Functional Roles of Kv1 Channels in Neocortical Pyramidal Neurons

D. Guan, J. C. F. Lee, M. H. Higgs, W. J. Spain, and R. C. Foehring

Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Tennessee; and Departments of Neurology and Physiology and Biophysics, University of Washington, Veterans Administration Puget Sound Health Care System, Seattle Washington

Submitted 1 September 2006; accepted in final form 9 January 2007

Guan D, Lee JCF, Higgs MH, Spain WJ, Foehring RC. Functional roles of Kv1 channels in neocortical pyramidal neurons. J Neurophysiol 97: 1931–1940, 2007. First published January 10, 2007; doi:10.1152/jn.00933.2006. Pyramidal neurons from layers II/III of somatosensory and motor cortex express multiple Kv1 α-subunits and a current sensitive to block by α-dendrotoxin (α-DTX). We examined functional roles of native Kv1 channels in these cells using current-clamp recordings in brain slices and current- and voltage-clamp recordings in dissociated cells. α-DTX caused a significant negative shift in voltage threshold for action potentials (APs) and reduced rheobase. Correspondingly, a ramp-voltage protocol revealed that the α-DTX–sensitive current activated at subthreshold voltages. AP width at threshold increased with successive APs during repetitive firing. The steady-state threshold width for a given firing rate was similar in control and α-DTX, despite an initially broader AP in α-DTX. AP voltage threshold increased similarly during a train of spikes under control conditions and in the presence of α-DTX. α-DTX had no effect on input resistance or resting membrane potential and modest effects on the amplitude or width of a single AP. Accordingly, experiments using AP waveforms (APWs) as voltage protocols revealed that α-DTX–sensitive current peaked late during the AP repolarization phase. Application of α-DTX increased the rate of firing to intracellular current injection and increased gain (multiplicative effects), but did not alter spike-frequency adaptation. Consistent with these findings, voltage-clamp experiments revealed that the proportion of outward current sensitive to α-DTX was highest during the interval between two APWs, reflecting slow deactivation kinetics at −50 mV. Finally, α-DTX did not alter the selectivity of pyramidal neurons for DC versus time-varying stimuli.

INTRODUCTION

Slowly inactivating, voltage-gated K⁺ current in neurons could be attributable to expression of several channel types, including the Kv1, Kv2, Kv3, and Kv7 families. How is this potential molecular diversity used by neurons to regulate excitability? What are the functional consequences of expression of these different types of channels? In this study, we investigated functional roles of channels containing Kv1 α-subunits in supragranular pyramidal cells from somatosensory and motor cortex of rats.

Previously (Guan et al. 2006), we used single-cell RT-PCR, immunocytochemistry, and whole cell recordings with specific peptide toxins to show that individual pyramidal cells express multiple Kv1 α-subunits, which were localized both to somatodendritic and to axonal cell compartments. All cells expressed a current sensitive to α-dendrotoxin (α-DTX; blocks channels containing Kv1.1, Kv1.2, or Kv1.6 α-subunits; Harvey and Robertson 2004). Previously, similar currents were observed in many cell types (Coetzee et al. 1999), including layer V pyramidal neurons (Bekkers 2000a,b, Bekkers and Delaney 2001; Kornfeld and Sakmann 2000) and mixed populations of neocortical pyramidal cells (Dong et al. 2003; Foehring and Surmeier 1993; Locke and Nerbonne 1997; Zhou and Hablitz 1996). Very similar biophysical properties were reported for α-DTX–sensitive currents in on-cell or nucleated patch recordings from neocortical pyramidal cells in acute brain slices (Bekkers and Delaney 2001) and whole cell recordings from acutely dissociated pyramidal neurons (Guan et al. 2006).

Because Kv1 channels activate in the subthreshold voltage range in many cell types (e.g., Bekkers and Delaney 2001; Dodson et al. 2002; Shen et al. 2004; Slee et al. 2005), they are likely to play an important role in regulating cell excitability. In our previous study of pyramidal neurons, we found that the α-DTX–sensitive current activated more rapidly and at more negative potentials than the α-DTX–insensitive current and was first observed at voltages near action potential (AP) threshold (Guan et al. 2006). All cells expressed an α-DTX–sensitive current with slow inactivation kinetics. A more rapidly inactivating component was variably expressed, reflecting heterogeneity of Kv1 subunit composition (Guan et al. 2006). Deactivation kinetics was voltage dependent, such that deactivation was slow (τ of 22–46 ms between −50 and −30 mV) at potentials similar to those traversed by interspike intervals (ISIs) during repetitive firing.

