INTRODUCTION

Spontaneous electrical activity plays a fundamental role in many aspects of nervous system development, including DNA synthesis and cell cycle exit (Barish 1998; Catalano et al. 1997; Dutton et al. 1993; Fields 2001; LoTurco et al. 1995), cell migration (Behar et al. 2001; Edgar and Price 2001; Flint et al. 1999; Komuro and Rakic 1992, 1993, 1998; Maric et al. 2001), formation and refinement of synaptic connections (Catalano and Shatz 1998; Herrmann and Shatz 1995; O’Leary et al. 1994; Penn et al. 1998; Shatz and Katz 1996; Shatz and Stryker 1988), and development of mature ion channel properties (Dallman et al. 1998; Moody 1998; Spitzer 1991; Spitzer and Ribera 1998). The existence and patterns of spontaneous activity in any given neuron early in its development depend on both the input it receives (from any existing synaptic inputs and the presence of nonsynaptically released transmitters in the extracellular space) (Haydar et al. 2000; Owens et al. 1996) and on its intrinsic ion channel properties.

It has been well established in invertebrate and nonmammalian vertebrate cells that the properties of ion channels present at early stages of development can be quite different from those in the mature state. These differences are critical in determining the existence of spontaneous electrical activity and in regulating its ability to mediate activity-dependent developmental events (Catalano et al. 1997; Dallman et al. 1998; Greaves et al. 1996; Gu and Spitzer 1995; Linsdell and Moody 1995; O’Dowd et al. 1988; Wong et al. 1993). Because the properties and types of ion channels present in a cell can change rapidly during development, understanding the roles of immature ion channel properties in activity-dependent development has required detailed maps of ion channel development (Dallman et al. 1998; Greaves et al. 1996). Such maps have not been drawn for developing neurons of the mammalian neocortex in sufficient detail to allow correlations to be determined between the patterns of ion channel development and the occurrence of critical developmental periods of spontaneous electrical activity. Here, we report measurements in neurons of mouse sensorimotor cortex of input resistance ($R_{in}$), inward Na$^+$ current ($I_{Na}$), outward K$^+$ current ($I_{K}$), and inwardly rectifying currents between embryonic day 14 (E14) and postnatal day 17 (P17). Our results show that each of these properties is regulated in a different way during embryonic and early postnatal development and suggest that early postnatal periods of spontaneous activity may in part be regulated by the developmental expression of currents that support repetitive firing ability in individual neurons.

The neurons that populate the layers of the mammalian neocortex arise in part from repeated divisions of precursors in the neocortical ventricular zone (VZ), a proliferative population that in rodents actively generates neuronal precursors during most of the latter third of embryonic development (Luskin et al. 1988; Takahashi et al. 1994). A secondary proliferative population, responsible for generating most cortical glia, persists much later in development (Goldman 1995; Levers et al. 2001). During neurogenesis, which in mouse occurs between about E11.5 and E17, depending on neocortical region, a steadily increasing fraction of the proliferative population exits the cell cycle in the VZ and migrates outward through the intermediate zone (IZ) to final destinations in the cortical plate (CP) (Caviness 1982; Shoukimas and Hinds 1978; Sidman and Rakic 1973). Presumptive neurons that exit the cell cycle at earlier days occupy the deeper cortical layers, and those that exit later migrate through these to form the more superficial layers. This radial migratory process appears to generate the pyramidal cell population (Anderson et al. 1999;...
cerebral cortices were removed to ice-cold postnatal ACSF [post-
ACSF, which contained (in mM) 115 NaCl, 4.3 KCl, 2CaCl2, 2
MgCl2, 0.28 MgSO4, 0.22 KH2PO4, 0.85 NaHPO4, 27 NaHCO3,
and 25 mM-glucose (Beier and Barish 2000)] bubbled with carbogen. Of-
lactory lobes and brain stem were removed, and cerebral cortices were
mounted directly on a metal pan for slicing.

Coronal slices (200 µm) were cut using a Vibratome 1000 (Tech-
nical Products International, St. Louis, MO), removed from the agar if
embedded, and allowed to recover in oxygenated room temperature
preACSF or postACSF for 60–90 min before recording.

