Roles of specific $K_v$ channel types in repolarization of the action potential in genetically identified subclasses of pyramidal neurons in mouse neocortex

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**Pathak D, Guan D, Foehring RC.** Roles of specific $K_v$ channel types in repolarization of the action potential in genetically identified subclasses of pyramidal neurons in mouse neocortex. *J Neurophysiol* 115: 2317–2329, 2016. First published February 10, 2016; doi:10.1152/jn.01028.2015.—The action potential (AP) is a fundamental feature of excitable cells that serves as the basis for long-distance signaling in the nervous system. There is considerable diversity in the appearance of APs and the underlying repolarization mechanisms in different neuronal types (reviewed in Bean BP. *Nat Rev Neurosci* 8: 451–465, 2007), including among pyramidal cell subtypes. In the present work, we used specific pharmacological blockers to test for contributions of $K_v$1, $K_v$2, or $K_v$4 channels to repolarization of single APs in two genetically defined subpopulations of pyramidal cells in layer 5 of mouse somatosensory cortex (*etv1* and *glt*) as well as pyramidal cells from layer 2/3. These three subtypes differ in AP properties (Grob A, Meyer HS, Schmidt EF, Heintz N, Sakmann B, Krieger P. *Cereb Cortex* 20: 826–836, 2010; Guan D, Armstrong WE, Foehring RC. *J Neurophysiol* 113: 2014–2032, 2015) as well as laminar position, morphology, and projection targets. We asked what the roles of $K_v$1, $K_v$2, and $K_v$4 channels are in AP repolarization and whether the underlying mechanisms are pyramidal cell subtype dependent. We found that $K_v$4 channels are critically involved in repolarizing neocortical pyramidal cells. There are also pyramidal cell subtype-specific differences in the role for $K_v$1 channels. Only $K_v$4 channels were involved in repolarizing the narrow APs of *glt* cells. In contrast, in *etv1* cells and layer 2/3 cells, the broader APs are partially repolarized by $K_v$1 channels in addition to $K_v$4 channels. Consistent with their activation in the subthreshold range, $K_v$1 channels also regulate AP voltage threshold in all pyramidal cell subtypes.

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**somatosensory cortex; potassium channel; AmmTx3; dendrotoxin; guanidionixotin**

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**THE ACTION POTENTIAL (AP)** is a fundamental feature of neurons that serves as the basis for long-distance signaling in the nervous system. The number, frequency, and timing of APs can serve as a neural code (Larkum et al. 1999b; Mainen and Sejnowski 1995; Singer 2001; Williams and Stuart 2000). In neocortical pyramidal neurons, APs are initiated in the axonal initial segment and then propagate along the axon as well as backpropagate into the soma and dendrites (Kole and Stuart 2012; Martina et al. 2000; Palmer and Stuart 2006; Shu et al. 2007; Stuart et al. 1997). In general, the time course of the AP determines the temporal pattern of voltage changes that gate voltage-dependent ionic conductances and the shape of the AP regulates $Ca^{2+}$ entry (e.g., Bean 2007; Stewart and Foehring 2001). In turn, intracellular $Ca^{2+}$ concentration ([Ca$^{2+}$]) controls many cellular processes, including activation of $Ca^{2+}$-dependent conductances (e.g., Andrade et al. 2012; Schwindt et al. 1988b), activation of enzymes and signaling pathways, and induction of synaptic plasticity. The basics of AP form are similar in most neurons (Bean 2007): the interplay between $Na^+$ channels, leak channels, and voltage-gated $K^+$ channels regulates voltage threshold (which is dynamic), the AP upstroke is primarily due to gating of voltage-gated $Na^+$ channels, and repolarization of the AP is dominated by $K^+$ conductances and inactivation of $Na^+$ conductance. Repolarization mechanisms may also contribute to the fast afterhyperpolarization.

There is considerable diversity, however, in the appearance of APs and underlying repolarization mechanisms in different neuronal types (reviewed by Bean 2007). For example, fast-spiking cells (e.g., GABAergic interneurons in cortical regions: Rudy et al. 1999; neurons in auditory brain stem: Gan and Kaczmarek 1998; Wang et al. 1998; cerebellar Purkinje cells: Martina et al. 2007) are repolarized by high-threshold $K_v$3 channels with rapid activation/deactivation kinetics. Cortical pyramidal neurons typically do not express $K_v$3 channels (Martina et al. 1998; Massengill et al. 1997; Rudy et al. 1999) and thus must use different mechanisms to repolarize the AP. For example, $Ca^{2+}$-dependent BK channels are important for AP repolarization in somas of CA1 pyramidal cells (Poolos and Johnston 1999; Storm 1987) but do not play such a role in distal dendrites of CA1 pyramidal cells (Poolos and Johnston 1999) or in neocortical pyramidal cell somas (Guan et al. 2015; Lorenzon and Foehring 1992; Pineda et al. 1998; Schwindt et al. 1988a, 1988b).

There is also considerable variability in AP properties among neocortical pyramidal cells. Large layer 5 pyramidal cells from the motor cortex of cats (Betz cells) have very narrow APs, and repolarization is due to multiple voltage-gated $K^+$ channels (which have not been characterized as to their genetic or molecular basis; Schwindt et al. 1988a, 1988b). In rodent neocortex, pyramidal neurons have been classified on the basis of projection target as IT type (intratelencephalic: project within the telencephalon) or PT type (project beyond the telencephalon, including the pyramidal tract; Dembrow et al. 2010; Reiner et al. 2003; Shepherd 2013; Suter et al. 2013). A consistent finding is that PT-type cells have narrower APs with more rapid repolarization, whereas IT-type cells have broader APs with slower repolarization (reviewed in Guan et al. 2015; Shepherd 2013).

Neocortical pyramidal cells can also be classified by genetic differences. For example, consider two lines of mice that express enhanced green fluorescent protein (EGFP) in cells expressing the genes *etv1* or *glt* (Doyle et al. 2008; Gong et al. 2007). In mouse somatosensory cortex, *etv1* cells are a subset
of IT-type neurons located primarily in superficial layer 5 and glt cells are a subset of PT-type neurons, primarily located in deep layer 5 (Bishop et al. 2015; Groh et al. 2010; Guan et al. 2015). glt cells have narrower and more rapidly repolarizing APs than etvl or layer 2/3 cells (Groh et al. 2010; Guan et al. 2015). Morphology and projection targets also differ for these cell populations (Groh et al. 2010). Most previous work on the ionic basis for AP repolarization in rodent central neurons used relatively nonspecific pharmacological agents [e.g., 4-aminopyridine (4-AP), tetraethylammonium (TEA)] and thus could not definitively identify the roles of specific channel types. Pyramidal neurons express several types of K+ channels that could potentially shape AP properties. There are transient and persistent components to outward K+ currents in rodent pyramidal neurons (Bekkers 2000; Foehring and Surmeier 1993; Kornogreen and Sakmann 2000; Locke and Nerbonne 1997).

