Postnatal development of A-type and Kv1- and Kv2-mediated potassium channel currents in neocortical pyramidal neurons

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Guan D, Horton LR, Armstrong WE, Foehring RC. Postnatal development of A-type and Kv1- and Kv2-mediated potassium channel currents in neocortical pyramidal neurons. J Neurophysiol 105: 2976–2988, 2011. First published March 30, 2011; doi:10.1152/jn.00758.2010.—Potassium channels regulate numerous aspects of neuronal excitability, and several voltage-gated K+ channel subunits have been identified in pyramidal neurons of rat neocortex. Previous studies have either considered the development of outward current as a whole or divided currents into transient, A-type and persistent, delayed rectifier components but did not differentiate between current components defined by α-subunit type. To facilitate comparisons of studies reporting K+ currents from animals of different ages and to understand the functional roles of specific current components, we characterized the postnatal development of identified Kv channel-mediated currents in pyramidal neurons from layers II/III from rat somatosensory cortex. Both the persistent/slowly inactivating and transient components of the total K+ current increased in density with postnatal age. We used specific pharmacological agents to test the relative contributions of putative Kv1- and Kv2-mediated currents (100 nM α-dendrotoxin and 600 nM stromatoxin, respectively). A combination of voltage protocol, pharmacology, and curve fitting was used to isolate the rapidly inactivating A-type current. We found that the density of all identified current components increased with postnatal age, approaching a plateau at 3–5 wk. We found no significant changes in the relative proportions or kinetics of any component between postnatal weeks 1 and 5, except that the activation time constant for A-type current was longer at 1 wk. The putative Kv2-mediated component was the largest at all ages. Immunochemistry indicated that protein expression for Kv4.2, Kv4.3, Kv1.4, and Kv2.1 increased between 1 wk and 4–5 wk of age.

Kv channel; somatosensory cortex; voltage clamp; A current; delayed rectifier

At birth, the rodent somatosensory system is immature, and somatosensory cortex undergoes dramatic changes over the first postnatal month. Neocortical pyramidal cells are generated prenatally in an “inside-out” laminar pattern (Caviness 1982; Caviness et al. 2009; Takahashi et al. 1994), and pyramidal cell morphology and physiology mature after birth (Bahrey and Moody 2003; Beique et al. 2004; Blue and Parnevalas 1983; Christophe et al. 2005; Kasper et al. 1994a, 1994b; Kriegstein et al. 1987; Krist 1978; Lorenzon and Foehring 1993; Maravall et al. 2004a, 2004b; McCormick and Prince 1987; Metherate and Aramkis 1999; Oswald and Reyes 2008; Tyzio et al. 2003). Repetitive firing behavior also changes with age (Bahrey and Moody 2003; Christophe et al. 2005; Kasper et al. 1994a, 1994b; Lorenzon and Foehring 1993; Maravall et al. 2004a, 2004b; McCormick and Prince 1987; Metherate and Aramkis 1999; Oswald and Reyes 2008; van Brederode et al. 2000; Zhang 2004). In mature pyramidal neurons, voltage-gated K+ channels contribute to the filtering and integration of synaptic inputs, as well as shaping firing rates and pattern (Guan et al. 2007a; Higgs and Spain 2009; Schwindt et al. 1988). Developmental changes in these channels should therefore play significant roles in the maturation of pyramidal cell function.

Previous electrophysiological studies of development have considered the outward current as a whole, divided currents into transient A-type and persistent delayed rectifier (DR) components (Bahrey and Moody 2003; Hamill et al. 1991; Zhou and Hablitz 1996a), or separated current components based upon inactivation kinetics (Foehring and Surmeier 1993; Locke and Nerbonne 1997a, 1997b). Much of the literature also concerns data from immature animals over limited age ranges that vary between laboratories. Little is known, however, about development of current components identified by molecular composition.

Several voltage-gated potassium channel (Kv) subunits and currents have been identified in the pyramidal neurons of juvenile rat cortex (Bekkers 2000a, 2000b; Guan et al. 2005, 2006, 2007b; Hamill et al. 1991; Korngreen and Sakmann 2000; Nerbonne et al. 2008; Norris and Nerbonne 2010; Yuan et al. 2005), and developmental changes in action potential (AP) and firing properties suggest that Kv channel subunits may be developmentally regulated. To help understand the functional roles of specific current components and to facilitate comparisons between studies reporting K+ currents from animals of different ages, we systematically characterized the expression of the total potassium current and the relative contributions of putative Kv1- and Kv2-mediated current and
A-type current during the first 5 wk of postnatal development of pyramidal cells from rat somatosensory cortex.

METHODS

Tissue Preparation

These studies were performed on Sprague-Dawley rats aged P3–P38. All procedures were approved by the Animal Care and Use Committee, University of Tennessee Health Science Center. Briefly, the animal was placed into a sealed plastic container in which gauze soaked with isofluorane was placed under a fiberglass screen floor. The rats were anesthetized with isofluorane until they were areflexive. The animals were decapitated, and the brain was removed and held in ice-cold cutting solution. The cutting solution contained (in mM) 250 sucrose, 2.5 KCl, 1 NaH$_2$PO$_4$, 11 dextrose, 4 MgSO$_4$, 0.1 CaCl$_2$, 15 HEPES (pH = 7.3–7.4; 300 mosmol/kgH$_2$O). Four hundred-micrometer coronal slices of the frontal-parietal regions were cut with a vibrating tissue slicer (World Precision Instruments, Sarasota, FL). The slices were then transferred to a mesh surface in a chamber containing artificial cerebrospinal fluid (aCSF), which was continuously bubbled with a 95% O$_2$–5% CO$_2$ (carbogen) mixture at room temperature. The aCSF contained (mM) 125 NaCl, 3 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, and 20 dextrose (pH = 7.4; 310 mosmol/kgH$_2$O).

