was within the physiological range of Cl^- concentrations in adult neurons. As predicted Goldman-Hodgkin-Katz rectification exaggerated the outward rectification of the I - V curve (Fig. 4A,B). The slope conductance was low at resting membrane potential, but rapidly increased at near threshold voltages (-50 mV to -40 mV) (Fig. 4C). This has important ramifications for signal processing as it implies that the tonic conductance will have a greater effect on EPSPs at threshold.

We next asked whether the rectification of the signal mediated by tonically active GABA_A Rs is still present when the extracellular GABA concentration is increased by either uptake blockade or addition of GABA to the perfusate. Inhibiting GABA uptake by blocking GAT1 transporters with SKF-89976A (30 μM) resulted in a significant increase in the magnitude of the tonic current at -70 mV (baseline: -19.0 ± 7.7 pA, $n = 8$; SKF-89976A: -83.5 ± 22.3 pA, $n = 8$; $p = 0.016$, t test) (Fig. 5A,C). When GAT1 was blocked, the picrotoxin-sensitive component continued to show outward rectification, albeit less marked than under control conditions (Fig. 5D). Rectification was also diminished by adding GABA to the perfusion solution at a concentration (20–30 μM) that gave approximately the same shift in holding current at resting membrane potential as observed for 2 μM GABA in hippocampal culture (Scimemi et al., 2005) and as that resulting from inhibition of GAT1 (Fig. 5B–D). The decrease in the rectification index (Fig. 5D) at high GABA concentrations

Figure 2. Tonic GABA_A -mediated currents in hippocampal pyramidal cells display marked outward rectification. **A**, GABA_A receptor block by picrotoxin produces a positive shift in holding current in pyramidal cells voltage clamped to -70 mV. Representative traces (left) and time course of normalized tonic current following application of picrotoxin (PTX; right; $n = 7$). **B**, I - V relationship of the tonically active GABA_A Rs obtained from the difference in holding current during voltage ramps before and after perfusing picrotoxin (see Materials and Methods; $n = 7$; black trace represents averaged I - V curve, gray area is SEM). **C**, Similar rectification was obtained by measuring steady-state holding currents at different voltages using a step protocol (see Materials and Methods; $n = 4$).

indicates relative loss of rectification due either to recruitment of less rectifying GABA_A Rs or to a dependence of GABA_A R rectification on GABA concentrations (these possibilities were not examined further).

Tonic inhibition alters offset, not gain, in pyramidal cells

In vivo neurons constantly receive inputs from tens of thousands of synapses and their membrane potential fluctuates as a result of such background “synaptic noise”. The finding that tonically active GABA_A Rs exhibit profound outward rectification suggests that they will have a greater effect on EPSPs at threshold than close to resting membrane potential. How do tonically active GABA_A receptors affect neuronal integration of EPSPs?

Because CA1 pyramidal cells convey information at low firing frequencies by the ensemble pattern and timing of neuronal firing (McNaughton et al., 1983; Wilson and McNaughton, 1993), the I - O function of CA1 pyramidal neurons was estimated by measuring the probability of firing in response to synchronous firing of multiple excitatory afferents (Azouz, 2005; Wolfart et al., 2005; Carvalho and Buonanno, 2009). To control the amplitude of the excitatory conductance with high reproducibility, this was simulated by dynamic clamp (g_{SEPS}) rather than by electrical stimulation. AMPA, NMDA, and GABA_B receptors were blocked with NBQX (25 μM), APV (50 μM), and CGP52432 (5 μM), respectively.

Without simulated background synaptic noise, the variation of the membrane potential was low (RMS 0.11 ± 0.01 mV, $n = 4$). As a result, the I - O function, relating spiking probability to the amplitude of the injected excitatory synaptic conductance

Figure 3. Rectification is time independent. **A**, Reversing the voltage ramp does not change the rectification of the tonically active GABA_ARs. Top, Example of a recording when the cell was slowly depolarized from -70 mV to $+40$ mV (ramp-up) and then hyperpolarized back to -70 mV (ramp-down). The I - V relationships of the up and down ramps are identical (bottom). **B**, Example of the time course of the picrotoxin-sensitive current obtained with a step command from -60 mV to $+40$ mV (the fast capacitance artifact was removed). The full trace is shown in the inset. Dashed vertical lines indicate time points at which the magnitude of tonic current was measured. PTX, Picrotoxin.