The persistent currents are due to strong expression of K1.1, K2 (the largest component), and K7 channels (Bekkers and Delaney 2001; Bishop et al. 2015; Guan et al. 2006, 2007a, 2007b, 2011a, 2011b, 2013, 2015; Murakoshi and Trimmer 1999). In addition, Nerbonne and colleagues used genetic approaches to reveal that most of the transient, A-type current in rat neocortical cells is due to K4.2 and K4.3 channels (Carrasquillo et al. 2012; Norris and Nerbonne 2010; Yuan et al. 2005; see also Guan et al. 2011b), with a contribution from K1.4 channels.

We previously found that in layer 2/3 pyramidal neurons from rats K1.1 channels did not affect AP width or repolarization (Guan et al. 2007a), and we used a dominant-negative approach to show that K2.1-containing channels did not play a role in AP repolarization in rat neocortical pyramidal neurons (Guan et al. 2013). We also showed that AP repolarization in mouse layer 2/3, etvl, or glt cells is not Ca2+ dependent (Guan et al. 2015), consistent with previous studies on neocortical pyramidal cells (Lorenzon and Foehring 1992; Pineda et al. 1999; Schwindt et al. 1988a, 1988b). In addition, the kinetics of K7 channels are too slow to influence single APs of neocortical pyramidal cells (layer 2/3; Guan et al. 2011a) and other pyramidal cell types (Aiken et al. 1996; Gu et al. 2007; Hu et al. 2007; Marrion 1997; Mateos-Aparicio et al. 2014; Prescott and Sejnowski 2007; Storm 1989; Yue and Yaari 2004). On the basis of these findings, we hypothesized that K4.4 channels may be the primary channels involved in repolarizing APs in neocortical pyramidal cells. We also asked whether the underlying mechanisms are pyramidal cell subtype dependent.

METHODS

We studied layer 2/3 pyramidal neurons and layer 5 pyramidal neurons from two bacterial artificial chromosome lines of mice, each of which expresses EGFP in a different subpopulation of layer 5 pyramidal neurons (Gong et al. 2002, 2003, 2007; Guan et al. 2015). In the somatosensory cortex of Tg(etvl-EGFP)/BZ192Gsat/Mmucd (etvl) mice, EGFP is primarily expressed in pyramidal neurons from superficial layer 5 (Groh et al. 2010; Guan et al. 2015). The etvl gene is a transcription factor that has been shown to be involved in neurogenesis in the olfactory bulb (Stemman et al. 2003) and circuit formation in the spinal cord (Arber et al. 2000). In somatosensory cortex of Tg(Gl Gl252d-EGFP)/BN20Gsat/Mmmnc (glt) mice, glt-EGFP is primarily expressed in a subset of deep layer 5 pyramidal neurons (Groh et al. 2010; Guan et al. 2015), although some EGFP+ cells were also observed in superficial layer 5. The glt gene is a glycosyl transferase (Gong et al. 2003, 2007). We maintain breeding colonies of both mouse lines (Swiss-Webster background), which were originally obtained from the Mutant Mouse Regional Resource Centers (MMRRC) of the GENSAT project.

The present studies were performed on juvenile mice from 2 to 4 wk of age. All procedures were approved by the Animal Care and Use Committee, University of Tennessee Health Science Center. The animals were anesthetized with isoflurane until they were areflexic. 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 bubbled with O2 for 30 – 60 s. This solution contained (in mM) 250 sucrose, 2.5 KCl, 1 NaH2PO4, 11 glucose, 4 MgSO4, 0.1 CaCl2, 0.4 ascorbate, 0.6 sodium pyruvate, and 15 HEPES (pH 7.3 – 7.4, 300 mosM). The brain was then sliced into 300-μm-thick coronal sections with a vibrating tissue slicer (Vibroslice, Campden Instruments).

Slice recordings. Slices were placed in a recording chamber on the stage of an Olympus BX50WI upright microscope and bathed in artificial cerebrospinal fluid (aCSF) bubbled with 95% O2-5% CO2, delivered at 2 ml/min, and heated with an in-line heater (Warner Instruments, Hamden, CT) to 33 ± 1°C (measured with a thermometer in the bath adjacent to the slice). The aCSF contained (in mM) 125 NaCl, 3 KCl, 2 CaCl2, 2 MgCl2, 1.25 NaH2PO4, 26 NaHCO3, and 20 glucose (pH 7.4, 310 mosM). For most of the experiments with toxins, we used a recirculating bath system in which a peristaltic pump (Gilion Minipuls 3) was used for controlling both the inflow (~2 ml/min) and the outflow of carbogenated aCSF.

All slice current-clamp recordings were done in the presence of 6,7-dinitroquinoxaline-2,3-dione (DNQX, 20 μM) to block α-aminopyridine-3-hydroxy-5-methylisoxazol-propionic acid (AMPA) receptors, d-(-) -2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM) to block N-methyl-d-aspartate (NMDA) receptors, and picrotixin [100 μM; to block γ-aminobutyric acid type A (GABA_A) receptors] to minimize the influence of effects of the pharmacological agents on fast synaptic transmission presynaptic to the recorded cell. Pharmacological agents were added directly to the aCSF. Most reagents were purchased from Sigma-Aldrich (St. Louis, MO). In addition, α-dendrotoxin (DTX) and guangtixotoxin-1E (GxTx) were purchased from Alomone Labs (Jerusalem, Israel), DNQX, D-AP5, and picrotixin were purchased from Tocris Bioscience (Ellisville, MO), and AminTx3 was purchased from Smartox Biotechnology (Saint-Martin-d’Hères, France). For experiments with DTX, GxTx, or AminTx3, 0.2% bovine serum albumin was added to saturate nonspecific binding sites.