Acute Isolation of Neurons

The supragranular layers (I–III) of primary somatosensory cortex were dissected from brain slices into 2- to 3-mm-wide pieces. Four to eight tissue pieces were then transferred to oxygenated aCSF (35°C) with added enzyme (Sigma protease type XIV, 1.2 mg/ml; Sigma Chemicals, St. Louis, MO). After 10–30 min of incubation in enzyme, the tissue pieces were washed with sodium isethionate solution, which consisted of (in mM) 140 sodium isethionate, 2 KCl, 4 MgCl$_2$, 0.1 CaCl$_2$, 23 dextrose, 15 HEPES (pH = 7.3; 300 mosmol/kgH$_2$O). This solution and the enzyme-treated tissue pieces were triturated with three successively smaller fire-polished pipettes to release individual neuronal somata. The supernatant from each trituration step was collected, transferred to a fresh container, and plated onto a plastic dish (Nunc, Rochester, NY) on an inverted microscope stage. Neuronal somata. The supernatant from each trituration step was collected, transferred to a fresh container, and plated onto a plastic dish (Nunc, Rochester, NY) on an inverted microscope stage. The slices were then transferred to a mesh surface in a chamber containing artificial cerebrospinal fluid (aCSF), which was continuously bubbled with a 95% O$_2$–5% CO$_2$ (carbogen) mixture at room temperature. The aCSF contained (mM) 125 NaCl, 3 KCl, 2 CaCl$_2$, 2 MgCl$_2$, 1.25 NaH$_2$PO$_4$, 26 NaHCO$_3$, and 20 dextrose (pH = 7.4; 310 mosmol/kgH$_2$O).

Electrophysiology

Whole cell patch-clamp recordings were performed on acutely dissociated pyramidal neurons at room temperature (20–22°C). Pyramidal cells were identified by soma shape and the presence of a single apical dendrite (of <25 μm in length). A multibarrel array of glass capillaries (500-μm outer diameter) was used to apply external recording solutions. External solutions were changed by moving the active barrel (from which the solution flows) so as to center the flow upon the recorded cell. The external solution for isolating the outward K$^+$ current contained (mM) 140 sodium isethionate, 3 KCl, 1 MgCl$_2$, 12 dextrose, 10 HEPES, 1.9 CaCl$_2$, 0.001 tetrodotoxin (TTX) for blocking Na$^+$ channels, and in most experiments, 0.1 CdCl$_2$ for blocking Ca$^{2+}$ channels.

Voltage-clamp recordings were made with a DAGON 8900 (Minneapolis, MN) amplifier. Corning 8250 capillary glass (Garner Glass, Claremont, CA) was used to create electrodes on a model P-87 Flaming/Brown micropipette puller (Sutter Instruments, Novato, CA). Electrodess were fire-polished and filled with an internal solution consisting of (mM) 85 potassium methylsulfate, 63 KOH$_2$, 2 MgCl$_2$, 30 HEPES, 2 adenosine triphosphate disodium (ATP), 0.2 guanosine 5’-triphosphate sodium salt (GTP), 15 creatine phosphate, 0.1 leupeptin, 10 1,2-bis(2-aminophenoxy)ethane-N,N’,N”-N”-tetraacetic acid (BAPTA: pH = 7.2; 270 mosmol/kgH$_2$O). Electrode resistances were between 10–18 MΩ. Series resistance was usually compensated by 70–90%. Cells with calculated series resistance errors of >5 mV were discarded [series resistance error (mV) = series resistance after compensation (MΩ) multiplied by peak current (pA)]. Membrane potentials were corrected for the measured liquid junction potential (+8 mV). K$^+$ currents were filtered at a cutoff frequency of 5 kHz and sampled at a rate of 20 kHz. Linear leak currents and capacitative artifacts were subtracted with an online P/4 or P/6 protocol. All measurements and recordings were conducted with pCLAMP 8 software (Axon Instruments, Union City, CA) within ~5 min after obtaining whole cell mode.

Current Isolation

We assessed the amplitude of outward K$^+$ currents by 500-ms steps to +10 mV from a holding potential (HP) of −70 mV. The test steps were repeated at intervals of 15 s. Unless otherwise noted, we included TTX (1 μM) and 100 μM Cd$^{2+}$ in the extracellular solutions to block Na$^+$ and Ca$^{2+}$ currents, respectively. The internal solution included 10 mM BAPTA to prevent activation of Ca$^{2+}$-dependent currents. Current components due to Kv1 or Kv2 subunits were isolated pharmacologically. All peptide toxins were obtained from Alomone Labs (Jerusalem, Israel). BSA (0.1%) was added to the external solutions to prevent peptides from binding to glass and plastic vessels. We previously determined dose-response relationships for the blockers, and we chose doses that were specific for the relevant subunits (Guan et al. 2006, 2007b). To study putative Kv1-mediated current (Guan et al. 2006), we applied 100 nM α-dendrotoxin (DTX, blocks current through channels containing Kv1.1, Kv1.2, or Kv1.6 subunits; Harvey and Robertson 2004) and 10–30 nM margatoxin (MTX, blocks Kv1.3-containing channels; Garcia-Calvo et al. 1993). The putative Kv1-mediated current was defined as the difference between currents recorded in control solution and currents in the presence of DTX plus MTX. The doses for DTX and MTX were previously measured this run-down and noted that after a stable period of 5 min after obtaining whole cell.

Run-down of current can be an issue in K$^+$ current recordings. We previously measured this run-down and noted that after a stable period of 3–5 min the current runs down. The current that runs down first has very slow activation kinetics compared with the putative Kv1 or Kv2 components (Guan et al. 2006, 2007a). All of our data for percent block were obtained within the initial stable period for current amplitude. In addition, we continually monitored series resistance (see above). Since recordings are typically <10 min, reported changes in cell input resistance after long recordings with K-methyl sulfate internals (Kaczorowski et al. 2007) are minimized.

