Role for the Subthreshold Currents $I_{\text{Leak}}$ and $I_H$ in the Homeostatic Control of Excitability in Neocortical Somatostatin-Positive Inhibitory Neurons

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Gibson, Jay R., Aundrea F. Bartley, and Kimberly M. Huber. Role for the subthreshold currents $I_{\text{Leak}}$ and $I_H$ in the homeostatic control of excitability in neocortical somatostatin-positive inhibitory neurons. J Neurophysiol 96: 420–432, 2006. First published May 10, 2006; doi:10.1152/jn.01203.2005. Cortical circuitry reconfigures in response to chronic (1–3 days) changes in activity levels. To understand this process, we must know the role played by inhibitory neurons because they crucially influence network properties by controlling action potential generation and synaptic integration. Using pharmacological blockade of activity in neocortical organotypic slice cultures, we examined the activity-dependent regulation of membrane excitability in a specific inhibitory neuron subtype: the somatostatin-positive (SOM+) neuron. Chronic action potential blockade (TTX, 2.5 days) resulted in increased excitability in SOM+ neurons. This result is consistent with a homeostatic process to maintain the average firing rate of SOM+ neurons at a particular level. Excitability changes were not ascribed to changing cell size or alterations in voltage-dependent sodium current. Instead, the excitability increase was largely the result of a decrease in the density of two subthreshold-dependent sodium current. Instead, the excitability increase was not ascribed to changing cell size or alterations in voltage-firing rate of SOM/H11001 neuron. Chronic action potential blockade (TTX, 2.5 days) resulted in increased excitability in SOM+ neurons. This result is consistent with a homeostatic process to maintain the average firing rate of SOM+ neurons at a particular level. Excitability changes were not ascribed to changing cell size or alterations in voltage-dependent sodium current. Instead, the excitability increase was largely the result of a decrease in the density of two subthreshold currents: a passive leak current ($I_{\text{Leak}}$) and H-current ($I_H$). The downregulation of these currents increased excitability mostly through a decrease in membrane input conductance. The coadaptation of $I_{\text{Leak}}$ and $I_H$ enabled a change in input conductance while helping to preserve membrane potential. Evidence indicated that $I_{\text{Leak}}$ was probably mainly mediated by K+. At earlier culture ages, this adaptation was superimposed on developmental changes, whereas at older ages, the same types of induced alterations occurred but with no developmental component. Together with other studies, these data indicate that both inhibitory and excitatory neurons increase membrane excitability with chronic reduction in activity, but through different mechanisms.

INTRODUCTION

Neocortical activity levels are chronically altered during sensory map plasticity (Horton and Hubel 1981), neuronal circuit maturation (Turrigiano and Nelson 2004), and certain pathological conditions, such as epilepsy or stroke. To understand how neural circuit properties are altered in these situations, we must know the cellular alterations occurring in different cell types.

Across various species, most adaptations in neuronal properties in response to chronic activity level changes (minutes to days) appear to be homeostatic (Davis and Goodman 1998; Marder and Prinz 2002; Turrigiano and Nelson 2000). Regulation of neocortical excitatory neurons is consistent with the homeostatic maintenance of activity levels at a particular set point. In general, a reduction in circuit activity results in changes at excitatory neurons that would promote more activity in excitatory neurons. For instance, in response to long-term activity suppression (days), excitatory synapses increase in strength (Desai et al. 2002; Lissin et al. 1998; Murthy et al. 2001; Turrigiano et al. 1998), whereas inhibitory synaptic drive generally decreases (Hendry and Jones 1988; Kilman et al. 2002; Marty et al. 2000; Micheva and Beaulieu 1995). Membrane excitability is enhanced mainly through upregulation of voltage-dependent sodium channels (VDSCs) and downregulation of voltage-dependent potassium (K+) channels, but not through subthreshold currents (Desai et al. 1999b; but see Liu 2004). More “acute” forms of homeostatic plasticity (minutes) involve the subthreshold current $I_H$ (Fan et al. 2005; van Welie et al. 2004).

Inhibitory neurons critically influence cortical circuit properties (Chagnac-Amitai and Connors 1989; Hensch 2004; Silfio 1974; Whittington and Traub 2003), but little is known about their activity-dependent regulation. Cortical inhibitory neurons are divided into subtypes with distinct anatomical, biochemical, and electrophysiological properties (Gonchar and Burkhalter 1997; Gupta et al. 2000; Kawaguchi and Kubota 1997). In dissociated culture, activity blockade for 2 days using the VDSC blocker, tetrodotoxin (TTX), induces increased excitability in inhibitory neurons, but neither the inhibitory neuron subtype nor the mechanism underlying this change was known (Desai et al. 1999a). We investigated activity-dependent regulation of membrane excitability in one inhibitory subtype: the somatostatin-positive (SOM+) neuron. This subtype targets the distal dendrites of excitatory neurons, and thus more likely controls synaptic input rather than spike timing in excitatory neurons (Di Cristo et al. 2004; Miles et al. 1996; Somogyi et al. 1998). Accordingly, alterations in SOM+ neurons could alter synaptic integration and plasticity.

Intrinsic membrane excitability is affected by various current types and many of these have been described in SOM+ neurons: VDSCs, potassium currents, and H-current ($I_H$) (Lien et al. 2002; Maccarelli and McBain 1996; Martina et al. 2000; Santoro et al. 2000). In other neuron types, each of these currents has been demonstrated to be either acutely or chronically (long-term) modified by activity, and consequently to affect neuron excitability (Aizenman et al. 2003; Brickley et al. 2001; Chen et al. 2001; Coulter et al. 1989; Desai et al. 1999b; Fan et al. 2005; Nelson et al. 2003; Shah et al. 2004; Sourdet et al. 2003; Turrigiano et al. 1995; van Welie et al. 2006 2004). We demonstrate that excitability in neocortical SOM+ neurons is homeostatically regulated in the long term (2.5 days), and...
this regulation appears to be mediated to a significant extent by subthreshold currents, specifically an $I_{\text{leak}}$ and $I_H$.