Pyramidal neurons in layer 2/3 and layer 5 were visualized with infrared-interference contrast (IR-DIC) videomicroscopy (Dott and Ziegglänsberger 1990; Stuart et al. 1993) using a ×40 (0.8 NA) Olympus water-immersion objective and an IR-sensitive camera (Olympus OLY-150 or DAGE-MTI), etvl or glt pyramidal cells were visually identified by the presence of EGFP epifluorescence with an FITC filter. Layer 5 recordings were directed within the main band of EGFP+ cells in layer 5 in each animal. In etvl neurons, recordings were biased toward the most superficial EGFP+ cells, and for glt neurons recordings were biased toward deeper EGFP+ cells. We previously showed that in somatosensory cortex, etvl neurons have a more superficial (and narrower) expression pattern in layer 5. glt neurons are found deeper in layer 5 (Guan et al. 2015), but there is overlap with the etvl distribution (Bishop et al. 2015; Guan et al. 2015). We switched between IR-DIC and epifluorescence to determine cell type and to obtain a gigaohm seal. Electrode position was controlled with Sutter ROE-200 manipulators and PC-200 controller or Luigs-Neumann manipulators and controller. Whole cell patch-clamp recordings were acquired with an Axon Multiclamp 700A or Multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA) and pCLAMP 9 or 10 software. For current-clamp recordings, the data were digitized at 20–50 kHz and filtered at 10 kHz. We recorded with J Neurophysiol · doi:10.1152/jn.01028.2015 · www.jn.org
Table 1. Comparison of single action potential parameters between cell types

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Layer 2/3</th>
<th>etvl</th>
<th>glt</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP, mV</td>
<td>89.6 ± 1.1 (51)</td>
<td>87.3 ± 1.1 (50)</td>
<td>92.2 ± 0.8 (48)*</td>
</tr>
<tr>
<td>Vm, mV</td>
<td>-54.29 ± 0.68 (51)</td>
<td>-53.96 ± 0.63 (50)</td>
<td>-56.65 ± 0.52 (48)*†</td>
</tr>
<tr>
<td>HW, ms</td>
<td>1.02 ± 0.04 (51)</td>
<td>1.01 ± 0.05 (50)</td>
<td>0.66 ± 0.05 (48)*†</td>
</tr>
<tr>
<td>Width at threshold, ms</td>
<td>1.89 ± 0.10 (51)</td>
<td>2.04 ± 0.18 (50)</td>
<td>1.25 ± 0.06 (48)*†</td>
</tr>
<tr>
<td>dv/dt up, V/s</td>
<td>297.1 ± 10.08 (51)</td>
<td>300.1 ± 14.46 (50)</td>
<td>409.5 ± 12.25 (48)*†</td>
</tr>
<tr>
<td>dv/dt down, V/s</td>
<td>91.35 ± 4.04 (51)</td>
<td>94.9 ± 6.16 (50)</td>
<td>157.7 ± 8.12 (48)*†</td>
</tr>
<tr>
<td>RMP, mV</td>
<td>-72.6 ± 1.0 (51)</td>
<td>-69.5 ± 0.8 (50)†</td>
<td>-67.6 ± 0.6 (48)*†</td>
</tr>
<tr>
<td>Rm, MΩ</td>
<td>137.6 ± 12.1 (51)</td>
<td>101.7 ± 7.1 (50)†</td>
<td>88.9 ± 6.6 (48)*†</td>
</tr>
</tbody>
</table>

Data are means ± SE for number of cells in parentheses. AP, action potential amplitude; Vm, voltage threshold; HW, AP width at half-amplitude (from resting potential); dv/dt up, maximum rate of AP rise; dv/dt down, maximum rate of AP repolarization; RMP, resting membrane potential; Rm, input resistance. *glt significantly different from etvl (P < 0.05, unpaired t-test); †significantly different from layer 2/3 (1-way ANOVA + Tukey’s post hoc test).

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The role of Kv2 channels in AP repolarization

DTX for any cell type. In mouse layer 2/3 pyramidal cells, DTX caused significant broadening of the AP (AP HW: P < 0.0001, Figure 1, A and B) with an increase in width at threshold (P < 0.008, Table 2) and slowed the rate of AP repolarization (dV/dt down: P < 0.007; Table 2). The average change in AP HW was 21 ± 3% (n = 10 cells). DTX also had a small but significant hyperpolarizing effect on voltage threshold in layer 2/3 pyramidal cells (Fig. 1C, P < 0.04). AP amplitude and dV/dt for the AP upstroke were not affected in layer 2/3 cells by DTX.

In etv1 pyramidal cells, there was a similar significant increase in AP HW (25 ± 6%, P < 0.004, n = 8) and width at threshold (P < 0.008) and a significant decrease in dV/dt for repolarization (P < 0.008; Fig. 1, D and E, Table 2). There was also a small but significant hyperpolarizing shift in voltage threshold for the AP in etv1 cells (P < 0.02; Table 2, Fig. 1F). AP amplitude was unchanged after DTX (Fig. 1D). In glt cells there was a significant negative shift in voltage threshold for the AP in DTX (P < 0.03, Fig. 1I), but, in contrast to etv1 and layer 2/3 cells, no significant effects on AP HW, width at threshold, or dV/dt for repolarization were observed in glt cells (Fig. 1, G and H, Table 2).

Role for K_v2 channels. GxTx was found to be a relatively selective blocker of all K_v2 subtypes by Herrington et al. (2006). They found that in pancreatic beta cells 40–100 nM GxTx caused spike broadening and increased intracellular Ca^2+.

<table>
<thead>
<tr>
<th>Layer 2/3</th>
<th>AP, mV</th>
<th>V_th, mV</th>
<th>HW, ms</th>
<th>Width at V_th, ms</th>
<th>dV/dt up</th>
<th>dV/dt down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>90 ± 2</td>
<td>−55.3 ± 1.4</td>
<td>0.86 ± 0.03</td>
<td>1.54 ± 0.08</td>
<td>299 ± 13</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>DTX</td>
<td>90 ± 1</td>
<td>−57.1 ± 1.2*</td>
<td>1.04 ± 0.04*</td>
<td>1.92 ± 0.13*</td>
<td>281 ± 14</td>
<td>85 ± 5*</td>
</tr>
<tr>
<td>etv1</td>
<td>Control</td>
<td>86 ± 2</td>
<td>−55.6 ± 1.5</td>
<td>0.87 ± 0.08</td>
<td>1.35 ± 0.17</td>
<td>330 ± 34</td>
</tr>
<tr>
<td>DTX</td>
<td>86 ± 2</td>
<td>−57.5 ± 1.5*</td>
<td>1.07 ± 0.11*</td>
<td>1.84 ± 0.29*</td>
<td>297 ± 23</td>
<td>105 ± 24*</td>
</tr>
<tr>
<td>glt</td>
<td>Control</td>
<td>91 ± 2</td>
<td>−54.6 ± 1.1</td>
<td>0.52 ± 0.05</td>
<td>0.90 ± 0.07</td>
<td>449 ± 31</td>
</tr>
<tr>
<td>DTX</td>
<td>89 ± 2</td>
<td>−56.7 ± 1.1*</td>
<td>0.54 ± 0.04</td>
<td>0.96 ± 0.07</td>
<td>421 ± 31</td>
<td>187 ± 17</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE for 10 (layer 2/3), 8 (etv1), and 11 (glt) cells. DTX, α-dendrotoxin; AP, action potential amplitude; V_th, voltage threshold; HW, half-width (width at half-amplitude from resting potential); width at V_th = width of AP at voltage threshold; dV/dt up, rate of change of voltage for AP upstroke; dV/dt down, rate of change of voltage for AP repolarization. *Significant difference from matched controls in paired t-test (P < 0.05).