For experiments designed to study the rapidly activating and inactivating A-type current, we used an external recording solution without Cd$^{2+}$ present because Cd$^{2+}$ shifts the voltage dependence of both activation and inactivation of Kv4 channels to more depolarized potentials and slows their kinetics (Guan et al. 2007b; Song et al. 1998; Wickenden et al. 1999). Our initial method for isolating the
A-type current was via a voltage protocol. DTX and MTX were present in all recordings to block Kv1-mediated currents. A -40 mV HP was used to inactivate most of the outward current. After an initial period at -40 mV to allow the holding current to stabilize, a step is made to +10 mV for 500 ms. The cell is returned to -40 mV for 5 s, and then a prepulse to -70 mV (150-ms duration) is applied to allow preferential recovery of the A-type current. This prepulse duration is long enough to recover most of the A current (τA/recovery = 61 ± 5 ms, n = 3) but is too short to recover the majority of the DR currents (Foehring and Surmerek 1993; Guan et al. 2006, 2007b). We compared all age groups, using a test potential to +10 mV for 500 ms, repeated every 15 s. A-type current was defined as the difference between current during the test potential after the prepulse and current during a test pulse without the prepulse. Curve fitting was used to further separate the A current from DR currents (see RESULTS). In an additional set of experiments, we studied steady-state activation and inactivation of A-type current at 1 wk and 4–5 wk of age. In those experiments, 30 mM TEA replaced equimolar NaCl in the external solution to further isolate the A current (together with biophysics and curve fitting).

Immunocytochemistry

Animals (n = 4 at P6–P9 and n = 4 at P26–P38) were anesthetized with pentobarbital sodium (50 mg/kg ip). The anesthetized animals were transcardially perfused with 0.01 M sodium phosphate buffer plus 0.89% NaCl (PBS) followed by PBS-buffered 4% paraformaldehyde and 0.2% picric acid. (For Kv4.2, we also used 4% paraformaldehyde without picric acid, postfixed for ~12 h, and washed overnight in 0.1 M PBS; Burkharter et al. 2006). Brains were removed, postfixed for ~12 h at 4°C, and then blocked and postfixed for ~24 h. Sections through the cortex were taken at 50 μm on a vibrating microtome (Leica VT1000S), rinsed in PBS, and incubated in 5% normal goat serum with 3% H2O2 for 1–2 h to reduce background staining. For Kv4.2, we also tried treatment with 0.5% sodium borohydride, followed by 0.3% H2O2 and then 50% ethanol (Burkharter et al. 2006). All antibodies used were monoclonal (NeuroMAB, Univ. of California, Davis, CA) and purchased from Antibodies Incorporated (Davis, CA). These included the K13/3/1 clone (Kv1.4), K57/1 clone (Kv4.2), K75/41 clone (Kv4.3), and K89/34 clone (Kv2.1). Antibodies were first tested at various concentrations (1:100 to 1:1,000) in PBS and 0.5% Triton X-100 (PBS-TX). After this dilution screening, the same concentration for a particular antibody was then used to compare across ages.

After overnight incubation with the primary antibody (1:200 to 1:500), the sections were incubated overnight in a secondary antibody (1:200) consisting of goat anti-mouse (GAM)-Alexa Fluor 594 or rabbit anti-mouse Alexa Fluor 568 (for Kv4.2 only) conjugates, all at 4°C. The sections were rinsed in PBS-TX, mounted on gelatin-coated slides, and coverslipped with a solution of polyvinyl alcohol (PVA: 2.4 g), 1,4-diazabicyclo[2.2.2]octane (DABCO: 0.625 g), glycerin (6 g), PBS (12 ml), and 6 ml of distilled water (recipe courtesy of Bioengineering Confocal and Multiphoton Imaging Core Facility, University of Pennsylvania; http://www.seas.upenn.edu/~confocal/antifade.html#PVA-DABCO%20Mounting%20Media). These sections were viewed with a Zeiss 710 confocal microscope using either a DPH (for Alexa Fluor 568; 561 nm peak excitation) or a HeNe (for Alexa 594; 594 nm peak excitation) laser. The optical section thickness was 1–2 μm. Most sections had full penetrance of primary antibody (~50 μm). Sections were viewed singly or in stacks of 5–12 optical sections. For comparative purposes across age, laser settings (pinhole, power, gain, and pixel resolution) were made from just below saturation in the oldest animals, where fluorescence was brightest, and reused for other ages. Images were captured with Zen10 software and saved in native and tiff formats. The final confocal figures were made in Canvas 8, without alteration in dynamic range.

Statistics

Data are presented as means ± SD in the text and tables and as means ± SE in figures. Prism Software version 4 (GraphPad Software, San Diego, CA) was used for statistical tests of significance. Student’s t-test was used to compare means of two samples. One-way ANOVA was used to test differences for three or more groups. If the ANOVA was significant, we used the post hoc Tukey’s test to determine which specific means were different. P values < 0.05 were considered significant.

RESULTS

Our isolation procedure restricted recordings to pyramidal cells from layers II–III (see METHODS) of rat primary somatosensory cortex. We used acutely dissociated neurons to allow normal in situ development before cell harvest and to facilitate spatial control of voltage (by removing cell processes) and rapid exchange of extracellular solutions. Because these cells have had the axon and dendrites removed, our conclusions are restricted to currents in the soma and proximal (<25 μm) apical dendrites. Total potassium current was studied in 140 neurons. The effects of combined Kv1 blockers (DTX + MTX) were studied in 109 cells. ScTx-sensitive current was studied in 56 cells. Some of the included Kv1 and Kv2 data from animals aged 4–5 wk were taken from our previous work (Guan et al. 2006, 2007a, 2007b). A-type currents were studied in the absence of extracellular Cd2+ in an additional group of 80 cells.

For analysis, we grouped the data according to animal age: 7 ± 3 days was considered week 1, 14 ± 3 days = week 2, 21 ± 3 days = week 3, 28 ± 3 days = week 4, and 35 ± 3 days = week 5. We used peak current and time to peak (TTP) to characterize the total potassium current and toxin-sensitive current at +10 mV and used the current at 500 ms to describe the “steady-state” amplitude of these currents. Figure 1 characterizes the population of neocortical pyramidal cells that we studied. Diameters of cell somata were measured with a graduated ocular scale under the microscope (n = 87). Whole cell capacitance was measured from the current in response to a 10-ms step from −70 mV to −80 mV as Q/10 mV (where Q is the total charge displaced by the step: the time integral of the current). For the recorded cells, there were no significant differences between age groups for soma diameters or whole cell capacitance (Kruskal-Wallis test; Fig. 1, A and B), suggesting that cells that survived the dissociation conditions and our selection process were of similar size at all ages. Membrane capacitance increased with soma diameter (Fig. 1B), and the histograms of soma diameters (P > 0.24) and membrane capacitance (P > 0.65) were normally distributed (D’Agostino and Pearson omnibus normality test; Fig. 1, C and D).