waveform (g_{sEPSP}), was very steep (supplemental Fig. S3A,C, available at www.jneurosci.org as supplemental material). It resembled a step function, with a small increase in input conductance resulting in a change of firing probability from 0 to 1. Consistent with previous studies in other neurons (Fellous et al., 2003; Shu et al., 2003; Wolfart et al., 2005), addition of background synaptic noise (g_{noise} , simulated by injecting a pseudo-random stochastic train of synaptic waveforms) increased the detection of low amplitude inputs, thus decreasing both the slope and the threshold of the I-O function. We explored the effect of varying the noise amplitude by scaling the underlying mean unitary synaptic conductances. As expected, the impact on the slope of the I-O relationship varied with the amplitude of noise (supplemental Fig. S3B-D, available at www.jneurosci.org as supplemental material).

Figure 4. Rectification of tonically active GABA_ARs is more pronounced with a physiological intracellular Cl^- concentration. **A**, I - V plot obtained using a step protocol with 8 mM intracellular Cl^- ($n = 4$). Data points are fitted with a Boltzmann function. Representative trace demonstrating no shift in holding current at -74 mV (top). **B**, The rectification due to unequal concentration of Cl^- inside and outside neurons cannot fully account for the rectification of GABA_ARs. The predicted I - V curve due to Goldman-Hodgkin-Katz rectification (red line) superimposed with normalized experimental curve (black) demonstrating significant additional rectification due to receptor properties. **C**, Slope conductance mediated by tonically active GABA_ARs calculated for the fitted I - V curve in **A**. PTX, Picrotoxin.

We next asked how tonically active GABA_ARs affect the I-O relationship. To increase the tonic GABA_AR-mediated conductance, we added 20 – 30 μ M GABA to the perfusion solution (which reduces but does not abolish outward rectification) (Fig. 5D). This resulted in a rightward shift of the I-O function (the input conductance estimated to give a 50% probability of action potential generation shifted from 12.5 ± 1.5 nS to 15.8 ± 1.9 nS; $n = 11$; $p = 0.00022$; paired t test). However, it had no significant effect on the slope (control, 0.205 ± 0.029 nS $^{-1}$; in GABA, 0.241 ± 0.037 nS $^{-1}$; $n = 11$, $p = 0.14$) (Fig. 6A–C).

Conversely, blocking GABA_ARs with picrotoxin (100 μ M) resulted in a leftward shift in the I-O function beyond baseline,

Figure 5. Rectification persists when the extracellular GABA concentration is increased. **A, B,** Application of picrotoxin (PTX) in the presence of the GAT1 blocker 30 μ M SKF-89976A ($n = 8$, **A**) or 20–30 μ M GABA ($n = 9$, **B**) reveals a shift in the normalized holding current at -70 mV (left), and continued rectification (right). **C,** Summary plot of the effects of GABA application or blockade of GABA transport on the holding current at -70 mV. Increased GABA concentration in the slice leads to a similar increase in the tonic current following addition of GAT1 blocker or 20–30 μ M GABA. **D,** Summary plot of RI (see Materials and Methods) with either the GAT1 blocker or 20–30 μ M GABA. In the I - V plots, black traces represent mean values, while gray areas are SEM; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, t test.

confirming that receptors active in the absence of added GABA also affect neuronal firing (the amplitude of the excitatory input that results in 50% probability of spiking changed from 14.1 ± 1.6 nS in control to 12.6 ± 1.4 nS in picrotoxin; $n = 15$; $p = 0.001$; paired t test) (Fig. 6A–C). Despite this marked effect of the tonic conductance on the offset of the I - O function, there was again no effect on its slope (control, 0.222 ± 0.020 nS $^{-1}$; in picrotoxin, 0.229 ± 0.022 nS $^{-1}$; $p = 0.6$; paired t test; $n = 15$).