Fig. 1. Effects of 100 nM α-dendrotoxin (DTX) on the action potential. Action potentials (APs) were elicited in the presence of 20 μM DNQX, 50 μM AP-5, and 100 μM picrotoxin to block fast synaptic transmission via AMPA, NMDA, or GABA_A receptors, respectively. A: representative traces for single APs in a layer 2/3 pyramidal neuron before and after application of 100 nM DTX. Inset: same APs at longer time base. B: summary data for AP half-width (AP HW: width of the AP at one-half amplitude relative to the resting potential) in layer 2/3 pyramidal cells. C: summary data for AP voltage threshold (Threshold) in layer 2/3 pyramidal cells. D: representative traces for single APs in an etv1 pyramidal neuron (layer 5) before and after application of 100 nM DTX. Scale bars also apply to A and G. Inset: same APs at longer time base. E: summary data for AP half-width (AP HW) in etv1 pyramidal cells. F: summary data for AP voltage threshold (Threshold) in etv1 pyramidal cells. G: representative traces for single APs in a glt pyramidal neuron (layer 5) before and after application of 100 nM DTX. Inset: same APs at longer time base; scale also applies to insets in A and D. H: summary data for AP half-width (AP HW) in glt pyramidal cells. I: summary data for AP voltage threshold (Threshold) in glt cells. *Significant difference between control and drug (paired t-test, P < 0.05).
Ca\(^{2+}\) levels. In that preparation the IC\(_{50}\) for K\(_{2}\) channels was 10–50 times lower than for K\(_{4}\). Similarly, Liu and Bean (2014) observed \(<10\%\) block of the K\(_{4}\)-mediated A current in CA1 pyramidal neurons with 100 nM GxTx (the A current is K\(_{4}\) mediated in CA1 pyramidal cells; Kim et al. 2005). We found that 100 nM GxTx had no effects on RMP [layer 2/3 (10 cells): −71 ± 3 mV control vs. −70 ± 3 mV GxTx; etv1 (13 cells): −69 ± 2 mV control vs. −68 ± 2 mV GxTx; glt (11 cells): −69 ± 2 mV control vs. −70 ± 2 mV GxTx] or input resistance [layer 2/3 (9 cells): 101 ± 19 MΩ control vs. 103 ± 19 MΩ GxTx; etv1 (11 cells): 110 ± 15 MΩ control vs. 124 ± 17 MΩ GxTx; glt (11 cells): 94 ± 15 MΩ control vs. 100 ± 16 MΩ GxTx] for any of the cell types we tested. We also found that 100 nM GxTx had no effect on single APs in any pyramidal cell subtype (Fig. 2, Table 3). We previously showed that GxTx did block K\(_{2}\)-mediated currents in mouse neocortical pyramidal neurons at this dose (Bishop et al. 2015). These data indicate little to no role for K\(_{2}\) channels in AP repolarization in neocortical pyramidal cells (at least for a single AP in response to a brief current injection; the function of K\(_{2}\) channels becomes more important with high-frequency firing: Du et al. 2000; Guan et al. 2013; Johnston et al. 2008; Liu and Bean 2014). The lack of effect of GxTx is consistent with our previous conclusion, based on use of genetic manipulation of K\(_{2},2.1\) channels in rat pyramidal cells, that K\(_{2}\) channels play little role in AP repolarization (Guan et al. 2013).

**Role for K\(_{4}\) channels.** Given the effects of K\(_{1}\) blockers on AP width and repolarization (in layers 2/3 and etv1 only) and the lack of effect of K\(_{2}\) blockers, we hypothesized that the dominant outward current involved in AP repolarization in mouse neocortical pyramidal cells would be due to K\(_{4}\) channels. A major role for K\(_{4}\) channels in AP repolarization would likely have gone undetected with analysis of only single APs.

Figure 2. Effects of 100 nM guangxitoxin-1E (GxTx; blocks K\(_{2}\) channels) on the action potential. A: representative traces for single APs in a layer 2/3 pyramidal neuron before and after application of 100 nM GxTx. GxTx had no effect on the AP in these cells. Inset: same APs at longer time base. B: summary data for AP half-width (AP HW) in layer 2/3 pyramidal cells. C: summary data for AP voltage threshold (Threshold) in layer 2/3 pyramidal cells. D: representative traces for single APs in an etv1 pyramidal neuron (layer 5) before and after application of 100 nM GxTx. GxTx had no effect on the AP in these cells. Scale bars also apply to A and G. Inset: same APs at longer time base. E: summary data for AP half-width (AP HW) in etv1 pyramidal cells. F: summary data for AP voltage threshold (Threshold) in etv1 pyramidal cells. G: representative traces for single APs in a glt pyramidal neuron (layer 5) before and after application of 100 nM GxTx. GxTx had no effect on the AP in these cells. Inset: same APs at longer time base; scale also applies to insets in A and D. H: summary data for AP half-width (AP HW) in glt pyramidal cells. I: summary data for AP voltage threshold (Threshold) in glt cells.

Table 3. **Effects of K\(_{2}\) blocker guangxitoxin on action potential parameters: effects of 100 nM guangxitoxin vs. same-cell control**

<table>
<thead>
<tr>
<th>Layer 2/3</th>
<th>AP, mV</th>
<th>(V_{\text{th}, \text{m}}), mV</th>
<th>HW, ms</th>
<th>Width at (V_{\text{th}, \text{m}}), ms</th>
<th>dV/dt up</th>
<th>dV/dt down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94 ± 2</td>
<td>−53.2 ± 0.7</td>
<td>0.86 ± 0.07</td>
<td>1.59 ± 0.16</td>
<td>338 ± 18</td>
<td>113 ± 13</td>
</tr>
<tr>
<td>GxTx</td>
<td>92 ± 2</td>
<td>−54.1 ± 0.9</td>
<td>0.87 ± 0.09</td>
<td>1.69 ± 0.21</td>
<td>339 ± 19</td>
<td>120 ± 20</td>
</tr>
<tr>
<td>etv1</td>
<td>92 ± 2</td>
<td>−51.2 ± 1.0</td>
<td>0.80 ± 0.02</td>
<td>1.34 ± 0.04</td>
<td>355 ± 11</td>
<td>108 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>90 ± 1</td>
<td>−50.5 ± 1.5</td>
<td>0.77 ± 0.02</td>
<td>1.28 ± 0.04</td>
<td>341 ± 12</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>GxTx</td>
<td>88 ± 2</td>
<td>−57.3 ± 1.1</td>
<td>0.65 ± 0.04</td>
<td>1.13 ± 0.08</td>
<td>388 ± 20</td>
<td>163 ± 13</td>
</tr>
</tbody>
</table>

Data are means ± SE for 10 (layer 2/3), 13 (etv1), and 11 (glt) cells. GxTx, guangxitoxin; AP, action potential amplitude; \(V_{\text{th}, \text{m}}\), voltage threshold; HW, half-width (width at half amplitude from resting potential); width at \(V_{\text{th}, \text{m}}\), width of AP at voltage threshold; dV/dt up, rate of change of voltage for AP upstroke; dV/dt down, rate of change of voltage for AP repolarization. No significant differences were found.