**METHODS**

**Slice culture and pharmacological treatments**

We performed all experiments using GIN (GFP-expressing inhibitory neuron) mice, which express enhanced green fluorescent protein (EGFP) in only a subset of SOM+ neurons (Oliva et al. 2000) (Jackson Laboratories). The preparation of interface cultures was based on a previous study (Stoppani et al. 1991). Mice (P6) were anesthetized with halothane in a manner consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. The brain was removed and then dissected in a HEPES-based buffer containing kynurenic acid (1 mM) to obtain a square sheet of somatosensory neocortex, 2–3 mm on each side, which was subsequently sliced into 400-μm slices with a McIlwain chopper. Slices were transferred to 0°C culture medium and then plated onto semiporous membranes (Millecill, Millipore) in warmed culture medium. Slices were kept at 5% CO$_2$/35°C. Culture medium was exchanged the next day and every 2 days thereafter. The first two exchanges involved adding a mitotic inhibitor to the culture medium (FUDR, 35 μM; uridine, 80 μM). Culture medium (based on Musleb et al. 1997) was 20% adult horse serum (Hyclone, defined, SH 30074,02) and 80% MEM (GIBCO, 51200–020) and contained the following (in mM): 1 glutamine (Glutamax, Invitrogen), 0.7 ascorbic acid, 0.5 MgSO$_4$, 12.9 dextrose, 5.3 NaCO$_3$, 30 HEPES, and 1 μg/ml bovine insulin (Sigma), pH 7.3, 310 mOsm. Chronic TTX (2 μM) treatments were refreshed with new drug once per day. Indicating that our results were not an artifact of drug application, effects were identical whether sucrose (2 μM) was added to the control cultures. Usually, TTX was added to controls 10 min before removal from the insert to control for any excitability phenomena occurring on transferring the slice to the recording chamber. For any experiments that involved sodium currents, all inserts were transferred into medium containing 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 2-amino-5-phosphonopantoic acid (AP5) for ≥10 min before recording to washout TTX applied during treatment. All experimental comparisons required slices in the same plate obtained from the same animal. Slice culture age was termed “equivalent day” (ED), which is the sum of “days in vitro” and the postnatal age of the dissected mouse.

**Electrophysiology**

Whole cell recordings on layer 2/3 neurons were performed at 22 and 32°C (as indicated) in a submerison recording chamber (Gibson et al. 1999). Recordings were performed with IR-DIC visualization (Stuart et al. 1993) using a Nikon E600FN microscope and a CCD camera (Hamamatsu), and most recordings lasted <15 min. Recorded cells were typically about 15 μm below the slice surface. At 32°C, we observed a gradual depolarization of about 6 mV over the first 10 min. Therefore any experiments that critically depended on resting potential were performed within the first 5 min to assure properties in the more natural, initial state of the cell. Resting membrane potential and series resistance were continuously measured to monitor recording stability. Cell capacitance was always measured at recording onset to provide a rough estimate of cell size and to normalize currents (filtered at 30 kHz, sampled at 50 kHz). Capacitance and input resistance were measured in voltage clamp with a 400-ms, −10-mV step from a −60-mV holding potential. The capacitance was obtained by multiplying the series resistance by the fastest time constant of a double-exponential decay calculated fitted to the first 20 ms of the capacitive transient decay induced by a voltage step. Input resistance (or conductance) was based on the average voltage in the 80- to 130-ms window after step onset. Data were not corrected for junction potential.

**Excitability measurements**

Current-clamp recordings were performed at 32°C unless stated otherwise. Threshold current for firing a spike was determined using a series of depolarizing current steps of 600-ms length at 25- or 50-pA intervals. Threshold voltage for firing a spike was defined as the membrane potential, in a 5-ms window preceding spike peak, at which the third derivative was a maximum (an inflection point). Firing frequency versus injected current plots (F–I plots) was made by measuring the initial firing frequency of a spike train evoked by a series of incrementing current steps.

**Voltage-ramp protocols and analysis examining $I_{\text{leak}}$**

Voltage-clamp recordings were performed at 22°C in “Basic Block” artificial cerebrospinal fluid (ACSF) with 2.5 mM Cs" and accepted if series resistance was <15 MΩ and compensation/prediction were >85%. Holding potential was −60 mV and a step was applied to −20 mV for 6 s to allow sufficient relaxation to a steady-state “standing outward current.” This was followed by a voltage ramp down to −100 mV for 1.5–3 s (ramp durations indicated in Figs. 4 and 5). Statistical difference between average traces (P value, each cell was a sample) was the lower of “treatment only” and “treatment–voltage interaction” terms as obtained in a repeated-measures ANOVA. If these average traces were different, then their difference trace (subtraction of traces between the two groups) was termed statistically significant. Thus any $P$ values referring to a difference trace means the traces used in the subtraction were statistically different. Difference traces were always remapped into current versus voltage (I–V) plots, and statistical analysis on all ramp data was applied only to current obtained during the ramp. The SE for each point in the difference current was the square root of the sum squared of the SE values from the original two points (Zar 1999). If specific voltages during the ramp were compared, a Fisher’s LSD (protected least-significant difference) multicomparison test was applied.

When difference currents were obtained from two different external solutions in the same cell (all experiments in Fig. 5), the cell-to-cell variability in the external solution conditions was correlated. For determining a statistically significant effect on this type of difference current between treatment groups (control vs. TTX-treated), ramp currents from each cell were normalized to the first external solution condition (i.e., 1 mM [K] in Fig. 5, A and B). In effect, error bars exist only for current traces collected during the second external solution condition, and a repeated-measures ANOVA was again used to compare traces between treatment groups.

To assess the ionic identity of currents in I–V plots, passive currents for K$^+$ and Cl$^-$ were calculated according to Goldman–Hodgkin–Katz (GHK) current equations (Johnston and Wu 1995), adjusted for junction potentials, and normalized to the peak current at the voltage ramp apex (see Figs. 4, 5, and 9). A repeated-measures ANOVA was used to determine whether the experimental data were different from the predicted passive currents ($P > 0.05$ indicated that data matched the predicted passive current).

**H-current protocols and analysis**

Voltage-clamp recordings were performed at 22°C unless stated otherwise and accepted if series resistance was <15 MΩ and compensation/prediction were >85%. When “Basic Block” ACSF was used, 1 mM BaCl$_2$ was added. $I_H$ was measured during a 2-s, hyperpolarizing voltage step. Even though steps were immediately preceded by a series of smaller, −10-mV steps for the subtraction of leak currents [akin to a p4 protocol (Bezanzilla and Armstrong 1977)], we found this was not effective in isolating a clear $I_H$. This may have been a result of insufficient voltage clamp of dendritic current or some other nonlinear currents being modulated. Because repeated application of hyperpolarizing steps to −110 or below disrupted recording
stability, we restricted our data collection to one to two traces per condition when such steps were required. All I_H measurements were normalized to total membrane capacitance. Dual-exponential fits for current activation were performed with the generalized reduced gradient (GRG2) nonlinear optimization code (Excel software, Microsoft).

Unless stated otherwise, we measured I_H with respect to the local maximum during the step that represented the difference between the maximum current extent during the voltage step (usually within 200 ms of step onset) and that immediately before step offset (see Fig. 7A, arrows). We preferred this method for its simplicity, and amplitudes were nearly 79% less compared with I_H isolated using pharmacological blockers (Cs⁺, ZD7288; n = 11, 10; see RESULTS).