For comparison with earlier studies, we first studied the total outward potassium current as a function of postnatal age (Fig. 2). Currents were small at 1 wk (Fig. 2A) and increased with age. Peak current (and current density: current/whole cell capacitance) increased significantly with age (Fig. 2, A and C). The biggest increase was seen from week 1 to week 2, and a plateau was approached by 3–5 wk. When currents were scaled to the same peak amplitude, the currents generally superimposed (Fig. 2B). With this protocol, a fast, transient component was evident in many cells at all ages (virtually all cells have an A-type current that is revealed with a recovery from inactiva-
tion protocol as in Fig. 5). The ratio of the transient current (peak current – current at 500 ms) to the persistent current (current at 500 ms) was unchanged with age (0.12 ± 0.08 at week 1, 0.13 ± 0.09 at week 2, 0.09 ± 0.06 at week 3, 0.11 ± 0.08 at week 4, and 0.11 ± 0.05 at 5 wk).

Development of Specific Current Components

Slowly inactivating currents. Previously, we demonstrated isolation of putative Kv1- and Kv2-mediated currents from 4- to 5-wk-old rats with specific toxins (Guan et al. 2006, 2007a, 2007b). We assume here that the sensitivities of specific potassium channels to the toxins do not change over the age range tested. Test steps to +10 mV (500 ms) from a HP of −70 mV were repeated every 15 s, and toxin-sensitive currents were determined as subtracted currents. We used DTX (100 nM) together with 10–30 nM MTX to block putative Kv1 channel currents and 600 nM ScTx to block the putative Kv2 channel currents (METHODS; Fig. 3).

Kv1 mediated. Almost all cells at all ages expressed putative Kv1-mediated current (Fig. 4, Table 1). This current was very small at 1 wk and increased in amplitude with age (Fig. 4, A and C). This current made up 5–8% of the whole current at +10 mV, with no significant changes in this percentage with age (Table 1). Peak current and current density significantly increased during postnatal development (Fig. 4C; Table 1). Again, a large increase in amplitude occurred between weeks 1 and 2, with little change between weeks 3 and 5. At all ages, some cells expressed both transient and persistent components to the putative Kv1-mediated current. χ2 Analysis indicates that the percentage of cells with a transient component did not...
change with age (P = 0.71; week 1: 3/13 cells, week 2: 4/13 cells, week 3: 7/20 cells, week 4: 16/48 cells, week 5: 9/15 cells). When scaled to the same peak amplitude, currents from 1-wk and 5-wk cells superimposed (Fig. 4B), suggesting no changes in kinetics with age. Accordingly, we found no significant changes in the ratio of transient to persistent current, TTP, or activation time constant as a function of age (Table 1).

Kv2 mediated. All cells at all ages expressed a putative Kv2-mediated (ScTx sensitive) current (Fig. 4D; Table 2). This was the largest current component at all ages (Table 2). Similar...
Allow the holding current to stabilize, a two-pulse protocol was used. Table 1. Putative Kv1 current as a function of age

<table>
<thead>
<tr>
<th>Age</th>
<th>C_m</th>
<th>Peak Amp</th>
<th>Density</th>
<th>SS Amp</th>
<th>SS Density</th>
<th>Peak %</th>
<th>SS %</th>
<th>TTP</th>
<th>( \tau_{act} )</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>13.9 ± 3.7</td>
<td>143 ± 170*</td>
<td>10.1 ± 10.0</td>
<td>101 ± 116</td>
<td>7.2 ± 7.0</td>
<td>5.8 ± 5.2</td>
<td>4.8 ± 4.7</td>
<td>16.8 ± 8.2</td>
<td>2.9 ± 1.0</td>
<td>13</td>
</tr>
<tr>
<td>Week 2</td>
<td>13.8 ± 3.0</td>
<td>423 ± 115*</td>
<td>31.5 ± 8.6*</td>
<td>293 ± 59*</td>
<td>22.1 ± 6.1*</td>
<td>8.8 ± 2.9</td>
<td>7.0 ± 2.4</td>
<td>29.4 ± 10.6</td>
<td>4.0 ± 2.1</td>
<td>13</td>
</tr>
<tr>
<td>Week 3</td>
<td>13.4 ± 3.9</td>
<td>490 ± 275*</td>
<td>37.0 ± 21.0*</td>
<td>381 ± 237*</td>
<td>28.6 ± 17.3*</td>
<td>8.4 ± 3.8</td>
<td>6.8 ± 3.2</td>
<td>24.0 ± 16.0</td>
<td>3.9 ± 1.6</td>
<td>20</td>
</tr>
<tr>
<td>Week 4</td>
<td>14.1 ± 3.5</td>
<td>460 ± 286*</td>
<td>34.5 ± 22.6*</td>
<td>368 ± 257*</td>
<td>27.8 ± 20.1*</td>
<td>8.3 ± 4.5</td>
<td>6.9 ± 4.3</td>
<td>23.1 ± 10.8</td>
<td>3.8 ± 0.6</td>
<td>38</td>
</tr>
<tr>
<td>Week 5</td>
<td>13.9 ± 5.2</td>
<td>499 ± 286*</td>
<td>35.1 ± 12.7*</td>
<td>393 ± 282*</td>
<td>28.4 ± 14.9*</td>
<td>7.8 ± 2.9</td>
<td>6.2 ± 3.3</td>
<td>18.6 ± 4.1</td>
<td>4.3 ± 1.0</td>
<td>15</td>
</tr>
</tbody>
</table>

Values are means ± SD for n cells. Week 1 = 7 ± 3 days; week 2 = 14 ± 3 days; week 3 = 21 ± 3 days; week 4 = 28 ± 3 days; week 5 = 35 ± 3 days. α-Dendrotoxin (DTX, 100 nM) and margatoxin (MTX, 20 nM)-sensitive current. Holding potential (HP) = −70 mV. Step to +10 mV, C_m, whole cell capacitance (pF); peak amp, peak amplitude of current (pA); density, peak amplitude of current/whole cell capacitance (pA/pF); SS amp, peak amplitude of current at 500 ms after step onset (pA); SS density, current density at 500 ms after step onset/whole cell capacitance (pA/pF); peak %, (peak amplitude of DTX-sensitive/whole cell peak current) × 100; SS %, (amplitude of DTX-sensitive current at 500 ms/whole cell current at 500 ms) × 100; TTP, time to peak (ms); \( \tau_{act} \), activation time constant (ms). Fitting function = A(1 − exp(−t/\( \tau_{act} \))). *Significant difference from week 1.