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confirm the findings of Nerbonne and colleagues for unidentified layer 5 pyramidal cells from visual cortex of K₄,4.2- and K₄,4.3-knockout mice (Carrasquillo et al. 2012; Nerbonne et al. 2008; Norris and Nerbonne 2010).

Our initial approach was to examine APs before and after application of 4 mM 4-AP, since this readily available agent has been shown to block Kᵥ4 channels and A-type current in many cell types (e.g., reviewed in Coetzee et al. 1999; Hille 2001). Application of 4 mM 4-AP resulted in a significant depolarization from RMP in layer 2/3 (from −73 ± 2 mV to −66 ± 1 mV, n = 8, P < 0.004), etv1 (from −69 ± 2 mV to −65 ± 2 mV, n = 11, P < 0.01), and glt (from −67 ± 1 mV to −64 ± 2 mV, n = 9, P < 0.02) pyramidal cells. 4-AP had no effects on input resistance in layer 2/3 (8 cells: 137 ± 29 MΩ control vs. 135 ± 42 MΩ 4-AP) or glt (8 cells: 110 ± 21 MΩ control vs. 98 ± 20 MΩ 4-AP) cells but significantly increased input resistance in etv1 cells (from 77 ± 15 MΩ control to 98 ± 14 MΩ 4-AP, n = 7, P < 0.03).

Consistent with a role for Kᵥ4 channels in spike repolarization, AP HW was broadened by 4-AP in all three pyramidal cell types (Fig. 3; layer 2/3: P < 0.0001, etv1: P < 0.0001, glt: P < 0.003). A small but significant hyperpolarizing shift in AP voltage threshold was observed for glt cells (P < 0.03) but not layer 2/3 or etv1 cells (Fig. 3). The average increase in AP HW varied from 36 ± 5% in etv1 cells (n = 11) to 42 ± 7% in glt (n = 9) and 48 ± 6% in layer 2/3 pyramidal cells (n = 9). Width at threshold was significantly increased by 4-AP (P < 0.0001 for layer 2/3; P < 0.003 for etv1; P < 0.02 for glt) and dV/dt for repolarization was significantly decreased (P < 0.0001 for layer 2/3 and glt; P < 0.001 for etv1) in all three subtypes. These effects of 4-AP (broader APs and reduced dV/dt for repolarization) are consistent with a role for Kᵥ4 channels in AP repolarization in all cell types, although the effects on input resistance and RMP suggest additional actions of 4-AP on channels other than Kᵥ4. There was also a significant decrease of dV/dt for the AP upstroke with 4-AP in all cell types (P < 0.001 for layer 2/3; P < 0.007 for etv1 and glt), suggesting a possible influence of a 4-AP-sensitive current on the rate of AP repolarization, nonspecific effects of 4-AP on Na⁺ currents, or Na⁺ current rundown under our recording conditions.

We next tested the effects of BaCl₂, another agent reported to block A-type current relatively selectively in hippocampal pyramidal cell dendrites (Harnett et al. 2013; Losończy and Magee 2006) and heart (Li et al. 1998, 2000) at submillimolar doses. We found that 150 μM BaCl₂ caused a large depolarization in layer 2/3 (from −70 ± 1 mV to −61 ± 2 mV, n = 11, P < 0.0001), etv1 (from −73 ± 2 mV to −66 ± 1 mV, n = 8, P < 0.002), and glt (from −65 ± 1 mV to −57 ± 3 mV, n = 7, P < 0.02) pyramidal cells. Input resistance was also significantly increased by BaCl₂ in etv1 (from 137 ± 24 MΩ to 213 ± 38 MΩ, n = 7, P < 0.04) and glt (from 57 ± 8 MΩ to 115 ± 20 MΩ, n = 7, P < 0.02) cells but not layer 2/3 pyramidal cells (from 148 ± 14 MΩ to 187 ± 22 MΩ, n = 11, P < 0.08). We also found that BaCl₂ dramatically broadened the AP. This was true for width at threshold (layer 2/3 P < 0.0001; etv1 P < 0.001; glt P < 0.0001) and AP HW (layer 2/3 P < 0.0001; etv1 P < 0.02; glt P < 0.007) (Fig. 4). BaCl₂ also reduced the repolarization dV/dt (Fig. 4) in all three pyramidal cell subtypes (layer 2/3 and etv1 P < 0.0001; glt P < 0.0003). Spike broadening was especially dramatic with BaCl₂ [64 ± 8% in layer 2/3 (n = 11), 123 ± 33% in etv1 (n = 8), 338 ± 89% in glt (n = 7)].

Finally, we compared APs of the three pyramidal cell subtypes before and after application of the peptide blocker AmmTx3 (200 nM). Rudy and colleagues showed that this agent is highly selective for channels containing Kᵥ4 α-subunits in association with DPP6 or DPP10 auxiliary subunits (Maffie et al. 2013). We found that unlike 4-AP and BaCl₂, AmmTx3 had no effects on RMP (etv1 (10 cells): control −73 ± 0.1 mV vs. −73 ± 0.4 mV AmmTx3; glt (10 cells): control −73 ± 0.4 mV vs. −74 ± 0.3 mV AmmTx3; layer 2/3 (5 cells): control −71 ± 0.9 mV vs. −72 ± 0.5 mV AmmTx3) or input resistance [etv1 (10 cells): control 83 ± 9 MΩ vs. 81 ± 9 MΩ AmmTx3; glt (10 cells): control 91 ± 11 MΩ vs. 103 ±
17 MΩ AmmTx3; layer 2/3 (5 cells): control 273 ± 51 MΩ and 201 ± 42 MΩ AmmTx3) in any pyramidal cell type. AmmTx3 application resulted in significant AP broadening (HW and width at threshold) and significant reduction of dV/dt for repolarization in all pyramidal cell subtypes tested, with no changes in voltage threshold (Fig. 5, Table 4). The percent change in AP HW was 22 ± 5% in layer 2/3 cells (n = 11), 10 ± 3% in etv1 cells (n = 10), and 18 ± 4% in glt cells (n = 10). There was a trend for decrease of dV/dt for the AP upstroke with time in all cell types that was significant in glt cells (P < 0.004), suggesting a role for Kv4 channels in regulating the AP upstroke, possible nonspecific effects on Na+ channels, or rundown of Na+ channels under our recording conditions.