Steady-state, voltage-dependent activation was inferred by first inactivating I_H with a prestep (2 s), and then determining how much remained with a final test step to −110 mV (2 s) (Maccarelli and McBain 1996). The percentage reduction in I_H at this test step indicated the percentage activated during the prestep. Holding potential was −40 mV and bath solution was “Basic Block” ACSF.

Where indicated, I_H was also measured with a −40 mV step (2 s) from a potential of −60 mV in normal ACSF (with DNQX, AP5, and picrotoxin). This was an accurate measure of I_H because ZD7288 blocked 96 ± 2% of the slowly activating current (n = 4, see Fig. 10C). I_H in normal ACSF was larger than that measured in “Basic Block” external solution, making it easier to measure. Basic Block solution reduced I_H by 61 ± 7% (n = 5). Much of this was mediated by the 5 mM 4-AP which by itself reduced I_H by 46 ± 3% (n = 5). The rest of the block may have arisen from Ba²⁺ (van Welie et al. 2005). We cannot explain this reduction by 4-AP, but one possible reason is that a significant amount of I_H occurred in the dendrites where voltage-clamp control was weak, and with addition of these agents, the dendritic membrane was too depolarized for somatic hyperpolarization to activate I_H. This explanation is supported by abundant I_H in pyramidal neuron dendrites (Magee 1998), but no study has yet examined the localization of I_H in SOM+ dendrites.

**Na-current protocols**

Recordings were performed at 22°C and accepted if series resistance was <13 MΩ and compensation/prediction were >90%. Activation curves were based on the peak current evoked by incremental, 10-ms depolarizing steps (Fig. 9A); inactivation curves were derived from the maximum current evoked by a 10-ms step to 0 mV with varying 100-ms, prestep potentials (Desai et al. 1999b). All step protocols were preceded by fifteen 10-ms depolarizing steps that were one sixth the magnitude of the test step. All traces underwent leak subtraction before measurements were made. Subsequent application of TTX revealed that the leak subtraction method was accurate (n = 3).

**Electrophysiology solutions**

Normal ACSF contained (in mM): 126 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 26 NaHCO₃, 10 dextrose, and 2 CaCl₂. “Basic Block” ACSF was based on a previous study (in mM) (Maccarelli and McBain 1996): 110 NaCl, 5 KCl, 1 MgCl₂, 24 NaHCO₃, 10 dextrose, 10 TEA-Cl, 5 4-AP, 0.2 CaCl₂, 0.1 NiCl₂, and 0.001 TTX. “Basic Block” HEPES solution was (in mM): 110 NaCl, 5 KCl, 1 MgCl₂, 20 HEPES/Na-HEPES, 10 dextrose, 10 TEA-Cl, 5 4-AP, 0.2 CaCl₂, and 0.001 TTX. I_H, HEPES solution was as described (in mM) (Desai et al. 1999b): 10 NaCl, 120 choline-Cl, 3 KCl, 20 HEPES/Na-HEPES, 2 MgSO₄, 0.4 CaCl₂, 10 TEA-Cl, 5 4-AP, 14 dextrose, and 1.6 CaCl₂. All solutions were pH 7.4 (7.8 and 6.2 for acidification experiments) and all included (in μM): 20 DNQX, 100 picrotoxin, and 50 AP5. ACSF was saturated with 95% O₂-5% CO₂. The following were the pipette solutions (in mM): K-Meth = 125 Cs-methanesulfonate, 16 CsCl, 10 NaCl, 10 HEPES, 2.5 BAPTA, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris, 10 sucrose (pH 7.25, 290 mOsm); Cs-Meth = 125 Cs-methanesulfonate, 16 CsCl, 10 NaCl, 10 HEPES, 2.5 BAPTA, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris, 10 sucrose, 2 QX314-Cl, 10 TEA (pH 7.25, 290 mOsm); measured junction potentials were about 9 and 10 mV, respectively, in all the above external solutions (Neher 1992).

**Drugs**

For all membrane current measurements, fast synaptic transmission was blocked with the following: N-methyl-D-aspartate (NMDA) receptor antagonist AP5 (Sigma), the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPKA) kainate receptor antagonist DNQX (Sigma), and the γ-aminobutyric acid type A (GABA_ₐ) receptor antagonist picrotoxin (Sigma). Where stated, voltage-dependent sodium currents were blocked with tetrodotoxin (TTX; 1 μM, Sigma) and voltage-dependent potassium currents were blocked with tetraethylammonium chloride (TEA; 10 mM, Sigma) and 4-aminopyridine (4-AP; 5 mM, Sigma).

**Anatomical labeling**

Cells were filled with biocytin (Sigma) for 45 min using whole cell recording and subsequently placed in cold fixative. Slices were not resectioned because the cultures, in layer 2/3, had thinned to about 100–120 μm by the time of experimentation. After processing of tissue as described (Gibson et al. 1999), neurons were traced and projected into a single plane using Metavue software and, from this, total dendritic length, branch point number, and Scholl crosssections were measured (Jin et al. 2003; Scholl 1956). Fluorescent immunohistochemistry for somatostatin (#KO21, Santa Cruz Biotechnology, Santa Cruz, CA) was performed as previously described (Gibson et al. 1999) on fixed, unsectioned slice cultures in neocortical layers 2/3. The antibody did not penetrate >25 μm into the slice. Therefore we restricted our analysis to all focal planes above the deepest focal plane in which SOM+ could be clearly observed. GFP localization was performed with native EGFP signal.

**Analysis**

All statistics were performed using Statview (SAS Institute, 1998), and statistical significance was P < 0.05. Unless otherwise stated, we used an unpaired t-test or a one-way ANOVA followed by a multi-comparisons test (Fisher’s PLSD). Sample number (n) was cell number and was always given in the following order: (Control, TTX-treated, Before) or similar order if one group was omitted (i.e., Control, TTX-treated). All error bars are SE.