Because of its kinetics and voltage dependency, we predicted that the α-DTX–sensitive current would have minimal effects on resting membrane potential, input resistance, or AP amplitude or time course. In addition, the α-DTX–sensitive current is an important regulator of AP threshold and ISIs during repetitive firing in layer V pyramidal cells (Bekkers and Delaney 2001). We examined whether this was also the case for layer II/III cells. Further, to test how Kv1 channels influence firing rate, we used realistic action potential waveforms (APWs) to determine the percentage of outward current arising from Kv1 channels at various times during APs and ISIs. We also predicted that the α-DTX–sensitive current in pyramidal cells would have a role similar to that of Kv1 channels in auditory brain stem cells, where the main role of α-DTX–sensitive current is to make these cells responsive to time-varying inputs while filtering out DC inputs; i.e., these cells differentiate their synaptic inputs (Brew and Forsythe 1995; Kopp-Scheinpflug et al. 2003; Slee et al. 2005).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Address for reprint requests and other correspondence: R. C. Foehring, Department of Anatomy and Neurobiology, University of Tennessee, 855 Monroe Avenue, Memphis, TN 38163 (E-mail: rfoehrin@utmem.edu).
In the current study, we tested these hypotheses by examining the effects of α-DTX on the excitability of neocortical pyramidal cells in both acutely dissociated and acute slice preparations. We found that these channels activated in the subthreshold voltage range and contributed to rheobase (minimum current to elicit an AP with current injection of >500 ms) and the voltage threshold for action potentials. They also contributed a major portion of outward current during ISIs and regulated firing rate in current clamp. The α-DTX–sensitive current had modest effects on the amplitude and time course of single APs. α-DTX did not alter spike-frequency adaptation or selectivity to DC versus time-varying inputs.

Methods

These studies were performed on juvenile rats [Sprague-Dawley; for dissociated cells and microelectrode slice recordings: postnatal day (P) 28–P35; for whole cell slice P16–P20]. All procedures were approved by the Animal Care and Use Committee, University of Tennessee, Health Science Center. The animals were anesthetized with isoflurane until the animal was areflexive. Briefly, the animal was placed into a sealed plastic container into which gauze soaked with isoflurane was placed under a fiberglass screen floor. After anesthesia with isoflurane, the animal was decapitated and the brain was removed and dropped into ice-cold cutting solution for 30–60 s. This solution contained (in mM): 250 sucrose, 25 KCl, 1 NaH2PO4, 11 glucose, 4 MgSO4, 0.1 CaCl2, and 15 HEPES (pH = 7.3–7.4; 300 mOsm/L). The brain was then sliced into 300-μm-thick (slice recordings) or 400-μm-thick (dissociated cells) coronal sections using a vibrating tissue slicer (Vibroslice, Campden Instruments).

Slice recordings

For acute slice recording, slices were placed in a recording chamber on the stage of an Olympus BX50WI upright microscope. Slices were bathed in carbogenated artificial cerebrospinal fluid (aCSF) delivered at 2 mL/min and heated with an in-line heater (Warner Instruments, Hamden, CT) to 31–34°C. Pharmacological agents were prepared as a concentrated stock in double-distilled H2O and then thawed and further treatment to the supragranular layers (I–III). Two to three preparations. We found that these channels activated in the subthreshold voltage range and contributed to rheobase (minimum current to elicit an AP with current injection of >500 ms) and the voltage threshold for action potentials. They also contributed a major portion of outward current during ISIs and regulated firing rate in current clamp. The α-DTX–sensitive current had modest effects on the amplitude and time course of single APs. α-DTX did not alter spike-frequency adaptation or selectivity to DC versus time-varying inputs.

Microelectrode recordings

Several recordings were made with sharp microelectrodes pulled to resistances of 70–100 MΩ and filled with 3 M KCl. These recordings (33–35°C) used an Axoclamp 2A amplifier (Axon Instruments) and were made by running blind electrode tracks. We examined frequency–current (f–I) relations for these cells in response to DC current steps (4-s duration) and DC steps plus exponential-filtered Gaussian noise (τ = 3 ms, SD adjusted to elicit 4-mV voltage SD when applied with zero DC offset). The recovery period between steps was ±11 s. Data were filtered at 10 kHz and sampled at 20 kHz using an LTC-16 data acquisition board (InstruTECH, Port Washington, New York) connected to a Macintosh computer. Current commands and data acquisition were controlled by custom macros written in Igor Pro (by M. Higgs) and external operations written in C (kindly provided by Dr. Fred Rieke).

Data from microelectrode recordings were analyzed using custom macros written in Igor Pro. The effects of added noise on the f–I relation were separated into additive and multiplicative/divisive components by fitting the f–I relation for noisy current steps with a copy of the control (noiseless) f–I relation, f0(l), that was shifted vertically by an additive factor A along the firing-rate axis and multiplied by a gain factor 1 + ΔG

f0(l) = A(l) + ΔG

The effects of α-DTX on the f–I relation (with or without noise) were separated into a shift along the current axis and a multiplicative effect by fitting the f–I relation obtained in the presence of α-DTX with a copy of the control f–I relation, f0(l), that was shifted along the current axis by Δl and multiplied by a gain factor 1 + ΔG

fDTX(l) = (1 + ΔG)f0(l − Δl)

Acute dissociations

Neurons were isolated according to previously published methods (Lorenzo and Foehring 1995). The primary somatosensory and primary motor cortices were dissected from the slices and then transferred to a mesh surface in a chamber containing aCSF at 32°C for 1 h. We used the same aCSF described earlier for brain slices. Slices were then transferred to a holding chamber at room temperature (RT) (aCSF, 1–12 h) until dissociated.