**Voltage-clamp recordings**

Pipettes were pulled to a resistance of 8–12 MΩ from 50 µl hemat-
ocrit glass capillary tubes using a Narishige two-stage puller (PP-83
and PP-830, Japan), coated with silicone elastomer (Sylgard 184;
Dow Corning, Midland, MI), and filled with potassium internal solu-
tion [potassium methylsulfate, which contained (in mM) 113 KMSO4
(ICN Biomedicals), 20 KCl, 10 HEPES, 2 MgATP, 3 Na-ATP, and
0.2 Na-GTP, pH to 7.25; or potassium gluconate, which contained (in
mM) 100 KGluc, 0.5 EGTA, 5 MgCl2, 40 HEPES, 2 Na-ATP,
and 0.3 Na-GTP, pH to 7.25]. In a few cases, 3,000 MΩ biotin dextran
(2%; Molecular Probes, Eugene, OR) or Neurobiotin (2%; Vector
Laboratories, Burlingame, CA) were added to the recording pipette
for later confirmation of cell morphology.

Voltage-clamp experiments were performed using the whole cell
patch-clamp technique (Hamill et al. 1981). All recordings were made
at room temperature (24–26°C) in pre- or postACSF. Slices from
the somatosensory cortex were chosen for recording and were placed in
a 1.2-ml recording chamber and perfused at a rate of 0.6 ml/min with
carbogen bubbled pre- or postACSF.

Individual cell somas were visualized with an upright Axioskop
(Zeiss, Germany) using a water immersion ×63 objective with DIC
optics (Fig. 1C). Cells within a region ≤200 µm dorsal to the striatal
border were targeted prenatally and within 300 µm dorsal to the
caudatopallial angle postnatally. In prenatal recordings, IZ cells lo-

cated in the middle of the lateral IZ, and CP cells located in the middle
of that region were targeted (Fig. 1A). In postnatal recordings, cells
with a pyramidal morphology were targeted (Fig. 4A).

Pipettes were lowered onto individual visualized cells, keeping
constant positive pressure to avoid clogging. Pressure was then turned
off when the cell was touched, and a seal >4 GΩ usually formed
within 2–10 s. A holding potential of −60 mV was applied, and brief
pulses of suction were applied until the membrane inside the pipette
ruptured. Recordings were made using a List EPC-7 (Heka Elek-
tronik, Lambrecht/Pfalz, Germany) or Axopatch 1-D (Axon Instru-
ments, Foster City, CA) amplifier. The resulting currents were filtered
at 1 kHz and recorded and analyzed using pCLAMP8 software (Axon
Instruments).

Most cells were also held under current clamp to investigate
activity.

**Histology**

Slices with cells filled with Neurobiotin or biotin dextran were fixed
in 4% paraformaldehyde in 0.1 M PBS (pH 7.2) for 1 h at room
temperature or overnight at 4°C. After four washes in PBS, the slices
were treated with 3% hydrogen peroxide for 10 min and rinsed in
0.3% Triton-X 100 in PBS (PBS-TX). Slices were incubated for 2 h
in an avidin-biotin horseradish peroxidase BSA solution (Vector
Laboratories ABC kit with 2% bovine serum albumin added) and
again washed four times in PBS-TX. Fills were developed with
diaminobenzidine and glucose oxidase, D-biotin, NiCl2, and

dehydrated through an ethanol series. Slices were cleared using ce-
darwood oil or xylene and mounted on slides in DPX (Fluka, Swit-
zerland). Images were acquired with a ZVIS3C75DE Digital Camera
system (Zeiss) mounted on an Axioskop upright microscope with

**Methods**

**Animals**

Timed pregnant C57Bl/6 mice were obtained from ATL, Kent,
WA. Pregnant females were killed on gestational day 13–18 (E13–
E18) by CO2 inhalation according to National Institutes of Health and
institutional guidelines. Both uterine horns were dissected out and
placed in ice-cold prenatal artificial cerebral spinal fluid [preACSF,
which contained (mM) 119 NaCl, 2.5 KCl, 1.3 MgCl2, 2.5 CaCl2, 1
NaH2PO4, 26.2 NaHCO3, and 11 D-glucose (Mooney et al. 1996);
unless otherwise noted, all chemicals were obtained from Sigma, St.
Louis, MO] bubbled with carbogen (95% O2, 5% CO2). Pups were
removed from the uterus and staged by visual inspection or crown
to rump length, and cerebral cortices were dissected out and placed in
ice-cold preACSF, keeping intact the section from olfactory lobes
through a portion of the brain stem. Some brains were then embedded
in a 3% solution of Type IX-A Ultra-low temperature gelling agarose
and dehydrated through an ethanol series. Slices were cleared using ce-
darwood oil or xylene and mounted on slides in DPX (Fluka, Swit-
zerland). Images were acquired with a ZVIS3C75DE Digital Camera
system (Zeiss) mounted on an Axioskop upright microscope with

Mione et al. 1997; Tan et al. 1998). More recent evidence
indicates that a large fraction, if not all, of the inhibitory
interneurons of the rodent cortex arise in the VZ of the gan-
glionic eminences, the precursors of the striatum in the adult
animal (Anderson et al. 1999, 2001; Parnavelas et al. 2000).