**RESULTS**

**Cellular properties of SOM+ neurons are normal in slice culture**

In slice culture, GFP was expressed almost exclusively in somatostatin-positive (SOM+) neurons: 88% (21/24) of all GFP neurons co-localized with somatostatin at ED 16 and 91% (20/22) at ED 21 (ED is “equivalent day”; see METHODS). This was consistent with immunohistochemistry in acutely prepared anatomical sections (Oliva et al. 2000). The electrophysiological profile of GFP+ neurons in culture was consistent with the SOM+ subtype previously described in acute slices. Resting potential was similar and input conductance was in the upper range (–57.4 ± 0.9 mV, 12.4 ± 0.9 nS, n = 25) (acute, –59 mV and 3.4 to 12.2 nS from Bacci et al. 2003; Cauli et al. 2000; Deans et al. 2001). With simultaneous whole cell recordings of neighboring GFP and non-GFP neurons, the GFP to non-GFP...
Activity blockade induces higher spontaneous activity

Activity blockade has commonly been used to assess the role of activity on circuit and neuronal properties. Chronic activity blockade (1–5 days) is known to increase the spontaneous activity of cultures when the blockade is removed (Corner and Ramakers 1992; Gorba et al. 1999; Seil and Drake-Baumann 1994; Turrigiano et al. 1998). To determine whether this was the case in our cultures, we applied TTX (2 µM) for 2.5 days starting at late ED 14. At ED 17, slices were transferred to the recording chamber and bathed in modified ACSF (in mM: 1.8 Mg\(^{2+}\), 1.8 Ca\(^{2+}\), 5 KCl; 32°C). Spontaneous action potential rate was indeed higher in TTX-treated slices (Fig. 1A) as observed in both burst and individual spike rates in SOM+ neurons (0.1 ± 0.1 vs. 10.6 ± 2.0 burst/min, \(P < 0.0008\); 0.6 ± 0.4 vs. 17.2 ± 2.5 spikes/min, \(P < 0.0007\); \(n = 5, 5\)). This spontaneous activity was ascribed to intermittent, highly synchronous, excitatory input from other neurons because it was blocked by the AMPA-receptor (AMPA-R) antagonist DNQX (20 µM). The large excitatory drive was clearly observed in voltage clamp.

The sparse activity in controls might be a concern because this would suggest that the effects of TTX are not specifically the result of blocking activity. To address this issue, we repeated this experiment using 2.5-day treatment with DNQX (20 µM) to chronically reduce activity. Recordings were performed in an ACSF in which [Mg\(^{2+}\)] more accurately reflected that in the culture medium (1.3 mM). Action potentials were observed extracellularly with cell-attached patch recordings. Again, both burst and spike rates in SOM+ neurons were increased with activity reduction (1.4 ± 0.4 vs. 3.7 ± 0.6 burst/min, \(P < 0.01\); 34 ± 14 vs. 179 ± 53 spikes/min, \(P < 0.05\); \(n = 5, 7\)). Clear spontaneous activity was observed in control slices that, as observed with simultaneous recordings of SOM+/non-GFP pairs, was highly synchronized among neighboring neurons (\(n = 3\)).

Increased excitability and decreased subthreshold conductance with activity blockade

Excitability was determined by plotting firing frequency as a function of injected current (\(F-I\) plot) and by measuring the minimum amount of current to evoke an action potential from rest (threshold current; see METHODS). SOM+ neurons experiencing chronic activity blockade were more excitable compared with same-age controls (\(n = 25, 30\)) because they fired more rapidly for a given amount of current injection (Fig. 2A). As observed in \(F-I\) plots, this was true over the entire range of injected current intensities (Fig. 2B). Threshold currents decreased by 49% with activity blockade (Fig. 2D). These changes are consistent with a homeostatic regulation of excitability.

TTX-treated neurons also had a 24% reduction in subthreshold input conductance (Fig. 2, C and D; 12.4 ± 0.9 vs. 9.4 ± 0.7 nS, \(P < 0.001\)) and they had a more depolarized resting potential (−57.4 ± 0.9 vs. −55.8 ± 0.7 mV, \(P < 0.03\)). Spike threshold voltage may have been slightly hyperpolarized, but this was not statistically significant (−40.8 ± 0.6 vs. −42.2 ± 0.4 mV; \(P = 0.053\); see METHODS). To control for a possible bias in our sampling, a subset of experiments were performed blind to the treatment history of the slices (\(n = 7, 11\)), and the same excitability and input conductance changes were observed (input conductance, \(P < 0.02\); \(F-I\) curve, \(P < 0.04\)). The same conductance decrease occurred when chronic activity reduction was performed with the AMPA-R antagonist DNQX (\(P < 0.001\); \(n = 7, 7\)). Assuming a simple ohmic process to achieve voltage threshold and using the numbers given above, the input conductance decrease would account for most of the decrease in threshold current compared with the depolarized resting potential (78 and 22%, respectively). The strong influence input conductance has on excitability is further supported by the positive correlation between input conductance and threshold current in both Control and TTX-treated cells (\(R = 0.69, P < 0.001\); \(R = 0.81, P < 0.001\); data set from Fig. 2B; Fisher’s r-to-z test, Statview). The same threshold current difference was observed when cells were held at −60 mV (\(P < 0.002\); \(n = 14, 13\)).

Is excitability decreased with activity-blockade or simply maintained at an earlier developmental stage? A subset of the above experiments included a before-treatment condition (\(n = 18, 19, 18\)). Threshold current required to evoke a spike was significantly decreased in TTX-treated compared with before-

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**FIG. 1.** Slice culture preparation is adequate studying homeostasis in somatostatin-positive (SOM+) neurons. A: while simultaneously recording from an excitatory and a SOM+ neuron, brief voltage steps were applied to the excitatory neuron to induce a train of presynaptic action potentials (top). Resulting excitatory postsynaptic potentials (EPSPs) in the SOM+ neuron (bottom) displayed strong facilitation just like that observed in acute slices. Scale bars: 25 mV (top), 0.5 mV (bottom), 100 ms. B: spontaneously generated action potentials (APs) were examined in single SOM+ neurons. AP rate was higher in tetrodotoxin (TTX)-treated cells as a result of intermittent, strong excitatory input. Scale (in A): 25 mV, 800 ms.
different (P < 0.02), suggesting an upregulation in excitability by activity blockade (Fig. 2E). On the other hand, except for the lowest current, the F-I plots were not different (Fig. 2F). Furthermore, no difference was observed for input conductance (P = 0.25). These data suggest that SOM+ neurons are still maturing in our experiments, and that activity blockade may partly involve a developmental arrest of some electrophysiological properties.