Individual sections from combined primary motor and primary somatosensory cortices were dissected from brain slices under a stereomicroscope using backlighting. The cortex was cut to restrict further treatment to the supragranular layers (I–III). Two to three cortex pieces at a time were transferred to oxygenated aCSF (35°C) with added enzyme (Sigma Protease type XIV, 1.2 mg/mL; Sigma Chemicals, St. Louis, MO). After 15–30 min of incubation in enzyme, the tissue was washed with sodium isethionate solution, which consisted of (in mM): 140 Na isethionate, 2 KCl, 4 MgCl2, 25 glucose, and 15 N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), 0.1 CaCl2, pH = 7.3 (adjusted with 1 N NaOH).

This solution was triturated using three successively smaller fire-polished pipettes to release individual neuronal somata. Supernatant from each trituration step (containing dissociated neurons) was transferred to a fresh container, plated onto a plastic petri dish (Nunc, Rochester, NY) on an inverted microscope stage, and allowed to settle for about 5 min. A background flow of about 1 mL/min of HEPES-buffered saline solution (HBSS) was then established. HBSS consisted of (in mM): 138 NaCl, 3 KCl, 2 MgCl2, 10 HEPES, 2 adenosine 5'-triphosphate (ATP), 0.2 guanosine 5'-triphosphate (GTP). Unless otherwise specified, 100 μM EGTA was added to the intracellular solution. Data were collected only from cells forming a 1-GΩ or tighter seal. Data were corrected for the measured liquid junction potential (10 mV).
Instruments (Novato, CA) Model P-87 Flaming/Brown micropipette puller. Electrodes were then fire-polished and filled with internal solution. The internal solution consisted of (in mM): 120 KMeSO₄, 15 KOH, 2 MgCl₂, 7.5 NaCl, 30 HEPES, 4 ATP, 0.2 GTP, 0.1 leupeptin, and 10 2-bis(2-aminophenoxy)ethane-N,N',N''-tetraacetic acid (BAPTA).

A multibarrel array of glass capillaries (OD ≈ 500 μm) in “sewer pipe” configuration was used to apply solutions. Six to ten capillaries were glued side by side and attached to a micromanipulator. Solutions were changed by moving the active barrel so the flow surrounds the cell from which recordings were made. Care was taken to regulate flow through the array to prevent flow artifacts.

Recordings were made with a Dagan 8900 (Minneapolis, MN) amplifier at RT (21–23°C). Series resistance compensation of 70 – 150% was typically achieved. Cells with calculated series resistance errors of ±5 mV were discarded [(V = I × R); series resistance error (V) = peak current (I) multiplied by uncompensated series resistance (R)]. Data were acquired using pClamp 8. Reported membrane potentials were corrected off-line for the liquid junctional potential (about 8 mV). Data acquisition and analysis were done using pClamp and Axograph software (Axon Instruments). Linear leak current and the capacitative artifact were digitally subtracted before analysis, using a P/4 or P/7 protocol.

Statistics

Prism (GraphPad Software, San Diego, CA) software was used to perform statistical tests of significance. The nonparametric Mann–Whitney U test (paired groups) was used to compare sample population data throughout and summary data are presented as means ± SD, unless noted otherwise. P values ≤0.05 were considered to be significantly different.

Sample population data are represented as scatterplots, histograms of mean ± SD, or as box plots (Tukey 1977). Box plots indicate the upper and lower quartiles as edges of the box, with the median represented as a line crossing the box. The stems indicate the largest and smallest nonoutlying values. Outliers are indicated by asterisks. Outlying values are >1.5 times the quartile boundaries and extreme outlying values are >3.0 times the quartile boundaries.

RESULTS

Slice recordings

To test whether the α-DTX–sensitive current regulates excitability in intact layer II/III pyramidal neurons at physiological temperatures (32–35°C), we used IR-DIC optics to visually identify pyramidal neurons from layers II/III of somatosensory cortex. An α-DTX–sensitive current was present in 4/4 cells tested (roughly 14% block) in response to a test step to +30 mV from a −70-mV holding potential in voltage clamp (data not shown). We do not have spatial voltage control of these dendritic neurons, so we did not attempt to study detailed biophysics in this preparation. The biophysical properties of α-DTX–sensitive currents obtained in our previous study using acutely dissociated neurons (Guan et al. 2006) were very similar to those obtained using nucleated patches from acute slices (Bekkers and Delaney 2001). We recorded from pyramidal cells in whole cell current-clamp mode and examined the effects of α-DTX on membrane potential, input resistance, the AP, and repetitive firing.