An alternative means of measuring the impact of tonic inhibition on the I - O function independent of its effect on offset is to measure the area under the curve (AUC) from spike probability = 0–1 (equivalent to the range over which a change in input has an impact on output). This yields a composite measure of the effects on the shape of the I - O function and on the input dynamic range without making assumptions about the distribution of the spike probability function (see Materials and Methods). The AUC value under baseline conditions (5.66 ± 0.45 nS) did not significantly change following GABA application (5.27 ± 0.76 nS;

$p = 0.7$; paired t test; $n = 11$). Block of GABA $_A$ Rs by picrotoxin also had no significant effect on the AUC (control, 6.13 ± 0.49 nS; in picrotoxin, 5.43 ± 0.45 nS; $p = 0.09$; paired t test; $n = 15$) (Fig. 6D).

We further confirmed that application of GABA had only a small effect on RMS noise (before GABA: 2.92 ± 0.18 mV; after GABA: 2.62 ± 0.15 mV; $p = 0.006$; paired t test; $n = 10$) (Fig. 6A₃,E). Blockade of GABA $_A$ receptors by picrotoxin had no significant effect on RMS noise compared with control (control: 3.10 ± 0.21 mV; in picrotoxin: 3.19 ± 0.21 mV, $p = 0.3$; paired t test; $n = 11$) (Fig. 6A₃,E).

Rectifying tonic inhibition thus affects the offset of the I - O relationship without altering its gain. However, is rectification necessary to prevent changes in the shape of the I - O relationship? To address this, we took advantage of the dynamic clamp method, and, rather than modulating GABA $_A$ receptor activation, we blocked GABA $_A$ receptors with picrotoxin (100 μ M) and injected a simulated constant (voltage-independent) tonic conductance to effect a similar offset to the experiments above (supplemental Fig. S4, available at www.jneurosci.org as supplemental material). As predicted, a constant tonic conductance changed the slope of the I - O relationship and altered the AUC.

Effects of rectification of tonically active GABA $_A$ Rs on the I - O relationship

The shape of the I - O function may also be influenced by EPSP decay, noise frequency as well as the I - V relationship of tonic inhibition. To dissect out the influence of each of these, we constructed a simple conductance-based model of a pyramidal neuron (see Materials and Methods). Using this model, we injected Poisson noise (using multiples of underlying unitary synaptic conductances to generate different levels of membrane potential fluctuations) into the soma (g_{noise}) and simulated depolarizing synaptic conductance with variable amplitude to mimic synchronous excitatory inputs (g_{SIIPSP}) (Fig. 7A). We first confirmed that the model reproduced the effects of changing the noise amplitude on the I - O slope and on the AUC (supplemental Fig. S5A, available at www.jneurosci.org as supplemental material). Since changing tonic inhibition will alter the apparent time constant for EPSP decay (by altering the membrane conductance), we asked to what extent this would affect the gain of the I - O function. Altering the EPSP conductance decay constant had a significant effect on offset but only had a minimal effect on the AUC (supplemental Fig. S5B, available at www.jneurosci.org as supplemental material). The mean frequency of the EPSPs generating noise only had an impact on the AUC at low frequencies; from 50 to 70 Hz, which corresponds to the values used experimentally to generate background noise (similar to that observed *in vivo*), the AUC was fairly stable (supplemental Fig. S5C, available at www.jneurosci.org as supplemental material). These observations suggest that the magnitude of membrane potential fluctuations resulting from changes in the underlying synaptic conductance is the major factor that affects the shape of the I - O relationship in our experiments.

We used our model to ask how rectifying tonic GABA $_A$ R-mediated inhibition affects the I - O function, and compared this to a hypothetical scenario in which tonic inhibition did not show outward rectification. The I - V curve of rectifying tonically active GABA $_A$ Rs was modeled as a voltage-dependent conductance based upon the results illustrated in Figure 4. For comparison, we modeled a voltage-independent tonic conductance (Fig. 7B). One crucial advantage of using such a model is that the magnitudes of the voltage-dependent tonic conductance and of the