Table 2. Putative Kv2 current as a function of age

<table>
<thead>
<tr>
<th>Age</th>
<th>C_m</th>
<th>Peak Amp</th>
<th>Density</th>
<th>SS Amp</th>
<th>SS Density</th>
<th>Peak %</th>
<th>SS %</th>
<th>TTP</th>
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</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>14.3 ± 3.8</td>
<td>1,633 ± 760*</td>
<td>115 ± 54†</td>
<td>1,408 ± 729*</td>
<td>99 ± 54</td>
<td>59.1 ± 18.4</td>
<td>57.5 ± 18.9</td>
<td>166 ± 70</td>
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<tr>
<td>Week 2</td>
<td>13.7 ± 3.7</td>
<td>3,224 ± 1,138*</td>
<td>239 ± 82*</td>
<td>2,579 ± 830*</td>
<td>194 ± 73</td>
<td>60.7 ± 10.1</td>
<td>54.6 ± 10.4</td>
<td>142 ± 69</td>
</tr>
<tr>
<td>Week 3</td>
<td>14.2 ± 3.7</td>
<td>4,090 ± 909*</td>
<td>303 ± 93*</td>
<td>3,242 ± 804*</td>
<td>245 ± 88</td>
<td>61.1 ± 9.7</td>
<td>52.3 ± 12.1</td>
<td>156 ± 75</td>
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<tr>
<td>Weeks 4–5</td>
<td>11.1 ± 2.2</td>
<td>3,989 ± 1,299*</td>
<td>357 ± 82†</td>
<td>3,491 ± 1,136†</td>
<td>322 ± 84†</td>
<td>65.6 ± 5.9</td>
<td>58.7 ± 6.6</td>
<td>182 ± 43</td>
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</table>

Values are means ± SD for n cells. Week 1 = 7 ± 3 days; week 2 = 14 ± 3 days; week 3 = 21 ± 3 days; weeks 4–5 = 25–38 days combined. Stromatoxin (ScTx, 600 nM)-sensitive current. HP = −70 mV. Step to +10 mV, C_m, whole cell capacitance (pF); peak amp, peak amplitude of ScTx-sensitive current (pA); density, peak amplitude of ScTx-sensitive/whole cell capacitance (pA/pF); SS amp, peak amplitude of ScTx-sensitive current at 500 ms after step onset (pA); SS density, ScTx-sensitive current at 500 ms after step onset/whole cell capacitance (pA/pF); peak %, (peak amplitude of ScTx-sensitive/whole cell peak current) × 100; SS %, (amplitude of ScTx-sensitive current at 500 ms/whole cell current at 500 ms) × 100; TTP, time to peak (ms). *Significant difference from week 1; †significant difference from week 2.
biophysical isolation and subsequent fit with Eq. 1 (Fig. 6A) in the same cells. The peak amplitudes were highly correlated (Fig. 6D; slope = 0.99, \( r^2 = 0.93, P < 0.05, n = 28 \)), as were the TTPs (\( r^2 = 0.93, P < 0.05, n = 28 \)), suggesting that both methods isolate the same A-type current component (Fig. 6D).

Isolation of A-Type Current by Fit to ScTx-Sensitive Current

ScTx has similar affinity for Kv2 subunits and Kv4 subunits (Escoubas et al. 2002). Thus in most cells the rising phase of the ScTx-sensitive outward current (tested in the presence of DTX, MTX, and 100 \( \mu \)M CdCl\(_2\)) was steeper than expected for Kv2-mediated current alone (e.g., Fig. 6E). We therefore also examined A-type current separated by fitting Eq. 1 to the ScTx-sensitive current (Fig. 6E). In these cells, the activation-inactivation of ScTx-sensitive current was well fit with Eq. 1, where \( I_{\text{DR}} \) is the Kv2-mediated persistent component and \( I_A \) is the A-type current. These data are consistent with a significant fraction of the A-type current being due to Kv4 subunits in these cells (cf. Norris and Nerbonne 2010).

Kinetics and Voltage Dependence of A Current

To study the kinetics and voltage dependence of A-type current in more detail, we studied additional cells at 1 wk and 4–5 wk, using modifications to the recovery from inactivation protocol used in Figs. 5 and 6. These cells were examined in the absence of extracellular Cd\(_2^+\) and in the presence of 30 mM TEA to block DR current. The cells were held at -40 mV to inactivate most of the potassium currents. We first used a two-pulse protocol to compare the currents in response to a step to +10 mV, with or without a 150 ms prepulse to -100 mV (to allow complete recovery of A-type current), followed by a 500-ms step to +10 mV (Fig. 7A, Table 4). At -100 mV, the recovery time constant was 36 ± 9 ms (\( n = 3 \)). Compared with experiments with recovery at -70 mV, currents were significantly larger after a step to -100 mV (Tables 3 vs. 4). In these cells, there was a small but significant difference in the time constant for activation with age: younger cells had a longer \( \tau \) (Fig. 7B, Table 4).

Steady-state activation was studied by varying the step after recovery (indicated by step C in protocol) at -100 mV to potentials between -40 mV and +50 mV (in 10-mV increments, Fig. 7C). Steady-state inactivation was studied by varying the recovery potential (indicated by step B in protocol) in 10-mV intervals between -110 mV and -40 mV (Fig. 7D). The resulting activation and inactivation curves are shown in Fig. 7E (1 wk) and Fig. 7F (4–5 wk). Half-activation voltages were -18 ± 1.0 mV at 1 wk and -19 ± 0.7 mV at 4 wk (corresponding slopes were 17 ± 0.3 and 17 ± 0.8 mV). The half-inactivation voltage was -82 ± 4.9 mV at 1 wk and -79 ± 2.5 mV at 4–5 wk (corresponding slopes were -8 ± 1.0 mV and -7.7 ± 0.8 mV). None of these values for half-activation or inactivation or slope differed significantly with age.