We examined membrane properties with whole cell recordings in current clamp in 29 cells. We found no changes in resting membrane potential or input resistance after α-DTX (Fig. 1; Tables 1 and 2). Similarly, there were no significant differences in AP amplitude or width at half-amplitude (half-width: Table 1; Fig. 3C). AP width at threshold was significantly broader in α-DTX (Table 1). There was a small but significant reduction in AP voltage threshold, defined as the voltage at which a sharp increase was observed in dV/dt (Fig. 1; Table 1). The effects of α-DTX were incompletely reversible over the time course of our experiments. We found no significant changes in AP parameters or repetitive firing in five cells where the protocols were repeated without the application of α-DTX (data not shown). We replicated the effect on voltage threshold (−44 ± 7 mV control vs. −46 ± 5 mV in α-DTX) and threshold width (5.9 ± 1.9 mS control vs. 6.5 ± 1.5 mS in α-DTX) in current-clamp recordings from acutely dissociated cells at RT (n = 3; Fig. 1D). In all three cells, the voltage remained depolarized after the AP in α-DTX.

We next tested whether α-DTX altered repetitive firing in response to 3-s current steps of various amplitudes (slice; Fig. 2). All cells tested fired in a regular spiking pattern (McCormick et al. 1985). In α-DTX, there was a significant reduction in rheobase, the minimum current required to elicit an action potential (≈500-ms current step; Fig. 1G, Table 2). The latency to the spike at rheobase was also significantly shorter in α-DTX (54 ± 17 ms) versus control (71 ± 21 ms; P < 0.03). In all three dissociated cells tested, the latency to first spike was also reduced in α-DTX (17 ± 11 ms control vs. 10 ± 7 ms in α-DTX). Application of α-DTX increased AP frequency at all current intensities (Fig. 2, A and B; Table 2). The rate increase was greater for large current injections, resulting in an increase in the f–I slope (increased gain: Fig. 2B; Table 2). Because spike-frequency adaptation (SFA) is greater at higher firing rates, we compared SFA with firing rates matched in control and α-DTX. We found that SFA was unaltered by α-DTX when the number of APs was matched for control and α-DTX (Fig. 2, D and E).

In control solution, spike threshold increased during a train of repetitive spikes, eventually reaching steady state within about 10 spikes (Fig. 3, A and B). In α-DTX, the initial voltage thresholds were significantly more negative but the change in threshold during the spike train was similar to that in control solutions (Fig. 3, A and B). These data suggest that the influence of α-DTX–sensitive current on threshold remains similar over time during firing and that other mechanisms are responsible for the increase in threshold during a train of APs.

Action potential waveforms

How do Kv1 channels regulate AP threshold, rheobase, and ISIs? To answer these questions, we used voltage-clamp experiments in pyramidal cells dissociated from supragranular layers of the combined primary motor and somatosensory cortices (see METHODS). To complement our previous data obtained using voltage steps, we used more realistic voltage waveforms to test for conditions where Kv1 channels contribute to outward currents. We tested activation of the α-DTX–sensitive current in acutely dissociated pyramidal neurons at RT using a slow voltage ramp (from −70 to −35 mV: 0.2 mV/ms), which ended in a mock action potential waveform (APW; not shown in figure). The ramp was similar in slope to the trajectory to the first AP during a long current step at rheobase (Fig. 4). During the slow ramp, an outward current became apparent at voltages positive to −55 ± 7.9 mV (n =
10). This current increased in amplitude up to the “threshold” voltage for the APW (−35 mV). Negative to −45 mV, current amplitude was small but was dominated by α-DTX–sensitive current. At −35 mV, the α-DTX–sensitive current amplitude was about 15 pA (median; n = 8), 48 ± 30% of the whole current.

Figure 5 illustrates currents in response to APWs. These experiments were designed to test whether α-DTX–sensitive current could contribute to shaping aspects of the spike. In three cells, we used an AP recorded at RT in the slice preparation as the voltage protocol (Fig. 5, A and D). In an additional nine cells, we combined voltage ramps to mimic an action potential at RT (Fig. 5, B and E). Similar data were obtained with the two protocols. A large outward current was activated by the APW and peak current was slightly reduced by α-DTX (Fig. 5, A and B; Table 3). The peak of the α-DTX–sensitive current (101 ± 106 pA) occurred later than the remaining current in all cells tested, during the falling phase of the spike (Fig. 5C; Table 3, P < 0.06, not significant). At the time for peak whole current, the α-DTX–sensitive current was responsible for roughly 10% of the current (Fig. 5F).

To illustrate the fraction of the whole current contributed by the α-DTX–sensitive current, we calculated the percentage, plotted it as a function of time, and superimposed the results to the APW used to elicit the current. This analysis revealed that the α-DTX–sensitive current becomes important late in repolarization and especially after the spike (Fig. 5, D and E; Table 3). These findings predict that α-DTX–sensitive current would not play a large role in AP repolarization but might regulate the ISI if a second spike occurred within a few milliseconds after the first one.

We next used a voltage protocol with an initial shallow ramp followed by two APWs with 10 ms in between (total ISI = 50 ms) to mimic firing at 73 Hz (Fig. 6, A and B). The voltage during the ISI was set at −50 mV to approximate a typical average interspike voltage trajectory during a high-conduction state (e.g., “up-state”) in vivo (Contreras 2004; Destexhe et al. 2003). A second protocol mimicked the interspike trajectory in response to DC current injection during a low-conductance state (e.g., slice or “down-state” in vivo) more closely by including an initial afterhyperpolarization (AHP)-like ramp, followed by a ramp to threshold for the second APW.