Figure 6. The tonic GABA_AR-mediated conductances in hippocampal pyramidal cells modulate the offset, but not the gain, of the I–O function. **A**, Application of GABA (20–30 μ M) in the presence of simulated synaptic noise (g_{noise}) shifts the I–O curve of a neuron to the right without affecting the slope of fitted sigmoid function. Subsequent application of picrotoxin (PTX, 100 μ M) shifts the curve in the opposite direction beyond the control values without a change in slope. Shown are sample traces of the neuron's response (**A**₁) at three data points marked by the rectangle (**A**₂). Neither activation nor blockade of the tonic GABA_AR-mediated conductance produces significant change in the membrane potential (V_m) distribution (**A**₃; data from the same cell). **B–E**, Summary data for changes in the offset (**B**) of the I–O function demonstrating a significant effect produced by application of GABA (20–30 μ M) or picrotoxin (100 μ M) ($n = 11$ and 15 , respectively). The change in the offset was measured as a change in the magnitude of input conductance that results in 50% probability of action potential generation. There were however no significant changes in the slope of spike probability curve (**C**; also measured at 50% probability of action potential firing) and normalized area under the curve (AUC) from spike probability = 0–1 (**D**). Changes in V_m RMS noise (**E**) with either GABA or picrotoxin application ($n = 11$ and 10 , respectively). $g_{noise}(2)$ and $g_{noise}(3)$ (supplemental Fig. S3, available at www.jneurosci.org as supplemental material) were used in experiments. Open circles in **B–E** are values for individual cells; ** $p < 0.01$; *** $p < 0.001$; paired t test.

constant, voltage-independent, conductance could easily be adjusted so that they resulted in identical offsets of the I–O function in the absence of noise (Fig. 7C, top). These offsets were comparable to those produced by elevated GABA in our experiments (Fig. 6). When simulated synaptic noise was added, the threshold for action potential generation shifted to smaller excitatory inputs (Fig. 7C, bottom). The AUC was decreased by the addition of a constant inhibitory conductance (58% of control). In contrast, it was affected much less by the addition of a voltage-dependent conductance to mimic outward rectifying GABA_ARs (88% of control) (Fig. 7D). The outcome was qualitatively identical over a range of noise amplitudes and simulated EPSP conductance decay time constants: in all cases, addition of a constant inhibitory conductance reduced both the offset and the AUC of the I–O, while a voltage-dependent conductance with a comparable effect on offset had a consistently smaller effect on the shape of the I–O curve. The difference between linear and rectifying tonic inhibition was also evident when excitatory background synaptic activity underlying noisy fluctuations of the membrane potential was balanced by simulated random inhibitory input (supplemental Fig. S6, available at www.jneurosci.org as supplemental material; see Materials and Methods for details).

Outward rectification thus enables tonic inhibition to affect offset, but reduces its impact on the shape of the I–O function over the input dynamic range (spike probability from 0 to 1). We tested this further in a more detailed model of a CA1 pyramidal cell (supplemental Fig. S7, available at www.jneurosci.org as supplemental material). The rectification of tonic inhibition resulted in a similar preservation of the shape of the I–O function, as observed in the simpler model.

This difference between the two tonic conductances can be explained by a lesser effect of the voltage-dependent conductance on low amplitude, subthreshold EPSPs. However, as the amplitude of the EPSPs increase there should be less difference between the effects of voltage-dependent and voltage-independent conductances. We tested these predictions by examining the effects of tonic conductances on small conductance (0.7 nS) and large conductance (12 nS) EPSPs. As anticipated, adding a voltage-independent tonic inhibitory conductance had a greater effect on the amplitude of small rather than large EPSPs and should therefore have a greater impact

on low amplitude noise rather than high amplitude coincident EPSPs (supplemental Fig. S8, available at www.jneurosci.org as supplemental material). Conversely, adding a voltage-dependent tonic inhibitory conductance affected large excitatory inputs to a greater extent than small ones. We then tested the effects of tonic conductances on the RMS of membrane noise over a range of peak conductances (at +40 mV) and found that there is considerable discrepancy between the effects of simulating nonrectifying and rectifying tonic inhibition (Fig. 7E). Rectifying tonic inhibition has a minimal effect on RMS noise over a large “maximal” conductance range, compared with the profound effect of even small increases in the constant tonic conductance. This is, however, not a fair comparison, as the same peak conductance of tonic voltage-dependent and voltage-independent conductances will have different effects on offset. We therefore calculated the effects of simulated tonic conductances carried by rectifying and nonrectifying GABA_ARs on the offset of the neuronal I–O function (without noise) and plotted the RMS noise in the presence of the tonic conductance against the offset of the I–O function (Fig. 7F). There was again a large discrepancy, with rectifying tonically active GABA_ARs having a much smaller effect on noise variance over a large range of offset. Therefore, outward rectification preserves noise variance despite greatly affecting the offset of the I–O function.