Immunocytochemistry

We compared cortex at 1 wk and 4–5 wk of age to test whether the developmental changes we observed for current components were accompanied by increased subunit protein expression. We found that Kv4.3 staining was found in the neuropil as well as somas and dendrites of pyramidal cells in all layers and that staining intensity was greater at 4–5 wk than in 1-wk-old postnatal animals (Fig. 8A and B). In our hands, staining for Kv4.2 was not detectable at 1 wk and was weak but evident as diffuse staining throughout cortex at 4–5 wk (data not shown). These data suggest an increase in Kv4.3 (and Kv4.2) protein with postnatal age, consistent with our electrical data. A similar increase in staining was observed with Kv1.4 (Fig. 8C), which has also been shown to contribute to A-type current in neocortical pyramidal cells (Norris and Nerbonne 2010). We also saw increased Kv2.1 staining intensity with age, although the pattern of clustered channels on soma/proximal dendrite was evident by 1 wk (Fig. 8D).

DISCUSSION

Voltage-gated K\(^+\) currents increase in amplitude with age in neocortical pyramidal neurons (Bahrey and Moody 2003; Ha-
Fig. 6. Separation of A-type current by biophysics (recovery from inactivation) + curve fitting or ScTx + curve fitting. A: traces from a pyramidal cell from a P28 animal. We isolated A-type current by a voltage protocol (inset, bottom), followed by further separation by fit to Eq. 1: $I(t) = I_{\text{rest}}(1 - e^{-t/\tau_a})e^{-t/\tau_c} + I_a(1 - e^{-t/\tau_c})e^{-t/\tau_c}$. The black trace is the original current trace, the green trace is the fitted A-type component, and the blue trace is the DR component. B: histogram for peak current density for the isolated A-type current as a function of age (current/Cm). *Significant difference from 1 wk. Number of cells: week 1, 10; week 2, 20; week 3, 12; weeks 4+5, 21. C: fit to whole current in response to step to +10 mV from -70 mV for the same cell as in A. The black trace is the whole current, the green trace is the fitted A-type component, and the red trace is the combined fit to the A-type and DR components. D: there was a significant correlation between the amplitude of A-type current isolated by curve fit from the whole current compared with biophysical separation (+ curve fit to remove residual DR). E: example of current sensitive to 600 nM ScTx in a pyramidal cell recorded from a p11 animal (black trace). The red trace represents the fit to the whole current using Eq. 1, the blue trace is the fit to the A-type component, and the green trace is the fit to the putative Kv2-mediated component (all data were obtained in the presence of DTX and MTX). F: histogram for density (peak I/Cm) of ScTx-sensitive A-type current (separated by fit to Eq. 1) as a function of age. *Significant difference from week 1; #significant difference from week 2. Number of cells: week 1; 9; week 2; 12; week 3; 11; weeks 4+5, 15.

Table 3. A-type current as a function of age: comparison of A-type current isolated by subtraction (recovery from inactivation) or fit to whole current in the same cells

<table>
<thead>
<tr>
<th>Age</th>
<th>Cm (pF)</th>
<th>Peak Amp (pA)</th>
<th>Density (pA/pF)</th>
<th>TTP (ms)</th>
<th>Fit Peak (pA)</th>
<th>TTP Fit (pA)</th>
<th>( \tau_{\text{act}} ) (ms)</th>
<th>( \tau_{\text{inact}} ) (ms)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>12.7 ± 2.0</td>
<td>322 ± 93</td>
<td>25.9 ± 8.0</td>
<td>3.2 ± 1.3</td>
<td>301 ± 86</td>
<td>3.0 ± 1.1</td>
<td>0.68 ± 0.32</td>
<td>18.6 ± 11.7</td>
<td>10</td>
</tr>
<tr>
<td>Week 2</td>
<td>13.2 ± 3.2</td>
<td>637 ± 346</td>
<td>50.3 ± 29.4</td>
<td>3.1 ± 0.8</td>
<td>568 ± 344</td>
<td>2.6 ± 0.7</td>
<td>0.65 ± 0.18</td>
<td>10.0 ± 5.3</td>
<td>20</td>
</tr>
<tr>
<td>Week 3</td>
<td>13.1 ± 5.8</td>
<td>1,020 ± 763*</td>
<td>74.0 ± 34.7*</td>
<td>3.3 ± 0.9</td>
<td>926 ± 711*</td>
<td>2.9 ± 0.8</td>
<td>0.67 ± 0.11</td>
<td>15.6 ± 15.1</td>
<td>12</td>
</tr>
<tr>
<td>Weeks 4–5</td>
<td>13.0 ± 3.6</td>
<td>971 ± 570*</td>
<td>74.9 ± 40.4*</td>
<td>3.0 ± 0.6</td>
<td>867 ± 535*</td>
<td>2.8 ± 0.8</td>
<td>0.64 ± 0.12</td>
<td>15.1 ± 8.5</td>
<td>21</td>
</tr>
</tbody>
</table>

Values are means ± SD for n cells. Week 1 = 7 ± 3 days; week 2 = 14 ± 3 days; week 3 = 21 ± 3 days; weeks 4–5 = 25–38 days combined. Recordings were made in the absence of extracellular Cd²⁺. Step to +10 mV (recovery from inactivation protocol; see text). Cm, whole cell capacitance (pF); Peak Amp, peak amplitude of the subtracted A current (pA); TTP, time to peak for the A current/whole cell capacitance (pA/pF); TTP, time to peak for subtracted current (ms); Fit Peak, peak current determined by fit of Eq. 1 (pA); TTP fit, TTP determined by fit of Eq. 1 (ms); \( \tau_{\text{act}} \), activation time constant (ms); \( \tau_{\text{inact}} \), inactivation time constant (ms). Data were obtained in the presence of 100 nM DTX and 30 nM MTX. This procedure removes residual DR current (after biophysical separation). *Significant difference from week 1.