**Table 1.** Slice data: first AP during 3-s current injections

<table>
<thead>
<tr>
<th></th>
<th>RMP</th>
<th>AP</th>
<th>Width</th>
<th>HW</th>
<th>Up</th>
<th>Down</th>
<th>Vth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−73 ± 4.8 (n = 14)</td>
<td>101 ± 5.7 (n = 14)</td>
<td>4.9 ± 1.6 (n = 14)</td>
<td>2.7 ± 0.8 (n = 14)</td>
<td>159 ± 64 (n = 14)</td>
<td>34 ± 14 (n = 14)</td>
<td>−39 ± 5.9 (n = 20)</td>
</tr>
<tr>
<td>DTX</td>
<td>−73 ± 4.9 (n = 14)</td>
<td>98 ± 6.2 (n = 14)</td>
<td>6.1 ± 2.9 (n = 14)*</td>
<td>2.6 ± 0.6 (n = 14)</td>
<td>158 ± 62 (n = 14)</td>
<td>34 ± 19 (n = 14)</td>
<td>−41 ± 5.8 (n = 20)*</td>
</tr>
</tbody>
</table>

Values are means ± SD; values of n (whole cell recordings) are in parentheses. RMP, resting membrane potential (mV); AP, action potential amplitude from resting membrane potential (mV); HW, AP width at half amplitude [(peak − RMP)/2] (ms); Width, AP width at Vth (ms); Vth, voltage threshold for AP initiation (mV). Statistical significance: *P < 0.05.
Table 2. Slice data: 3-s current injections

<table>
<thead>
<tr>
<th></th>
<th>Rn</th>
<th>Rheobase</th>
<th>f-I Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>202 ± 72</td>
<td>160 ± 74</td>
<td>0.088 ± 0.05</td>
</tr>
<tr>
<td>DTX</td>
<td>215 ± 69</td>
<td>108 ± 47</td>
<td>0.110 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SD; values of n (whole cell recordings) are in parentheses. Rn, input resistance = Vf/MJ; Rheobase, lowest current to elicit an AP (pA); f-I (frequency–current) slope, between 1 and 2 s firing (Hz/pA). Statistical significance: *p < 0.05.

The main conclusions do not differ for data obtained from these two protocols: the α-DTX–sensitive current constituted a high percentage of the current during the time course of the ISI (Fig. 6, B and D). This percentage increased throughout the ISI for the protocol mimicking the high-conductance state and increased to an early peak and then remained high for the protocol that mimicked the low-conductance state. These data are consistent with our previous finding that deactivation τ values for the α-DTX–sensitive current are voltage dependent (Guan et al. 2006), with deactivation relatively slow at these voltages. Further, these data suggest that α-DTX–sensitive currents constitute a large percentage of outward current during subthreshold voltage ramps and during ISIs and thus can play important roles in regulating voltage threshold, rheobase, and ISIs.

α-DTX–sensitive current and filtering of inputs

In neurons of the auditory brain stem, the α-DTX–sensitive current prevents repetitive firing to pure DC current while allowing high-frequency spiking to time-varying stimuli (Brew et al. 2003; Dodson et al. 2002; Rothman and Manis 2003c; Slee et al. 2005). To test whether similar filtering effects occur in pyramidal neurons, we examined the effects of α-DTX on the selectivity of firing to DC current injections with or without somatic noise (see METHODS). Pyramidal cells fire more rapidly in response to a wide range of DC current injections when the DC stimulus is accompanied by broadband noise. Previous studies showed that addition of noise caused a gain increase that was greater in layer II/III cells than that in layer V pyramidal cells (Higgs et al. 2006).

Rundown of mechanisms regulating firing behavior is a concern during whole cell recordings. Accordingly, we performed these experiments using KCl-filled sharp microelectrodes (n = 6 cells). These experiments reproduced the key findings of the effects of α-DTX from whole cell recordings. There was no change in resting membrane potential or input resistance. Voltage threshold (~57 ± 9 mV in α-DTX vs. ~50 ± 10 mV control) and rheobase (330 ± 231 pA in α-DTX vs. 446 ± 206 pA) were significantly decreased. The firing rate significantly increased in α-DTX, with a gain increase of 21% (Fig. 7).

The present studies confirmed that addition of current noise significantly increased firing rate and gain in layer II/III pyramidal cells (by 12 ± 12%; Fig. 7). Gain changes by α-DTX were measured by fitting the “shift and multiply” equation in METHODS—that is, as a multiplication and a leftward shift along the current axis. Gain changes caused by noise were measured by fitting the “add and multiply” equation; i.e., they could be separated into a multiplicative effect and an additive shift along the firing rate axis. Note that the fit parameters do not give absolute gains, but only relative gain changes. Thus rather than using a t-test to compare control and experimental groups to attain P values, we used two-tailed Z tests to compare the relative gain changes to a null hypothesis (mean = 0, SD = observed SD). The effects of α-DTX were not altered by the presence of noise (the effects of α-DTX on gain were significant in the presence of noise: 25 ± 29%). In the presence of α-DTX the increased gain resulting from noise did not reach significance (11 ± 14%, P < 0.07). We conclude that, unlike in neurons of the auditory brain stem, the α-DTX–sensitive current did not alter the selectivity of pyramidal neurons for noisy (time-varying) versus DC inputs.
DISCUSSION