Although we focus on subthreshold currents, we briefly examined action potentials for clues to altered suprathreshold activity. An inward current activated at hyperpolarized potentials (Pape and Westbrook 1996; Robinson and Siegelbaum 2003) and the 

We first characterized the Cs+-insensitive component by applying voltage ramps (−20 to −100 mV, 3 s) to investigate currents as a function of voltage. Under these recording conditions, resting potential was clearly higher in TTX-treated SOM+ neurons (−55.5 ± 1.3 ms vs. −47.8 ± 1.1 mV). Activity blockade induced a clear change in current traces (Fig. 4A), which included a 43% decrease in the “standing-outward current” (measured at −20 mV, steady state; P < 0.0003). From these traces, we derived the net current that accounted for differences induced by activity blockade, which we call the “activity-dependent current.” The average TTX-treated trace...
was subtracted from the average control trace to attain a difference trace, and subsequently the difference trace was transformed into a current versus voltage ($I-V$) plot (Fig. 4C). This derived activity-dependent current represented a 25% decrease in input conductance (based on slope at −60 mV), which indicated that $I_{\text{Leak}}$ played a large role in the activity-dependent conductance alterations described above (see DISCUSSION for details). Even though this $I_{\text{Leak}}$ was not different from a predicted passive potassium leak current as calculated from the GHK equation (see METHODS), it had a reversal potential ($E_R$) 20 mV depolarized to that predicted for a pure K$^+$ current ($E_K = -120$ mV). The $E_R$ for $I_{\text{Leak}}$ may explain the more depolarized resting potential of TTX-treated SOM$^+$ neurons in normal ACSF.

To determine whether activity blockade induces a simple, isolated downregulation of $I_{\text{Leak}}$, we also made comparisons with cells before treatment. There was a clear developmental component in the experimental time window in which we observe these activity-dependent alterations (Fig. 4C). While the $I_{\text{Leak}}$ change was an isolated downregulation, we would expect the before and control traces to be identical and their difference trace to be a horizontal line at 0, but this was not the case. Instead, a difference trace (Control − Before) revealed a developmentally regulated current of unclear identity. In addition, there appears to be an activity-independent alteration of a DC, nonohmic current as indicated by the Before − TTX difference trace. In two other experiments, this nonohmic component contributed an even higher proportion of the Before − TTX difference trace (data not shown). The identity of this nonohmic current is unknown, but at the very least, these data indicate that activity-dependent alterations in $I_{\text{Leak}}$ are superimposed on other developmental changes in intrinsic membrane currents.

$I_{\text{Leak}}$ at least partly mediated by potassium

In a subset of experiments in Fig. 4, we subsequently altered extracellular [K$^+$] to 10 mM to see whether the activity-dependent current would shift as predicted for a K$^+$-mediated current. The $I-V$ plot shifted in the depolarizing direction as predicted (Fig. 5A; $P < 0.002$, $n = 9, 8$), but the shifted current was not identical to that predicted for K$^+$ involvement ($P < 0.03$). The $E_R$ shift was only 35% of the predicted shift. These data suggest that $I_{\text{Leak}}$ is partly mediated by K$^+$.

We next examined a role for Cl$^-$. Experiments were identical except the pipette solution was altered by substituting 26 mM K-Meth with equimolar KCl for a total of 35 mM [Cl$^-$]. If Cl$^-$ contributed to $I_{\text{Leak}}$, we would expect a distinct depolarizing shift in the reversal potential, although this was not the case (Fig. 5B). The activity-dependent current was no different from that predicted for a passive K$^+$ current ($P = 0.82$) but different from a predicted passive Cl$^-$ current ($P < 0.0001$, $n = 8, 8$). On washing in 10 mM K$^+$, the difference trace shifted to a similar extent as observed in Fig. 5A. The $E_R$ shift was 57% of that predicted for the K$^+$-mediated current, a higher contribution than observed above, which was probably a result of high cell-to-cell variability. Therefore Cl$^-$ played a minimal role.

Even though the $I-V$ plot shifts with extracellular [K$^+$], alterations indicate that K$^+$ is only partly involved; other characteristics of $I_{\text{Leak}}$ suggest that K$^+$ is the main ion: lack of any contribution by Cl$^-$, reversal near $E_K$, and slight outward rectification. Perhaps attempts to alter extracellular [K$^+$] are obstructed by buffering in our cultured slice preparation as is observed for pH in acute slices (Chesler et al. 1994; Voipio and Kaila 1993; see DISCUSSION). Some members of the two-pore (2P)–domain family of potassium channels have been demonstrated to mediate $I_{\text{Leak}}$ in various cell types (Goldstein et al. 2001; Lesage 2003). We wanted to determine whether the activity-dependent $I_{\text{Leak}}$ displayed characteristics of two members of this family whose existence and function has been demonstrated in CNS neurons: TASK1 and TASK3 (Brickley et al. 2001; Meuth et al. 2003; Talley et al. 2000; Washburn et al. 2002).
Acidification has been shown to block TASK channels (Brickley et al. 2001; Meuth et al. 2003; Talley et al. 2000). Acidification (pH 7.8–6.2) significantly changed ramp-induced currents obtained from both control and TTX-treated SOM+ neurons (P < 0.0001, P < 0.03, n = 15, 17). Acidification modulated more than one type of ionic current, and this was evident in our pH 7.8 – pH 6.2 difference currents, which had E_r values of −60 and −38 mV for Control and TTX, respectively. In spite of this apparent nonspecificity, acidification still clearly altered activity-dependent I_{leak} (P < 0.0001) and reduced its standing outward current by 56% (Fig. 5C; P < 0.0002, n = 15, 17).

One mM Ba^{2+} blocks 80% of TASK1-mediated current (Han et al. 2002; Talley et al. 2000). Barium (1 mM) clearly altered ramp currents in both control and TTX-treated SOM+ neurons (P < 0.0001, P < 0.0001; n = 19, 19). Unlike acidification, “no Ba^{2+} – Ba^{2+}” subtraction traces demonstrated a specific block of a potassium leak current. Upon Ba^{2+} application, we observed a 60% reduction in the activity-dependent standing outward current (P < 0.0007, Fig. 5D). In summary, both acidification and Ba^{2+} block suggest a large contribution of K^{+} to I_{leak}.

Decreased H-current (I_{H}) with activity blockade

Based on initial voltage-ramp experiments suggesting that a Cs^{+}-sensitive current, reminiscent of I_{H}, was activity-dependent (data not shown), we examined regulation of I_{H}. Cortical SOM+ neurons are known to display I_{H} (Lupica et al. 2001; Maccaferri and McBain 1996). We first established that I_{H} existed in our cultured SOM+ neurons. Hyperpolarizing steps down to < −60 mV elicited a clear, slowly activating inward current (Fig. 6A). The slowly activating current was blocked by I_{H} blockers (93 ± 3% reduction by 2.5 mM CsCl and 95 ± 1% by 100 μM ZD7288; n = 11, 10; Fig. 6A) (Kilb and Luhmann 2000; Maccaferri and McBain 1996). When traces collected under blocked conditions were subtracted from those under control conditions, only the slowly activating inward current, characteristic of I_{H}, was isolated (Fig. 6B). Based on tail currents, the I_{H} reversal was similar to that reported previously (−28 ± 6 mV; n = 3).