Discussion

We have shown that, in CA1 pyramidal cells, tonic GABA_AR-mediated inhibition exhibits marked outward rectification, and that this is also a feature of the late component of synaptic GABA_AR-mediated currents. The degree of outward rectification moreover depends inversely on the extracellular GABA concentration. Such rectification results in larger conductances at action potential threshold rather than subthreshold inputs. Consistent with this, tonic conductances alter offset while preserving the shape and gain of the I–O function, which depend upon the amplitude and frequency of synaptic noise.

GABA_A receptor rectification

GABA_ARs can exhibit both outward and inward rectification (Weiss, 1988; Birnir et al., 1994; Gage and Chung, 1994; Yoon, 1994; Burgard et al., 1996; Eghbali et al., 1997; Mennerick et al., 2001; Pytel et al., 2006). Studies of recombinant receptors in different expression systems indicate that the degree of rectification depends upon the subunit composition of the GABA_ARs, with many receptor subtypes showing no or minimal rectification (Verdoorn et al., 1990; Burgard et al., 1996). Using modeling and experimental data, rectification has been attributed to voltage-

Figure 7. Rectifying tonic GABA_AR-mediated inhibition decreases its shunting effect on the background synaptic noise and thus favors control of offset over modulation of gain. **A**, Similar to the experimental paradigm, the modeled pyramidal cell receives two inputs simulating variable EPSPs (g_{EPSP}) and stochastic synaptic noise (g_{noise}). Traces of the V_m at the cell soma resulting from the introduction of g_{EPSP} and g_{noise} separately and combined (lower trace). **B**, An example of I–V curves for rectifying and nonrectifying inhibition simulated in the pyramidal cell model by introducing a tonic inhibitory conductance with a reversal potential $E_i = -65$ mV and $g_{\text{max}} = 0.0001$ S/cm². **C**, The conductances of rectifying ($g_{\text{max}} = 15$ nS, at +40 mV) and nonrectifying ($g_{\text{const}} = 3$ nS) tonic inhibition were adjusted to give the same offset of the I–O function in the absence of background synaptic noise (top). The effects of these “equivalent” rectifying and nonrectifying inhibitions on the I–O function were then compared in the presence of synaptic noise (bottom). **D**, The I–O curves are plotted without offset to compare the range of synaptic inputs that lead to the generation of action potentials. The curves are aligned to match the maximum spike probability point. The bar chart shows that rectification preserves the AUC values, in contrast to a constant tonic conductance, which decreases the AUC. The unitary excitatory conductance in the noise templates was $0.0021 \mu\text{S}$ for all simulations [corresponding to $g_{\text{noise}}(3)$ in experiments]. **E**, The shunting effect of a constant tonic conductance on subthreshold noise is greater than that of rectifying inhibition. RMS is plotted against tonic conductance at +40 mV (g_{max}). Upper traces illustrate the effects of rectifying and nonrectifying tonic inhibition on RMS with $g_{\text{max}} = 4$ nS. **F**, The difference between the effects of rectifying and nonrectifying tonic inhibition on the variation of membrane potential is apparent even when their respective g_{max} values are matched to produce the same offset of I–O function in the absence of noise.

dependent deactivation of GABA_ARs (Mellor and Randall, 1998) though other mechanisms, such as voltage dependence of desensitization, conductance and opening time may also play a role (Birnir et al., 1994; Yoon, 1994). More recently, the outward rectification of GABA_ARs at low GABA concentrations has been suggested to be secondary to an increased GABA binding rate and a larger opening rate at depolarized potentials (Pytel et al., 2006). However, the question whether tonic GABA_AR-mediated inhibition shows a similar voltage dependence has not been previously addressed. Here we have demonstrated a marked outward rectification which occurs with almost equal intracellular and extracellular Cl[−] concentrations and is further exaggerated by a physiologically low intracellular Cl[−] concentration (due to Goldman–Hodgkin–Katz rectification). Increasing the extracellular concentration of GABA lessened the outward rectification.

A similar phenomenon has been observed for currents recorded in excised patches from neurons in cultures. Lesser rectification of GABA_ARs at higher GABA concentrations has been suggested to be secondary to a lesser effect of increased GABA binding with saturating GABA concentrations and consequently an increased role of voltage-dependent desensitization (Pytel et al., 2006). We cannot, however, exclude the possibility that higher GABA concentrations recruit receptors with less marked rectification or no rectification at all (Birnir et al., 1994; Burgard et al., 1996).