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Cd\textsuperscript{2+} sensitive and ScTx sensitive, consistent with this component being primarily due to Kv4 \(\alpha\)-subunits (Norris and Nerbonne 2010). As with the putative Kv1- and Kv2-mediated currents, the A-type current increased in amplitude with development. There was no significant change in inactivation kinetics with age, although we found a significantly longer activation time constant at 1 wk vs. 4–5 wk. Our qualitative immunohistochemical data were consistent with an increase in protein levels for Kv1.4, Kv2.1, Kv4.2, and Kv4.3 subunits between 1 wk and 4–5 wk of age. There were no significant changes in the relative proportions of any current component tested, suggesting a mature pattern by the end of week 1.

Our electrical data were obtained from acutely dissociated pyramidal cells from superficial layers of somatosensory cortex. The truncated processes of these cells facilitated spatial control of voltage but limit our conclusions to somatic and proximal dendritic membrane. Whole cell recordings are subject to washout of cellular constituents and run-down of currents during prolonged recordings, but our recordings were largely restricted to 10 min after break-in. Our findings are also dependent on the specificity of the pharmacological agents and antibodies used. We previously tested these pharmacological agents and found them to be selective at the doses used (Guan et al. 2006, 2007); however, this remains a concern. The monoclonal antibodies used were all reported not to stain cortex of knockout animals, suggesting their specificity (Burkhalter et al. 2006). Our voltage protocols for isolating A-type current are biased toward channel types with relatively rapid recovery from inactivation; thus our findings are likely biased toward detection of Kv4-mediated versus Kv1-mediated.
Table 4. A-type current as a function of age: isolation with TEA and recovery from inactivation at $-100 \text{ mV}$

<table>
<thead>
<tr>
<th>Age, days</th>
<th>$C_m$ (pF)</th>
<th>Peak Amp (pA)</th>
<th>Density (pA/pF)</th>
<th>TTP (ms)</th>
<th>Fit Peak (pA)</th>
<th>TTP Fit (ms)</th>
<th>$\tau_{act}$ (ms)</th>
<th>$\tau_{inact}$ (ms)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.4 ± 1.3</td>
<td>13.0 ± 4.4</td>
<td>929 ± 452</td>
<td>55.9 ± 17.1</td>
<td>2.6 ± 0.3</td>
<td>649 ± 301</td>
<td>2.6 ± 0.4</td>
<td>0.68 ± 0.13</td>
<td>8.9 ± 1.3</td>
<td>8</td>
</tr>
<tr>
<td>29.5 ± 2.2</td>
<td>11.9 ± 5.7</td>
<td>1,662 ± 813</td>
<td>146.9 ± 77.0</td>
<td>2.3 ± 0.2</td>
<td>1,248 ± 504</td>
<td>2.5 ± 0.2</td>
<td>0.57 ± 0.08*</td>
<td>11.3 ± 0.4</td>
<td>7</td>
</tr>
</tbody>
</table>

Values are means ± SD for n cells. *Week 1 = 7 ± 3 days; weeks 4–5 = 25–38 days combined. A-type current isolated by subtraction (recovery from inactivation). Recordings were made in the absence of extracellular Ca$^{2+}$ and in the presence of 100 nM DTX, 30 nM MTX, and 30 mM TEA. Currents were elicited with a voltage step to +10 mV (recovery from inactivation protocol; see text). $C_m$, whole cell capacitance (pF); peak amp, peak amplitude of the A current (pA); density, peak amplitude of the A current/whole cell capacitance (pA/pF); TTP, time to peak (ms); fit peak, peak current determined by fit of Eq. 1 (pA) (this procedure removes residual DR current (after biophysical separation)); TTP fit, TTP determined by fit of Eq. 1 (ms); $\tau_{act}$, activation time constant (ms); $\tau_{inact}$, inactivation time constant (ms). *Significant difference from week 1 ($P < 0.05$).

A-type current (which recovers much more slowly from inactivation; Bett and Rasmussen 2004).

Previous work showed that a persistent, DR current is present in neocortical neurons during early embryonic development and A-type current before E21 (Bahrey and Moody 2003; Mienville and Barker 1997). There is a subsequent postnatal increase in DR and A-type currents in pyramidal cells (Bahrey and Moody 2003; Hamill et al. 1991). In the first few postnatal days, the A-type current and the combined DR current increase with different developmental schedules (Bahrey and Moody 2003). Consistent with our electrophysiological and immunochemical data, Kv1.1 protein expression is present early in embryonic development, is low at birth, and rises dramatically at the end of the second postnatal week (P12–P15; Hallows and Tempel 1998). Similarly, Kv2.1 protein expression in cortex is present early in embryogenesis and gradually increases from birth until ~P30 (Trimmer 1993). In layer V pyramidal neurons, Kv4.2 and Kv4.3 mRNA expression increases from P5–P7 to P28–P30 (Christophe et al. 2005).

The general pattern of increased K$^+$ current density with development is also observed in other neuron types, for example, motoneurons (Martin-Caraballo and Greer 2000), CA1 pyramidal neurons (Falk et al. 2003; Spiegelman et al. 1992), Xenopus spinal neurons (Harris et al. 1988), cochlear nucleus pyramidal neurons (Bortone et al. 2006), and striatal medium spiny neurons (Surmeier et al. 1991). Unlike neocortical pyramidal cells, CA1 pyramidal cells and medium spiny cells exhibited a reduction in the ratio of A-type current to the persistent current with age (Spiegelman et al. 1992; Surmeier et al. 1991) that was associated with expression of a novel slowly inactivating current component after the first postnatal week (Surmeier et al. 1991). Our data indicate that any changes in relative expression of A-type versus DR current in neocortical pyramidal cells occur before the end of week 1.