The magnitude of the effects of 4-AP and BaCl2 (as well as effects on RMP and input resistance not seen with AmmTx3) led us to directly test whether BaCl2 or 4 mM 4-AP selectively blocked the A-type component of the current in neocortical pyramidal cells or if these agents also have effects on the sustained current (Kv1, Kv2, Kv7 mediated). We compared the effects of the blockers on the early peak current (dominated by A-type current in most cells) vs. current at the end of a 500-ms step to +10 mV (operationally defined as “steady-state” current). We tested outside-out patches from both etv1 and glt pyramidal cells or if these agents also have effects on the

Fig. 4. Effects of 150 μM BaCl2 on the action potential. A: representative traces for single APs in a layer 2/3 pyramidal neuron before and after application of 150 μM BaCl2. Inset: same APs at longer time base. B: summary data for AP half-width (AP HW) in layer 2/3 pyramidal cells. C: summary data for AP voltage threshold (Threshold) in layer 2/3 pyramidal cells. D: representative traces for single APs in an etv1 pyramidal neuron (layer 5) before and after application of 150 μM BaCl2. Inset: same APs at longer time base. E: summary data for AP half-width (AP HW) in etv1 pyramidal cells. F: summary data for AP voltage threshold (Threshold) in etv1 pyramidal cells. G: representative traces for single APs in a glt pyramidal neuron (layer 5) before and after application of 150 μM BaCl2. Inset: same APs at longer time base; scale also applies to A and G. Inset: same APs at longer time base; scale also applies to insets in A and D. H: summary data for AP half-width (AP HW) in glt pyramidal cells. I: summary data for AP voltage threshold (Threshold) in glt cells. *Significant difference between control and drug (paired t-test, P < 0.05).
cells. The results were similar for both cell types, so the data were pooled for the purpose of testing blocker specificity. For 4 mM 4-AP, the block of the peak current was 56 ± 16% (n = 6 cells) and the steady-state current (at 500 ms) was blocked by 8 ± 3% (n = 6) (Fig. 6, A and D). BaCl$_2$ (150 μM) blocked 56 ± 6% (n = 12) of the peak current and 48 ± 9% (n = 12) of the steady state current (Fig. 6, B and D). AmmTx3 blocked 42 ± 9% (n = 12) of the peak and 7 ± 2% (n = 12) of the steady-state current (Fig. 6, C and D).

These findings indicate that in neocortical pyramidal cells 150 μM BaCl$_2$ is not at all selective for A-type or K$_V$4-mediated currents but also substantially blocks other components of the K$^+$ current. Besides voltage-gated currents, BaCl$_2$ blocked leak currents underlying RMP and input resistance (see above). 4-AP is considered a relatively nonselective drug that blocks Kv channels at millimolar doses (including Kv2; Kirsch and Drewe 1993). We found that 4-AP (4 mM) affected a conductance active at RMP in our cells but surprisingly was relatively selective for the peak current vs. steady state, suggesting a greater degree of selectivity for A current than BaCl$_2$. One caveat is the known effects of 4-AP on slowing the inactivation kinetics of K$_V$4 currents (Jackson and Bean 2007; Thompson 1982). Thus slowing of remaining K$_V$4 current may make less apparent the block of K$_V$ currents that normally inactivate slowly.

We found that the recently characterized peptide blocker AmmTx3 (200 nM; Maffie et al. 2013) was selective for the peak transient A-type current over the steady-state current (and did not affect input resistance or RMP) in neocortical pyramidal cells. These voltage-clamp data with AmmTx3 also indicate that dipeptidyl peptidase-like-proteins (DPP6 or DPP10)...

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**Table 4. Effects of specific K$_V$4 channel blocker AmmTx3 on action potential parameters: effects of 200 nM AmmTx3 vs. same-cell control**

<table>
<thead>
<tr>
<th>Layer 2/3</th>
<th>AP, mV</th>
<th>$V_{th}$, mV</th>
<th>HW, ms</th>
<th>Width at $V_{th}$, ms</th>
<th>$dV/dt$ up</th>
<th>$dV/dt$ down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>88 ± 3</td>
<td>−58.3 ± 1.0</td>
<td>1.03 ± 0.06</td>
<td>1.97 ± 0.16</td>
<td>282 ± 19</td>
<td>86 ± 6</td>
</tr>
<tr>
<td>AmmTx3</td>
<td>86 ± 3</td>
<td>−58.1 ± 1.3</td>
<td>1.27 ± 0.11*</td>
<td>2.55 ± 0.29*</td>
<td>246 ± 20*</td>
<td>70 ± 7*</td>
</tr>
</tbody>
</table>

The effects of specific blockers for the A-type current. For 4 mM 4-AP, the block of the peak current was 56 ± 16% (n = 6 cells) and the steady-state current (at 500 ms) was blocked by 8 ± 3% (n = 6) (Fig. 6, A and D). BaCl$_2$ (150 μM) blocked 56 ± 6% (n = 12) of the peak current and 48 ± 9% (n = 12) of the steady state current (Fig. 6, B and D). AmmTx3 blocked 42 ± 9% (n = 12) of the peak and 7 ± 2% (n = 12) of the steady-state current (Fig. 6, C and D).

Data are means ± SE for 11 (layer 2/3), 10 (etv1), and 10 (glt) cells. AP, action potential amplitude; $V_{th}$, voltage threshold; HW, half-width (width at half-amplitude from resting potential); width at $V_{th}$, width of AP at voltage threshold; $dV/dt$ up, rate of change of voltage for AP upstroke; $dV/dt$ down, rate of change of voltage for AP repolarization. *Significant difference from matched controls in paired t-test (P < 0.05).
are associated with at least some of the Kv4 subunits in neocortical pyramidal cells of mice.

**DISCUSSION**

It has been confirmed numerous times since the pioneering studies of Hodgkin and Huxley (1945) that the AP upstroke in various types of neurons is due to influx of Na$^+$ through voltage-gated Na$^+$ channels and that spike repolarization is due to Na$^+$ channel inactivation and the activation of K$^+$ channels (Bean 2007; Hille 2001). Which specific types of K$^+$ channels underlie AP repolarization in different types of neurons is less well understood. In this study, we tested potential roles for Kv1, Kv2, and Kv4 channels in the repolarization of single APs recorded from the soma in three classes of pyramidal cells in mouse neocortex: two types of layer 5 cells genetically defined by expression of either evtl or glt and layer 2/3 pyramidal cells. We blocked Kv1 channels with DTX and Kv2 channels with GxTx at doses that we had previously determined to be selective with voltage-clamp experiments on rodent pyramidal cells. Additionally, we tested the selectivity of three putative Kv4 blockers (4-AP, BaCl$_2$, and AmmTx3) for A-type currents in voltage-clamp experiments using outside-out macropatches from pyramidal cell somas. We determined that only AmmTx3 was selective for the transient, A-type current in these cells.