In the same data set, we found that I_{H} was reduced with TTX treatment. This reduction was similar using both the local maximum measurement (64%; n = 9, 12, P < 0.02; Fig. 7A; see METHODS) and subtraction traces (60%, P < 0.03; obtained from blocker washins as described above). Because the local maximum method appeared adequate for measuring I_{H} alterations, it was thus always used.

Time constants for I_{H} activation were no different when obtained from local maximum and subtraction methods (P values of 0.20 and 0.90; 311 ± 21 and 2,774 ± 124 ms, from subtraction traces), and these time constants were similar to those observed in hippocampal SOM+ neurons in acute slices.

FIG. 5. Activity-dependent I_{leak} is at least partly mediated by a potassium conductance. All figures are I–V plots derived from voltage ramps of 3, 2, 1.5, and 2 s in A–D, respectively. A: an incomplete shift of I_{leak} when external [K^{+}] was increased indicates a partial contribution by K^{+}. Inset: traces are normalized to eliminate correlated error in both [K^{+}] conditions. This scaling causes highly variable behavior at midrange because of variability of zero crossings between [K^{+}]_leak conditions and between cells. B: with 35 mM [Cl^{-}] in the pipette, no alterations in I_{leak} properties were observed. C and D: I_{leak} is considerably reduced with acidification (pH 6.2) or 1 mM Ba^{2+}. Insets, C and D: normalized plots as described in A. In all panels, gray lines are predicted I–V plots for passive K^{+} and Cl^{-} conductances derived from the Goldman–Hodgkin–Katz equation. These prediction plots may be obscured by data. C and D performed in 3 and 1 mM [K^{+}]_leak, respectively.
However, it was not possible to determine whether kinetics were altered in TTX-treated SOM/H11001 neurons because $I_{H}$ was too small for an accurate measurement. We repeated measurements of $I_{H}$ under conditions that made both activation kinetics and amplitude more easily measured ($n$/H11005 13, 21; $-40$-mV step from $-60$-mV holding, normal ACSF, 32°C; see METHODS). We found a slight increase in the fast component (Fig. 7A) and no change in the slow component of activation with activity blockade. Amplitude was again decreased with activity blockade (45% drop), and this result was reproduced both with the experimenter blind to the groups ($P$/H11021 0.004, $n$/H11005 6, 11) and with 2.5-day treatment with the AMPA-R antagonist DNQX ($P$/H11021 0.003, $n$/H11005 7, 7). In summary, $I_{H}$ kinetics were altered relatively little, whereas amplitude was clearly decreased with activity blockade.

Differences in voltage-dependent activation could explain the decrease in $I_{H}$ (Chen et al. 2001). An accurate activation curve examining tail currents could not be obtained because we could neither get a maximum tail current nor repeatedly hyperpolarize cells to achieve this maximum (see METHODS). The former may possibly be explained by the poor voltage clamp of dendrites. Therefore a rough estimate of steady-state, voltage-dependent activation was obtained by measuring the percentage reduction in $I_{H}$ with different prestep potentials (see METHODS). This reduction represented the percentage of $I_{H}$ activated during the prestep. No change in voltage-dependent activation with activity blockade was observed even though $I_{H}$ magnitude was significantly decreased ($I_{H}$: $P$/H11021 < 0.0002; $n$/H11005 11, 11). C: $I_{H}$ amplitude is downregulated with activity blockade, but there also appears to be an age-dependent increase in $I_{H}$ [$n$/H11005 18, 15, 13; $-40$-mV step from $-60$-mV holding, Basic Block artificial cerebrospinal fluid (ACSF), 22°C]. $P$/H11021 < 0.02.

To determine whether the $I_{H}$ decrement was a result of either a developmental arrest or an active downregulation, we compared $I_{H}$ amplitude between control, TTX-treated, and before-treatment conditions (Fig. 7C). Although there was a clear $I_{H}$ reduction in TTX-treated neurons compared with either control or before groups, an age-dependent increase was also apparent. Thus there was a downregulation of $I_{H}$ superimposed on a maturational increase of $I_{H}$.

$I_{H}$ modulation of resting potential and excitability is altered with activity blockade

If $I_{H}$ were active at resting potential, it could play a critical role in controlling resting potential and excitability (Shah et al. 2004). Although our activation data suggest a minimal contribution at resting potential ($-55$ and $-60$ mV; see Fig. 7B), there may still be a significant $I_{H}$ contribution at these poten-
these data suggest decreased by a greater fraction in control neurons (cells. Using the reversal potential derived above, explain the slightly higher resting potential in TTX-treated polarization, indicating a significant contribution of contents of cells (series resistance high-resistance electrodes to better preserve the cytoplasmic that was not detected. We examined the effect of ZD7288 on resting potential and excitability in both control and TTX-treated SOM+ neurons. Recordings were performed with high-resistance electrodes to better preserve the cytoplasmic contents of cells (series resistance = 21.6 ± 1.1 MΩ; 22°C, 20 μM DNQX, 50 μM AP5).

In control SOM+ neurons, ZD7288 induced a 3-mV hyperpolarization, indicating a significant contribution of $I_{H}$ at resting potential (Fig. 8, A and B), which is consistent with previous studies (Lupica et al. 2001; Maccaferri and McBain 1996). To our surprise, no detectable change occurred in TTX-treated cells. Based on our $I_{H}$ measurements above, we expected a hyperpolarization nearly 40% of that observed for controls. Either $I_{H}$ was too low to be detected using ZD7288 or, contrary to our results above, there was a voltage-dependent activation shift in $I_{H}$. Based on the average induced hyperpolarization, input conductance, and membrane capacitance, the $I_{H}$ reduction at rest in TTX-treated SOM+ neurons was about 2 pA/pF, which closely matched the reduction in $I_{\text{leak}}$, about 3 pA/pF (Figs. 4 and 5). Because their reversal potentials are approximately equidistant from rest (about −95 and −28 mV), these data suggest $I_{\text{leak}}$ and $I_{H}$ changes offset each other to preserve resting potential. The greater reduction in $I_{\text{leak}}$ may explain the slightly higher resting potential in TTX-treated cells. Using the reversal potential derived above, $I_{H}$ at rest contributed 0.83 nS to total membrane conductance (10%).

We found that decreases in $I_{H}$ increased excitability in SOM+ neurons, as previously reported for pyramidal neurons (Shah et al. 2004). In control SOM+ neurons, ZD7288 washin caused a 14% decrease in threshold current, whereas no detectable change was observed with TTX-treated neurons (Fig. 8C). When excitability from a slightly more hyperpolarized potential of −70 mV was examined (Fig. 8D), the effects of ZD7288 were more dramatic, as expected for a hyperpolarization-activated current. Both control and TTX-treated SOM+ neurons became more excitable, but the decrease in threshold current was proportionately bigger in control neurons (36 vs. 22%, $P < 0.01$), consistent with their larger $I_{H}$. The greater increase in excitability in control neurons is consistent with the assertion that a decrease in $I_{H}$ with activity blockade promotes an excitability increase.