Several previous studies examined the effects of α-DTX on APs and firing in various cell types (e.g., Bekkers and Delaney 2001; Brew and Forsythe 1995; Faber and Sah 2004; Golding et al. 1999; Locke and Nerbonne 1997; Mitterdorfer and Bean 2002; Rothman and Manis 2003c). In layer V pyramidal cells, α-DTX–sensitive currents contribute to AP voltage threshold and firing rate (Bekkers and Delaney 2001). We recorded from layer II/III pyramidal neurons from rat somatosensory and motor cortex to test for functional roles of α-DTX–sensitive current (Kv1.1–, Kv1.2–, and Kv1.6-containing channels). Current-clamp recordings in the slice preparation were used to test effects of α-DTX on APs and repetitive firing. We used APWs and dissociated cells to identify potential roles for α-DTX–sensitive currents and to illustrate the contributions of α-DTX–sensitive current at various times during and after the APW. A few current-clamp experiments were conducted in dissociated cells to confirm key findings from slices (Fig. 1). Because Kv1 currents in medial nucleus of the trapezoid body (MNTB) neurons make the cells responsive to time-varying stimuli while filtering out DC inputs (Brew and Forsythe 2003; Slee et al. 2005), we also tested the role of α-DTX–sensitive current in the suprathreshold filtering characteristics of pyramidal cells.

Our previous study (Guan et al. 2006) suggested that the α-DTX–sensitive current should not contribute to resting potential (activation range) and should not have significant effects on AP repolarization (small percentage of current) after stimulation from negative resting potentials. Because the α-DTX–sensitive current activates at voltages near threshold, we predicted it would influence rheobase and voltage threshold. Also, the slow kinetics of deactivation at about −50 mV suggested that the α-DTX–sensitive current could influence ISIs and firing rate.

Our principal findings were as follows. 1) In dissociated cells, the DTX-sensitive current is activated by subthreshold ramps and by action potential waveforms. The α-DTX–sensitive current consistently activates at more negative potentials than the DTX-insensitive persistent current. The proportion of the whole current arising from α-DTX–sensitive current was larger after the repolarization of the APW, reaching a maxi-
sensitive current did not alter selectivity for DC versus time-

base, ISIs, and of 3 cells tested). Note that the percentage that was

peak currents occurred during the repolarization phase. C: summary box plots for time-to-peak current for the α-DTX-sensitive (DTX) and remaining, α-DTX-insensitive current (peak APW at 1.8 ms). α-DTX-sensitive current peaked later in time than the remaining current (protocol in B and E). D: AP used to elicit currents in A, superimposed with a record of the percentage of the whole current arising from α-DTX-sensitive current as a function of time (average of 3 cells tested). Note that the percentage that was α-DTX sensitive was greatest a few milliseconds after repolarization of the AP. E: voltage protocol used to elicit current in B. Gray trace indicates the proportion of the current that is α-DTX sensitive as a function of time. Dotted line indicates 0% of the whole current. Percentage of α-DTX-sensitive current was low during the AP and increased with time after repolarization of the APW. F: summary box plot for the percentage of the whole current contributed by the α-DTX-sensitive current at the time for peak whole current (protocol in B and E).

Effects on threshold

In response to our voltage-clamp ramp protocol, the α-DTX-sensitive current activated at subthreshold potentials and at more negative potentials than the DTX-insensitive current. The α-DTX-sensitive current accounted for about one half of the net outward current at −35 mV. Consistent with this finding, addition of α-DTX during current-clamp recordings in the slice preparation led to a significantly lowered rheobase as well as significantly lower voltage threshold for an AP. Bekkers and Delaney (2001) did not examine rheobase but reported that the α-DTX-sensitive current was active in the subthreshold voltage range and block resulted in a small but significant change in voltage threshold in layer V neocortical pyramidal neurons. Recordings with outside-out patches revealed that the α-DTX-sensitive current was largest in the soma and declined with distance along the apical dendrite (Bekkers and Delaney 2001). They suggested this indicated strategic placement of α-DTX-sensitive channels for influencing the initial segment and AP threshold.

We also found that α-DTX reduced the latency to the spike at rheobase. Our findings are similar to findings for α-DTX-sensitive currents in hippocampal pyramidal neurons (Wu and Barish 1992). Bekkers and Delaney (2001) showed that low doses of 4-AP (100 μM) blocked the same current component as α-DTX (1–2 μM). On the basis of 4-AP sensitivity, Locke and Nerbonne (1997) suggested that “D” current prolonged the voltage trajectory to the first action potential in acutely dissociated neocortical pyramidal neurons. For striatal medium spiny cells, models based on biophysical data suggest that blockade of Kv1.2 channels would reduce spike threshold by nearly 2 mV and decrease latency to the first AP (Nissenbaum et al. 1994; Shen et al. 2004).