Outward rectification adds greatly to the complexity of the analysis of tonic conductances, as the magnitude will depend upon not only the extracellular GABA but also the potential at which they are measured. In most studies, the tonic current is measured at hyperpolarized holding potentials (around the resting membrane potential), so significantly underestimating the magnitude of these conductances at action potential threshold.

Effects of rectification of the tonic conductance on noise and the I–O function

The temporal aspects of GABAergic signaling (e.g., slow versus fast) have distinct computational consequences (Crowley et al., 2009; Pavlov et al., 2009). A previous study in cerebellar granule cells found that tonic GABA_AR-mediated conductances affected both offset and gain of the I–O relationship with a noisy input (Mitchell and Silver, 2003). There are two notable differences between that study and ours. First, the tonic current was injected using dynamic clamp and was modeled as a voltage independent conductance. Second, the I–O function used the summation of random EPSPs to elicit firing so that the mean voltage of the neuron was relatively depolarized. Although we cannot comment on whether rectification of tonic inhibition is present in cerebellar granule cells, the method of measuring the I–O function results in membrane voltage fluctuations occurring near the threshold voltage. In this circumstance the rectification of tonic inhibition may be less important.

CA1 pyramidal cells are very different from cerebellar granule cells, which show little or no accommodation and which fire *in vivo* at high frequency in response to stimuli (Chadderton et al., 2004). The firing of a single CA1 pyramidal cell increases when the animal is at a particular spatial location and there is a change in the probability of that pyramidal cell firing during the theta cycle with a change in position (O'Keefe, 2007). The firing of a CA1 pyramidal cell can provide both rate (probability of firing) and time encoded (phase of the theta cycle at which the cell fires) information (O'Keefe, 2007). Here, we have analyzed the rate encoded information as the probability of firing following an excitatory input.

Similar to other systems, we found that the introduction of noise decreases the slope of the I–O function, because of summation of subthreshold signals with noise (Destexhe et al., 2001; Chance et al., 2002; Shu et al., 2003; Wolfart et al., 2005). As described by Holt and Koch (1997) and demonstrated in other computational studies (Hô and Destexhe, 2000) introducing shunting inhibition results in a constant offset current at firing threshold voltage. However in the presence of background synaptic input tonic inhibitory conductance would also shunt the noise and would therefore be expected to affect the gain. Yet the prominent outward rectification of the tonic GABA_A receptor-mediated conductance described here results in a minimal impact on subthreshold inputs (such as noise) and only has a significant effect at more depolarized potentials (at threshold voltage). This results in tonic GABA_A receptor conductances predominantly affecting offset while maintaining the shape of the I–O relationship.

What is the functional advantage of being able to change offset independently of gain? To optimize the encoding of information, the I–O function has to be adjusted to “match” the distribution of the stimulus (Carvalho and Buonomano, 2009). Therefore, a change of stimulus from a broad to a narrow range requires an associated change in the range of the I–O (manifest as a change in gain). Similarly, a change in the threshold of the stimulus would require a change in the offset of the I–O function to maximize information encoding. The observation that extracellular GABA increases during exposure to novel environments (Bianchi et al., 2003) raises the possibility that increases in tonic GABA_AR-mediated conductances may be advantageous during learning. The gain of the I–O relationship of pyramidal cells can change during synaptic plasticity, altering the sensitivity of the pyramidal cells to increases in input (Daoudal and Debanne, 2003; Carvalho and Buonomano, 2009). One possible consequence is output saturation at low input amplitudes, and so a loss of ability to discriminate these from inputs with higher amplitudes. An increase in tonic inhibition would increase the threshold of the input before the pyramidal cell would start firing, yet would maintain the sensitivity of the I–O relationship to a change in input. Consequently, the increased rate of spatial learning observed in animals in which CA1 pyramidal cell tonic inhibition is genetically or pharmacologically reduced (Collinson et al., 2002; Caraiscos et al., 2004; Attack et al., 2006; Dawson et al., 2006) may be at the expense of output saturation and so may result in paradoxical loss of input discrimination.

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