Fig. 8. Immunocytochemistry for Kv channels in rat somatosensory cortex during postnatal development. Higher-power images (B–D) were maximum-intensity projections from z-stacks containing the same number and thickness of optical sections for a given antibody. A: Kv4.3. A1: low-power view of stain for Kv4.3 at P9. A2: low-power view of Kv4.3 at P32. Note layer IV barrels (asterisks) and generally brighter staining in the older animal. Scale bar in A is 120 μm. B: Kv4.3: projections of 10 optical slices. B1: high-power view of layer III for Kv4.3 at P9. B2: Kv4.3 staining of layer III in a P32 animal. Note greater staining in the older animal. C: Kv1.4: projections of 13 optical slices. C1: high-power view (layer III) of Kv1.4 staining at P9. C2: Kv1.4 staining in a P32 animal. Note greater staining in the older animal. D: Kv2.1: projections of 12 optical slices. D1: high-power layer III staining in a P9 animal. D2: layer III staining in a P32 animal. The pattern of staining is similar at both ages. Staining intensity is greater in the older animal. Because the cellular detail in the 1 wk data was low, the brightness of the images for both ages in B1 and B2 were digitally brightened by 22%, images in C1 and C2 by 28%, and images in D1 and D2 by 22%. Scale bars for B–D are 20 μm.
In CA1 neurons (Spigelman et al. 1992) and motoneurons (Martin-Caraballo and Greer 2000) there were changes in voltage dependence and kinetics of the DR (and a Kv1-mediated component of A-type current in CA1 pyramids; Falk et al. 2003). In motoneurons, the A current has been reported to be large early and not increase with age (Gao and Ziskind-Conhaim, 1998) or to not increase with age (McCobb et al. 1990; Martin-Caraballo and Greer 2000).

Development of Excitability and Firing Patterns

It is difficult to evoke action potentials in embryonic cortical neurons, and little spontaneous activity is observed (Bahrey and Moody 2003; Crain 1952). With postnatal age, there is increased ability to spike, decreased AP threshold, increased AP amplitude, and decreased spike width (Bahrey and Moody 2003; Christophe et al. 2005; Kasper et al. 1994b; Kriegstein et al. 1987; Lorenzon and Foehring 1993; Maravall et al. 2004; McCormick and Prince 1987; Metherate and Aramakis 1999; Oswald and Reyes 2008). Spontaneous firing also increases with development (Bahrey and Moody 2003). In neocortical pyramidal neurons, voltage-gated Na+ currents (Bahrey and Moody 2003; Huguenard et al. 1988), Ca2+ currents (Lorenzon and Foehring 1995), and Ih (Bahrey and Moody 2003) also increase in density with development. Presumably, mature computational abilities of these cells require the postnatal increase of these inward currents, as well as Kv channels.

Consistent with our findings for Kv-mediated currents, the most dramatic changes in AP parameters occur between weeks 1 and 3, approaching adult values by weeks 3–5 (Lorenzon and Foehring 1993; McCormick and Prince 1987; Zhang 2004). Because of its relatively hyperpolarized activation range and fast kinetics (Foehring and Surmeier 1993; Yuan et al. 2005), the maturation of A-type current would be expected to lead to more rapid spike repolarization and narrower spikes. Lesser effects on spike parameters are expected for Kv1-mediated current (due to its small amplitude and slower kinetics; Bekkers and Delaney 2001; Guan et al. 2006, 2007a) or Kv2-mediated currents (depolarized activation range and slow kinetics; Guan et al. 2007b). However, Kv1-mediated currents contribute to spike threshold in mature pyramidal cells (Bekkers and Delaney 2001; Guan et al. 2007a).

Repetitive firing behavior also changes with age (Christophe et al. 2005; Kasper et al. 1994b; Lorenzon and Foehring 1993; Maravall et al. 2004b; McCormick and Prince 1987; Oswald and Reyes 2008). The greatest changes occur between weeks 1 and 3 (Bahrey and Moody 2003; Lorenzon and Foehring 1993; Locke and Nerboune 1997a; Maravall et al. 2004b; Metherate and Aramakis 1999). Both Kv1 (Guan et al. 2006b)- and Kv2 (Foehring et al. 2009)-mediated currents regulate interspike intervals in these cells. McCormick and Prince (1987) proposed that conservation of the ratios between different types of K+ (and other) channel types allows for rhythmic, regular spiking in most pyramidal cells at all but the earliest postnatal ages. Consistent with this proposal, we found that the percentage of the current due to each Kv current remained stable with age.

Postnatal Development of Somatosensory Cortex

During the time when voltage-gated currents are increasing most rapidly (weeks 1–3), the rodent somatosensory system undergoes dramatic changes that fine-tune pyramidal cell intrinsic properties and connectivity. Active whisker exploration begins during postnatal week 2 (P11–P13; Landers and Ziegler 2006; Woolsey and Wann 1976) and is mature at the end of the third week. Synaptogenesis also peaks in the second postnatal week (Micheva and Beaulieu 1996), although the number of excitatory and inhibitory synapses increases until P32 (after which it declines; De Felipe et al. 1997). An adult-like EEG is first observed at ~P10–P12 (Crain 1952; Snead and Stephens 1983). The reduced excitability of pyramidal cells in the first two postnatal weeks is correlated with immature K+ currents (present study; Lorenzon and Foehring 1993) and inward currents (Bahrey and Moody 2003; Huguenard et al. 1988; Lorenzon and Foehring 1995). These properties may limit pyramidal cell activity at ages prior to the mature expression of GABA-mediated synaptic inhibition, which begins at ~P8 (Agmon and O’Dowd 1992). Interestingly, in Kv1.1 knockout mice, seizure susceptibility manifests at ~2 wk of age (Rho et al. 1999). Several aspects of somatosensory cortical function exhibit plasticity and are sensitive to activity during critical periods of development (Feldman et al. 1999; Hentsch 2005). In particular, receptive field plasticity for L2/3 pyramidal cells is present until P10–P14 (Maravall et al. 2004a) and pyramidal cells undergo dendritic rearrangement in weeks 2–3 (Kasper et al. 1994a; Maravall et al. 2004a), becoming largely mature by about P21 (Blue and Parnevalas 1983; Kristt 1978; Miller 1981; Miller and Peters 1981; Wise and Jones 1976). These dendritic changes (Maravall et al. 2004a) and experience-dependent plasticity (Feldman et al. 1999; Hentsch 2005) are sensitive to levels and patterns of activity, which should in turn be affected by postsynaptic changes in somatic K+ currents.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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