On the basis of studies by the Nerbonne lab (Carrasquillo et al. 2012; Norris and Nerbonne 2010; Yuan et al. 2005), we hypothesized a major role for Kv4 channels in AP repolarization in neocortical pyramidal cells. We also hypothesized that the mechanisms underlying AP repolarization would be pyramidal cell subtype specific. In all three pyramidal cell subtypes tested, we found that the width of the AP and rate of AP repolarization were controlled by Kv4 channels. Blockade of Kv2 channels with GxTx did not affect any parameters of single APs in any cell type. The roles of Kv1 channels in shaping single APs were cell type dependent. In evtl cells and layer 2/3 cells (but not glt cells), block of Kv1 channels resulted in broader APs and reduced the rate of repolarization. AP voltage threshold was hyperpolarized by the Kv1 channel blocker DTX in all three subtypes of pyramidal neurons.

$K_{\text{v}1}$ DTX is a selective blocker of channels containing the Kv1.1, Kv1.2, or Kv1.6 subunits (Harvey and Robertson 2004). Consistent with a subthreshold activation range and relatively rapid activation kinetics (Guan et al. 2006) for Kv1 channels, we found that block of Kv1 channels with DTX resulted in a more hyperpolarized voltage threshold for single APs generated by brief current injections in both layer 5 pyramidal cell types and layer 2/3 cells. Previously, DTX-sensitive Kv1 channels were implicated in voltage threshold determination in rat layer 5 (Bekkers and Delaney 2001) and layer 2/3 (Guan et al. 2007b; Higgs and Spain 2011) neurons, as well as CA1 pyramidal neurons (Giglio and Storm 2014). It is interesting that Kv1 channels were more effective in regulating $V_{\text{th}}$ in our experiments than Kv4 channels, which activate even more rapidly and at more negative potentials (Carrasquillo et al. 2012; Guan et al. 2011b; Norris and Nerbonne 2010). Perhaps the strong expression of Kv1 channels at the axonal initial segment (Kole et al. 2007; Shu et al. 2007) grants these channels a greater role in regulating the voltage threshold in the soma. We found that in glt neurons block of Kv1 channels with DTX did not result in changes in AP width or rate of repolarization, consistent with results for somatic APs in rat layer 2/3 (Guan et al. 2007b) and rat and ferret layer 5 (Kole et al. 2007; Shu et al. 2007). In contrast, block of Kv1 channels broadened AP width and reduced repolarization rate in mouse evtl cells and layer 2/3 cells. In rat pyramidal neurons from layer 2/3 and layer 5, Kv1 channels were found to have little impact on AP repolarization in the soma (Guan et al. 2007b; Kole et al. 2007; Shu et al. 2007) but play a major role in the initial segment and axon (Kole et al. 2007; Shu et al. 2007). These findings suggest species differences in the role of somatic Kv1 channels or the influence of axonal Kv1 channels on somatic APs.

As a caveat, it should be noted that Kv1.4 channels have been shown to be a contributor to A-type current in pyramidal cells from rat visual cortex (Carrasquillo et al. 2012) and DTX would not block Kv1.4 subunits (unless they were expressed in heteromeric channels with Kv1.1, 1.2, or 1.6 $\alpha$-subunits). Kv1.4-containing channels would be 4-AP sensitive. Interestingly, Nerbonne and colleagues showed that genetic knockdown of Kv1.4 channels paradoxically increased the rate of AP repolarization (Carrasquillo et al. 2012) due to upregulation of Kv4 channels.

$K_{\text{v}2}$. We found that block of Kv2 channels with 100 nM GxTx did not affect AP parameters in any of the three mouse pyramidal cell subtypes, consistent with our previous findings in rat layer 2/3 and layer 5 cells with genetic manipulation of K2 channel expression and function (Guan et al. 2013). In concurrently conducted experiments, we showed that this dose of GxTx blocks substantial Kv2-mediated currents in both evtl and glt neurons (Bishop et al. 2015). These data are also consistent with the relatively slow activation kinetics and depolarized activation voltage range for Kv2,1-containing channels (Guan et al. 2007a; Murakoshi and Trimmer 1999). The lack of a GxTx effect on the AP also suggests minimal block of Kv3 channels by 100 nM GxTx in our hands. Liu and Bean (2014) observed modest spike broadening in CA1 pyramidal cells with GxTx. Honigsperger et al. (2013) also observed that GxTx blocked $I_h$ but slowed AP repolarization only after the first AP during repetitive firing in ERC stellate cells (layer 2). Both CA1 pyramidal cells and ERC pyramidal cells typically have broader APs than neocortical pyramidal cells, which may allow for the relatively kinetically slow Kv2 channels to contribute to repolarization.

$K_{\text{v}4}$. The rapid activation kinetics and relatively hyperpolarized activation range of Kv4 channels suggest that they could contribute to repolarization of the AP in neocortical pyramidal cells (Carrasquillo et al. 2012; Guan et al. 2011b; Norris and Nerbonne 2010). Our data with the selective Kv4 blocker AmmTx3 (200 nM), which slowed AP repolarization (and depolarization) and increased AP width, suggest that $K^+$ channels from the Kv4 family contribute to AP repolarization in layer 2/3 and both evtl and glt layer 5 neocortical pyramidal cells. AmmTx3 had no effect on RMP or input resistance, and our voltage-clamp experiments showed that AmmTx3 was selective for the initial transient, A-type current vs. the persistent current.