**TABLE 3. Action potential waveform data**

<table>
<thead>
<tr>
<th></th>
<th>Vfirst</th>
<th>Peak I, pA</th>
<th>% at APW</th>
<th>Peak Time</th>
<th>% DTX Peak</th>
<th>% DTX-insens Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTX-sensitive</td>
<td>−39.2 ± 18.3</td>
<td>262 ± 167*</td>
<td>10.9 ± 5.2*</td>
<td>1.0 ± 0.3</td>
<td>36.5 ± 36.1</td>
<td>10.9 ± 3.3*</td>
</tr>
<tr>
<td>DTX-insensitive</td>
<td>−37.8 ± 19.1</td>
<td>1,486 ± 815</td>
<td>90.5 ± 7.7</td>
<td>0.6 ± 0.4</td>
<td>64.5 ± 37.0</td>
<td>89.1 ± 3.3</td>
</tr>
</tbody>
</table>

Values are means ± SD; dissociated cells: n = 9 cells. Voltage protocol was as in Fig. 5E. Vfirst, voltage at which current first detected. Peak I, peak current for this component (pA). APW, action potential waveform (series of ramps from −70 to +30 mV and return to −70 mV (3.6-ms base width). % at APW Peak, percentage of the whole current arising from this component at a time corresponding to the peak of the AP waveform used as voltage stimulus. Peak Time, time (ms) after peak of APW (which occurred at 1.8 ms in protocol). % DTX Peak, percentage of whole current arising from this component at a time corresponding to peak DTX-sensitive current. % DTX-insens Peak, percentage of whole current arising from this component at a time corresponding to peak of the DTX-insensitive current. Significant difference: *P < 0.05.
Action potentials (APs)

Our experiments with APWs as voltage stimuli showed that the $\alpha$-DTX–sensitive current was a minor contributor to the initial outward current during the AP and at the peak of the APW the proportion was similar to that in response to long (200-ms) voltage steps (roughly 10%). We found that the proportion of the whole current arising from $\alpha$-DTX–sensitive current was much larger a few milliseconds after the repolarization of the APW. Consistent with these findings, we found that $\alpha$-DTX had minimal effects on AP amplitude, half-width, or repolarization rates measured with whole cell or sharp microelectrode recordings in the slice preparation. In contrast, $\alpha$-DTX caused a significant broadening of the spike at threshold or more negative voltages, resulting from slowing of the final phases of repolarization.

Locke and Nerbonne (1997) showed that 4-AP–sensitive current in acutely dissociated neocortical pyramidal neurons contributed to later phases of AP repolarization. Spain et al. (1991) reported similar effects of 4-AP on spike repolarization in cat Betz cells and Wu and Barish (1992) showed that $\alpha$-DTX caused AP broadening in CA1 pyramidal cells. Bekkers and Delaney (2001) found that $\alpha$-DTX did not change AP half-width in layer V pyramidal neurons. The $\alpha$-DTX–sensitive current also had little effect on AP repolarization in CA3 pyramidal neurons (Mitterdorfer and Bean 2002) or nodose ganglia neurons (Glazebrook et al. 2002). In dissociated CA3 neurons, the putative “D current” was activated before the peak of the AP, but not fully activated by an APW (Mitterdorfer and Bean 2002).

Repetitive firing

Our two APW protocols in dissociated cells showed that in neocortical pyramidal cells, the contributions of the $\alpha$-DTX–sensitive current reached a maximum during the interval between APWs. This was relatively insensitive to small variations in the interspike voltage trajectory (e.g., Fig. 3, A and B vs. $D$ and $E$) and is consistent with our previous finding (Guan et al. 2006) that voltage-dependent deactivation was slow at voltages corresponding to in vivo membrane potentials in the “up-state” (Cowan and Wilson 1994; Stern et al. 1997) and potentials traversed by ISIs during repetitive firing (Contreras 2004; Destexhe et al. 2003). These findings lead to the prediction that the $\alpha$-DTX–sensitive current would have a major role in regulating ISIs during repetitive firing. Our current-clamp
experiments in the slice preparation revealed that addition of 
\( \alpha\)-DTX leads to increased firing rate at all stimulus intensities. This resulted in an increased gain of spike output versus DC current input (increased \( f-I \) slope). We found that spike-frequency adaptation (SFA) was unaltered by \( \alpha\)-DTX. Effects of \( \alpha\)-DTX on SFA were not explicitly tested in previous studies of neocortical pyramidal neurons. In contrast, in amygdaloid pyramidal neurons, the \( \alpha\)-DTX-sensitive current regulates firing rate by enhancing SFA (Faber and Sah 2004).