No alteration in voltage-dependent sodium current

In excitatory neurons, upregulation of voltage-dependent Na$^+$ current is involved in the activity-dependent regulation of excitability (Desai et al. 1999b). To determine whether this is true for SOM+ neurons, we first measured Na$^+$ current amplitude with incremental voltage steps (Fig. 9A). These currents had both the voltage- and time-dependent characteristics of voltage-dependent Na$^+$ current (Desai et al. 1999b; Hamill et al. 1991; Huguenard et al. 1988) and were completely blocked by its specific blocker, TTX (1 μM, $n = 3$). We compared peak current at steps up to +10 mV between TTX-treated and
control SOM+ neurons and found no difference (Fig. 9B). Furthermore, there was no difference in the voltage dependence of activation (Fig. 9C).

Similar activity-dependent regulation in more mature SOM+ neurons

At ED 17, regulation of subthreshold currents in SOM+ neurons co-occurs with developmental changes in these currents. We wanted to determine whether this regulation was strictly an early developmental phenomenon by examining the same issue in older cultures (ED 25).

Again, we found a clear increase in excitability with activity blockade when examining both F–I curves (Fig. 10A) and threshold current (45% reduction, Fig. 10B). Again, input conductance and $I_{\text{leak}}$ decreased (24 and 44% reduction; Fig. 10, B and C). Unlike the earlier culture age, there were no detectable age-dependent alterations, although this is tempered by no observed difference between Before and TTX groups in the high end of the F–I curves. Statistically significant activity-dependent $I_{\text{leak}}$ was obtained for both Control − TTX and Before − TTX difference currents (Fig. 10D; $P < 0.0001$, $P < 0.03$), and both were not different from a predicted passive potassium current, although again $E_R$ was about 23 mV depolarized to this prediction. The contribution of $I_{\text{leak}}$ to excitability alterations was somewhat diminished compared with earlier ages, as observed in only a 21% reduction in standing outward current and a 11% conductance reduction (based on slope at −60 mV; Control vs. TTX-treated). Overall, a similar process appears to occur at later ages except with maturational processes mostly absent and a decreased contribution of $I_{\text{leak}}$ to conductance alterations.

DISCUSSION

Taken together with earlier reports, this study provides strong evidence that both neocortical excitatory and inhibitory neurons homeostatically regulate their average firing rate by membrane excitability in response to long-term changes in activity levels (Desai et al. 1999a,b). Our data indicate that subthreshold currents play the most significant role in this homeostatic process. We found a clear downregulation of a putative passive leak current ($I_{\text{leak}}$) and of $I_H$ with activity blockade. This was accompanied by a decrease in membrane conductance, a slight increase in resting potential, and—ultimately—increased membrane excitability. The decrease in conductance probably contributed most to the increased excitability.

To our knowledge, the activity-dependent regulation of excitability through an $I_{\text{leak}}$ has not been previously demonstrated. Cerebellar granule cells have been shown to upregulate a specific K+ -mediated $I_{\text{leak}}$, TASK1, to compensate for the genetic deletion of a tonic, GABAergic hyperpolarizing current (Bickley et al. 2001), but this finding is confounded both by developmental effects and the length of time over which the compensation occurred.

$I_H$ appears to be regulated in a number of plastic processes such as epilepsy (Chen et al. 2001; Shah et al. 2004), maintenance of invertebrate oscillatory circuitry (MacLean et al. 2003; Thoby-Brisson and Simmers 2002), and acute homeostatic regulation of excitability (Fan et al. 2005; van Welie et al. 2004). Here, we demonstrate that long-term regulation of $I_H$ occurs in more benign circumstances in mammalian neurons.

Our data cannot resolve whether the activity-dependent alterations in $I_{\text{leak}}$ and $I_H$ arise from changes in channel number or function. Most studies have shown that activity-dependent changes in both currents are at least partially mediated by protein expression (Brewster et al. 2002; Brickley et al. 2001; Fan et al. 2005; Shah et al. 2004). On the other hand, one study has demonstrated a shift in the voltage-dependent activation of $I_H$ in the epileptic rat (Chen et al. 2001).

Our interpretations have two notable limitations. First, although our experiments were intended to model processes in vivo, the complete suppression of action potentials used here is unlikely to occur in vivo. We used TTX to induce extreme, observable alterations in cellular properties that we subsequently interpreted based on a homeostatic model. Previous studies have shown that findings using this strategy can be reproduced with sensory deprivation in vivo (Desai et al. 2002; Maffei et al. 2004). Second, we could not determine whether alterations were occurring in the soma or dendrites. Furthermore, if changes occurred in the dendrites, measurements would not have been precise as a result of voltage-clamp error. Because we found no change in cell size or morphology, however, it is likely that this error was similar in the different treatment groups, and therefore our recordings were adequate for making comparisons.
The role of $I_{\text{Leak}}$ and $I_{\text{H}}$ in membrane conductance alterations

How much of the activity-dependent input conductance decrease (24%) in our young SOM+ neurons is contributed by $I_{\text{Leak}}$? This is difficult to measure because of the lack of a specific antagonist for the current. Furthermore, experimental conditions were very different when measuring overall input conductance versus the voltage ramps measuring $I_{\text{Leak}}$. Here, we make an approximate calculation. The average input conductance of control cells from all ramp experiments (Figs. 4 and 5) was 6.6 nS, and in these same experiments, the average decrease in input conductance with activity blockade was 20%. By virtue of the $I–V$ plots (Figs. 4 and 5), this difference was purely $I_{\text{Leak}}$. To scale for temperature differences between experiments, a $Q_{10}$ based on ion diffusion was applied (1.4; Hille 2001) and resulted in a modified 9.1-nS conductance in control cells (75% of the 12.4 nS reported in excitability experiments; Fig. 2D). These data suggest that $I_{\text{Leak}}$ accounted for nearly 63% \([0.2/0.24] \times 0.75\) of the subthreshold conductance decrease induced by activity blockade.

How much of the activity-dependent input conductance decrease was contributed by $I_{\text{H}}$? In our ZD7288 experiments, we estimated that $I_{\text{H}}$ contributed roughly 10% of the total conductance at rest in control neurons and that this was totally absent with TTX treatment. This would suggest that $I_{\text{H}}$ accounts for 42% \((0.10/0.24)\) of the conductance decrease. These approximate calculations for $I_{\text{Leak}}$ and $I_{\text{H}}$ above fully account for the conductance decrement observed with activity blockade.