Our voltage-clamp data also show that 150 $\mu$M BaCl$_2$ is not selective for the transient, A-type current. In addition, 4-AP (mM) and BaCl$_2$ also had effects on RMP and input resistance that likely reflect block of other $K^+$ channels besides Kv4. At the doses we used, 4-AP (and especially BaCl$_2$) had larger...
effects on APs than AmmTx3. This may be partly due to quantitatively larger block of the A current by 4-AP and BaCl\(_2\) at these doses but is also likely due to additional effects of 4-AP and BaCl\(_2\) on other K\(_v\) channels (e.g., K\(_v\)1, K\(_v\)2) and non-K\(_v\) K\(^+\) channels (e.g., BaCl\(_2\) is known to block inwardly rectifying GIRK and IRK channels; Coetzee et al. 1999). Interestingly, blocking other potential K\(_v\) targets of 4-AP and BaCl\(_2\) individually (e.g., K\(_v\)1 and K\(_v\)2) with more specific blockers did not cause significant AP broadening or slowing of repolarization. We interpret these data as follows: K\(_v\)4 channels provide a primary AP repolarizing conductance for all neocortical pyramidal cells. When K\(_v\)4 channels are blocked, this results in a broader AP. With the doses we used, 4-AP and BaCl\(_2\) blocked more A-type current than AmmTx3 and thus had greater effects of AP repolarization and width. Additionally, part of the effect of 4-AP and especially BaCl\(_2\) may reflect that when APs are broadened by K\(_v\)4 block, K\(_v\)1 and K\(_v\)2 channels will then be able to contribute to repolarization of the broader APs. Consistent with this interpretation, TEA and BaCl\(_2\) (400 \(\mu\)M) had much greater effects on AP repolarization in K\(_v\)4,2-knockout animals (Carrasquillo et al. 2012).

Our findings are consistent with studies from Nerbonne and colleagues (Carrasquillo et al. 2012; Norris and Nerbonne 2010) in which genetic manipulation of K\(_v\)4 expression/function significantly broadened APs from unidentified rat and mouse layer 5 pyramidal cells. They found that more prolonged reduction in K\(_v\)4 expression may also lead to remodeling of K\(^+\) currents and compensatory changes in AP repolarization (Nerbonne et al. 2008). Our data are also consistent with single-channel studies of Kang et al. (2000), which indicate that A-type channels are active during AP repolarization in neocortical pyramidal cells. K\(_v\)4 channels also play a prominent role in repolarization of APs in CA1 pyramidal cells (Andràsfalvy et al. 2008; Chen et al. 2006; Kim et al. 2005) and cerebellar granule cells (Shibata et al. 2000). A 4-AP-sensitive A-type current has also been shown to be the primary repolarizer of the AP in neonatal auditory spiral ganglion neurons (Jagger and Housley 2002), nodose ganglion type 1C neurons (Ducrue and Puizillout 1995), and rat vagal motoneurons (Sah and McLachlan 1992).

Functional implications. AP amplitude and width are important variables regulating potential maximum firing rates, the effectiveness of AP backpropagation into the dendrites, and the amount of Ca\(^{2+}\) entry into cells (Bean 2007). While this is obviously true in nerve terminals for regulating neurotransmitter release (Jackson et al. 1991), [Ca\(^{2+}\)]\(_i\) is also regulated by somatic and dendritic APs in neocortical pyramidal neurons (Abel et al. 2004; Helmcchen et al. 1996). Importantly, since most Ca\(^{2+}\) entry occurs during the repolarization of the AP and afterwards in these cells, the rate of repolarization strongly regulates Ca\(^{2+}\) entry (Stewart and Foehring 2001). Somatic [Ca\(^{2+}\)]\(_i\), is important for the activation of afterhyperpolarization currents, which regulate the temporal structure of pyramidal cell firing and somatic integration of inputs (Abel et al. 2004) as well as gene regulation and activation of enzymatic activity (Ghosh et al. 1994). Excessive Ca\(^{2+}\) entry and [Ca\(^{2+}\)]\(_i\) may lead to cell death after seizures or stroke or during neurodegenerative diseases (Roselli and Caroni 2015).

Recent work from our lab (Guan et al. 2015) and others (Avesar and Gulledge 2012; Brown and Hestrin 2009; Dembrow et al. 2010; Groh et al. 2010; Hattox and Nelson 2007; Larkman and Mason 1990; Le Bé et al. 2007; Mason and Larkman 1990; Schwindt et al. 1997; Sheets et al. 2011; Suter et al. 2013) has revealed differences in AP properties between pyramidal cells in different layers or with different genetic markers, anatomy, or projections. Most relevant to the present work, layer 5 pyramidal cells expressing the etv1 gene have much narrower APs and more rapid repolarization compared with etvl cells or layer 2/3 pyramidal cells (Groh et al. 2010; Guan et al. 2015). Groh et al. (2010) showed that etvl cells are a subset of IT-type neurons (Reiner et al. 2003; Suter et al. 2013) and etvl cells a subset of PT-type neurons (Reiner et al. 2003; Suter et al. 2013). etvl and PT-type pyramidal cells have narrower APs and exhibit fast, nonadapting firing patterns, and etvl and IT-type neurons have broader APs, fire slower, and exhibit strong spike frequency adaptation (Avesar and Gulledge 2012; Brown and Hestrin 2009; Dembrow et al. 2010; Groh et al. 2010; Guan et al. 2015; Hattox and Nelson 2007; Larkman and Mason 1990; Le Bé et al. 2007; Mason and Larkman 1990; Sheets et al. 2011; Suter et al. 2013).

We have shown that AmmTx3-sensitive (K\(_v\)4 \(\alpha\)-subunits associated with DPP6 or DPP10) channels play a critical role in repolarizing the APs of all of the pyramidal cell groups tested and that there are subtype-dependent differences in the effectiveness of K\(_v\)1 channels for AP repolarization. PT-type glt cells have narrower APs than IT-type etvl cells or layer 2/3 pyramidal cells (Groh et al. 2010; Guan et al. 2015). The relatively exclusive role for K\(_v\)4 channels in repolarization of glt cells likely reflects the rapid kinetics and relatively hyperpolarized activation range of K\(_v\)4 channels vs. K\(_v\)1 and K\(_v\)2 channels. The narrower APs in glt neurons might reflect greater expression or different biophysical properties of K\(_v\)4 channels in those cells vs. IT-type etvl or layer 2/3 cells. The role of K\(_v\)1 channels in etvl cells and layer 2/3 cells may be permitted by their relatively broader APs compared with glt cells. One might expect greater expression of K\(_v\)1 current in etvl and layer 2/3 cells. Another prediction is that glt cells may show more rapid spike broadening during repetitive activity than etvl or layer 2/3 cells because of the reliance of glt cells on the rapidly inactivating K\(_v\)4 channels. Differential mechanisms for AP repolarization also provide a substrate for differential modulation of APs by transmitters. We are currently testing these predictions.

Summary. We found that K\(_v\) channels of the K\(_v\)4 type repolarize the AP in all tested neocortical pyramidal cell subtypes but there are also subtype-specific differences in AP repolarization mechanisms. While only K\(_v\)4 channels are involved in AP repolarization in the rapidly repolarizing glt cells, the broader APs in layer 2/3 and etvl cells are also partially repolarized by K\(_v\)1 channels. Because of their activation in the subthreshold range, K\(_v\)1 channels play an important role in regulating AP voltage threshold in all pyramidal cell subtypes.

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