\( \alpha\)-DTX-sensitive currents affect repetitive firing in many neuron types. Bekkers and Delaney (2001) showed that \( \alpha\)-DTX resulted in increased firing in response to 1-s current injections in layer V pyramidal cells and Locke and Nerboune (1997) showed that 4-AP-sensitive current contributed to regulation of firing rate in acutely dissociated neocortical pyramidal neurons. Neither study explicitly tested for changes in gain or SFA. In nodose ganglia, \( \alpha\)-DTX caused increased firing rate and a reduced AHP (Glazebrook et al. 2002). For striatal medium spiny cells, models based on biophysical data suggest that blockade of Kv1.2 channels regulates the “up-state” but would have minimal effects on firing rate (Shen et al. 2004). Little effect of \( \alpha\)-DTX was observed at firing rates of <20 Hz (Shen et al. 2004). In Purkinje neurons, Kv1 channels maintain low frequencies of Na\(^+\) and Ca\(^{2+}\) spike output to optimize timing (McKay et al. 2005). In subicular pyramidal neurons, the 4-AP-sensitive current regulates burst firing behavior (Staff et al. 2000). The \( \alpha\)-DTX-sensitive current was also proposed to set the threshold for Ca\(^{2+}\) spikes in CA1 pyramidal cells (Golding et al. 1999).

The effect of \( \alpha\)-DTX-sensitive current is particularly dramatic in auditory MNTB cells, where Kv1 channels constitute 80\% of the outward current (Dodson et al. 2002) and Kv1 channels facilitate temporal precision of signaling (Brew and Forsythe 1995; Kopp-Scheinflug et al. 2003). These cells normally fire only one spike in response to long current injections but they can phase lock to repeated brief stimuli at several hundred Hertz. MNTB cells can be transformed to fire repetitively to long DC stimuli in the presence of \( \alpha\)-DTX (Dodson et al. 2002) or knock out of Kv1.1 (Brew et al. 2003). In neurons of the ventral cochlear nucleus (Rothman and Manis 2003a,b,c), a high density of \( \alpha\)-DTX-sensitive current was found to be responsible for phasic firing of type II neurons. In lower density (type I cells: <35\% of outward current), the \( \alpha\)-DTX-sensitive current promoted regular firing, spike repolarization, and reduced \( \tau_{\text{m}} \) at subthreshold potentials, thereby facilitating coincidence detection. The firing pattern of stellate cells is similar to that of neocortical pyramidal neurons. In these MNTB and other auditory brain stem cells, the main role of DTX-sensitive current is to make these cells responsive to time-varying inputs while filtering out DC inputs; i.e., they differentiate their synaptic inputs (Brew and Forsythe 1995; Kopp-Scheinflug et al. 2003; Slee et al. 2005).

We found that \( \alpha\)-DTX increased firing rate in pyramidal neurons. This increase could be decomposed into a leftward shift along the current axis and a multiplicative effect. Addition of broadband noise also increased firing rate (as in auditory brain stem neurons, but quantitatively less) but did not occlude the effects of \( \alpha\)-DTX. These results suggest that the contribution of \( \alpha\)-DTX-sensitive current to selectivity for time-varying input in pyramidal neurons is restricted to subthreshold mean input, during which the threshold elevation caused by this current will reduce firing to weakly fluctuating stimuli. Interestingly, direction selectivity of somatosensory pyramidal cells in vivo is enhanced by stimulus-dependent changes in spike threshold (Wilent and Contreras 2005). During suprathreshold input our results indicate that Kv1-containing channels contribute to regulation of firing rate and gain without altering selectivity for time-varying versus DC stimuli.

Consistent with an important role in regulating excitability in cortex, transgenic mice with Kv1.1 knockouts exhibit seizures (Rho et al. 1999; Smart et al. 1998) despite reports of only minor changes in single-cell physiology in CA3 (Smart et al. 1998) and layer V of neocortex (van Brederode et al. 2001). Kv1 subunits are highly expressed in neuropil and terminals (Guan et al. 2006; Shen et al. 1994; Wang et al. 1994). In Kv1.1 knockout mice, the frequency of spontaneous postsynaptic currents was increased in the mutant animals (van Brederode et al. 2001). The Kv1.1 knockout mice also show synaptic hyperexcitability in CA3 pyramidal neurons (Lopantsev et al. 2003). Mutations in Kv1.1 were also implicated in human epilepsy (Zuberi et al. 1999). Besides epileptiform activity, mutations in Kv1 subunits were also tied to human episodic ataxia (Adelman et al. 1995; Browne et al. 1994). Kv1 channels were also previously implicated in learning and memory. Drosoerpilka shaker mutants (Kv1 homologue) show memory impairment (Cowan and Siegel 1986) and antisense inhibition of Kv1.1 impaired memory in rats without effecting motor or sensory behaviors, but did not effect long-term potentiation in DG or CA3 (Meiri et al. 1997).

The diversity of clinical consequences of Kv1 mutations and contrast in function of channels containing Kv1 \( \alpha\)-subunits in auditory brain stem and neocortical pyramidal cells underscores the fact that channel function depends on context. Kv1 channel function varies by cell types, reflecting the different ion channel composition of the cells. In neocortical pyramidal cells, these channels have important effects on voltage- and current thresholds for spike initiation and strongly influence the time course of ISIs.

Acknowledgments
The authors express gratitude to S. Phillips and C. Robbins for technical assistance and to Dr. W. Armstrong for reading an earlier version of this manuscript.

Grants
This work was supported by National Institute of Neurological Disorders and Stroke Grant NS-044163 to R. C. Foehring and a Veterans Administration Merit Review grant to W. J. Spain.

References