The role of $I_{\text{Leak}}$ and $I_{\text{H}}$ in excitability alterations

We argue that the decrease in $I_{\text{Leak}}$ and $I_{\text{H}}$ with activity blockade results in an increase in excitability. The best evidence for this would be to block both currents and eliminate the difference in excitability. Unfortunately, we did not have a specific blocker for $I_{\text{Leak}}$. We did demonstrate that excitability differences were decreased with a specific blocker for $I_{\text{H}}$ (Fig. 8, C and D). Therefore we rely on this latter data and on the strong relationship between input conductance and excitability to support our assertion.

Regarding $I_{\text{H}}$, our data are consistent with studies asserting more $I_{\text{H}}$ decreases excitability (Shah et al. 2004; van Welie et al. 2004). We believe this is attributable to the proximity of the $I_{\text{H}}$ reversal potential \((-28 \pm 6 \text{ mV}, n = 3; \text{K-Meth pipette solution})\) to threshold potential \((-41 \pm 1 \text{ mV}, n = 25\)). This relationship means that $I_{\text{H}}$ contributes more shunting conductance to prevent depolarization than actually providing direct depolarization when membrane potential approaches spike threshold. This is substantiated by an analytical calculation of passive depolarizations in a membrane model using batteries and conductances (data not shown) (Koester and Siegelbaum 2000). We also believe there are circumstances where $I_{\text{H}}$ increases excitability (Chen et al. 2001) and the nature of the $I_{\text{H}}$ influence on excitability is context dependent.

The 49% decrease in threshold current compared with the 24% decrease in input conductance may appear inconsistent with the assertion that the downregulation of $I_{\text{Leak}}$ and $I_{\text{H}}$ mediate the excitability decrease (Fig. 2). If voltage threshold for spike generation and resting potential remained the same, then according to Ohm’s law, the percentage change in threshold current and input conductance should be equal.

With this in mind, the input conductance change can account for nearly 50% of the change in threshold current, although three additional factors suggest this percentage could be higher. First, we have already estimated that the conductance decrement and the resting potential increase would account for 78 and 22% of the threshold current change, respectively. This calculation accounts for 65% of the threshold decrement. In addition, we applied current steps in larger increments in control SOM+ neurons for faster data collection (100 vs. 50 pA). Therefore because of sampling error, our control values may be biased by an added 25 pA, which would mean the threshold current decrease is actually roughly 40%. This would increment the accounting to 80%. Third, a decreased membrane conductance decreases the amount of depolarizing current needed to evoke an action potential by virtue of a decreased threshold voltage (Noble 1966), but although our data suggested this to be true, it was not statistically significant (see results).

In summary, we can account for most of the discrepancy between threshold current and input conductance changes. This suggests that the major mechanism for excitability alterations in SOM+ neurons is centered in subthreshold current modifications. Alterations in voltage-dependent currents activated near spike threshold may explain the remaining discrepancy. The same arguments hold for data from older slices.

Coregulation of $I_{\text{Leak}}$ and $I_{\text{H}}$

Coregulation of $I_{\text{H}}$ and $I_{\text{Leak}}$ may provide a mechanism for regulating input conductance while preventing extreme membrane potential changes produced by regulation of either current alone. An activity-independent coregulation of $I_{\text{H}}$ and $I_A$ occurs in lobster pyloric dilator neurons to maintain circuit oscillatory behavior (MacLean et al. 2003), although to our knowledge, no activity-dependent coregulation of $I_{\text{H}}$ and a potassium current has been reported in mammals. In this study, the activity-dependent change in $I_{\text{H}}$ at resting potential closely matched the current provided by $I_{\text{Leak}}$, strongly suggesting these offsetting currents were critical for maintaining constant resting potential. A homeostatic regulation of resting potential may be important for the proper baseline activation of voltage-dependent currents.

Ionic identity of $I_{\text{Leak}}$

Extracellular alteration of $[K^+]$ induced an $I–V$ plot shift in the activity-dependent $I_{\text{Leak}}$ consistent with a nearly 40% contribution by $K^+$. However, the hyperpolarized reversal potential of $I_{\text{Leak}}$, its block by known $K^+$ channel blockers, and the absence of a $Cl^-$ contribution suggest the $K^+$ contribution may be higher. Both $Ca^{2+}$ and $Na^+$ are unlikely contributors because $Ca^{2+}$-channel blockers were used and the $-95$-mV reversal potential in the $I–V$ plots is very distant from that of $Ca^{2+}$ and $Na^+$. We speculate the less-than-expected shift in $I–V$ plots with $[K^+]_{\text{extr}}$ alterations may reflect buffering occurring within the slice like that observed for pH in acute slices (Chesler et al. 1994; Voipio and Kaila 1993). Such a buffering effect may be more pronounced in cultured slices because an astrocytic layer develops on the slice surface (Stoppini et al. 1991).
Implications for synaptic integration

Decreases in input conductance in response to activity blockade would increase the amplitude of postsynaptic potentials and their temporal summation (Johnston and Wu 1995), which in turn would homeostatically promote action potential firing. Interestingly, increased $I_h$ decreases excitatory postsynaptic potential size and temporal summation in hippocampal pyramidal neuron dendrites (Magee 1998). A similar process may occur in SOM+ neurons, although it is not known how much $I_h$ exists in SOM+ neuron dendrites. In the same long-term blockade paradigm used here, excitatory neuron homeostasis has been reported not to involve subthreshold alterations, and thus subthreshold synaptic integration would remain unchanged (Desai et al. 1999b). This suggests that transformation of synaptic input to action potential output may be differentially regulated in these two cell types.

Homeostatic regulation of inhibitory circuitry

A previous study demonstrated that inhibitory neurons become more excitable with activity blockade (Desai et al. 1999a). We think it likely these neurons were parvalbumin-positive neurons based on the electrophysiological criteria used and on the numbers of these neurons (roughly 50% of all inhibitory neurons) (Cauli et al. 2000; Kawaguchi and Kubota 1997). If this were the case, both parvalbumin-positive and SOM+ neurons regulate their activity levels consistent with a homeostatic process. From the excitatory neuron perspective, this regulation in inhibitory neurons would be nonhomeostatic because more inhibition is provided with activity blockade. Studies indicate that inhibitory synapses are homeostatically regulated (Kilman et al. 2002; Maffei et al. 2004) and may therefore compensate for the increased excitability in inhibitory neurons. Future studies examining the activity-dependent regulation of SOM+ inhibitory synapses targeting excitatory neurons are needed to better understand how total inhibitory drive originating from SOM+ neurons is regulated.

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