In various studies of membrane properties of the embryonic
rodent neocortex, proliferating populations have been found to
primarily express voltage-gated K+–Ca2+–activated K+ and small Na+ conductances when input resistances are sufficient
to permit detection of small currents (Bulan et al. 1994; Hal-
lows and Tempel 1998; Martin-Moutot et al. 1987; Mienville
and Barker 1997; Mienville et al. 1999; Noctor et al. 2002;
Picken Bahrey and Moody 2003). Na+ currents then increase
together throughout development as cells begin to migrate and differen-
tiate (Couraud et al. 1986; Hamill et al. 1991; Huguenard et
al. 1988; Luhmann et al. 2000; Mienville et al. 1994; Picken
Bahrey and Moody 2003; Villegas et al. 1994). Changing
levels of expression of L-type, low- and high-voltage-activated
(LVA and HVA) Ca2+ currents (Lorenzon and Foehring 1995;
Tarasenko et al. 1998), as well as voltage-gated inactivating
(Ih) and rectifier K+ currents (Bekkers 2000; Hamill et
al. 1991; Korngreen and Sakmann 2000; Mienville and Barker
1997), occur during both embryonic and postnatal develop-
ment. Cells of the developing cerebral cortex gain the ability to
generate activity as early as E18 (Hamill et al. 1991; Luhmann
et al. 1999), and there is some evidence that both Na+ and
outward K+ currents can be modulated by activity patterns
(Desai et al. 1999). Here we show that presumptive neurons
migrating through the IZ have relatively consistent electro-
physiological properties that change very little with develop-
mental stage: high-input resistance, delayed K+ currents, small
inward Na+ currents, and no hyperpolarization-activated cur-
rents. After they enter the CP, however, these properties begin
to change. Input resistance drops dramatically by P12. Na+ current
density increases while K+/H11001 current density increases while K+ current density remains constant. A slow, hyperpolarization-activated current resembling Ih appears abruptly at about P6. During the period in the
CP, neurons also gain the ability to fire repetitive action po-
tentials during long depolarizing stimuli, and many begin to
show spontaneous, repetitive action potentials.
Optronics software (Optronics, Goleta, CA). Images were stored using Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA).

Analysis methods

Input resistance ($R_i$) was calculated from the average of responses to voltage pulses to ±10 and ±20 mV from −60 mV. A triangle wave voltage command was then played to the cell, and capacitance measurements were calculated from the amplitude of the resulting square-wave current (Moody and Bosma 1985). Current density was calculated as peak current divided by the capacitance of the cell. Kinetics of activation and inactivation were determined by fitting exponential curves to the appropriate portions of the current trace within pCLAMP8 software. Histograms were created in Sigmaplot (SPSS Science, Chicago, IL), and statistics (descriptive and Student’s $t$-test) were done in Microsoft Excel (Microsoft, Redmond, WA). Figure data are shown as means ± SE, with $n$ values noted.

RESULTS

Development of $Na^+$ and $K^+$ currents in IZ cells

We recorded from 103 IZ cells in animals aged E13 to E18 (see Fig. 1B). Mean input resistance of the IZ cells did not change significantly across embryonic days, ranging from 7,350 ± 2,594 MΩ at E16 ($n = 17$) to 4,085 ± 714 MΩ at E17 ($n = 7$; $P = 0.44$; Fig. 1D). Capacitance also changed very little during these stages, although the difference between the maximum value of 6 ± 1 pF at E17 and the minimum of 4 ± 0.3 pF at E18 was marginally significant ($P = 0.046$; Fig. 1E).

Our previous work showed that in embryonic mouse cortex, dye coupling as seen in Fig. 1B is not associated with significant electrical coupling, so that voltage-clamp measurements are accurate for the recorded cell without resorting to uncoupling methods (Picken Bahrey and Moody 2003).

In previous work (Picken Bahrey and Moody 2003), we showed that the amplitude of the inward $Na^+$ current increased as presumptive neurons exit the cell cycle in the VZ and migrate into the IZ. This implies that $I_{Na}$ amplitude is determined by developmental stage of individual cells, i.e., by state of migration and time since cell cycle exit, rather than by chronological age of the embryo. If this is true, then mean $I_{Na}$ amplitude in IZ cells should be similar at different stages because the IZ is a constantly changing population of cells that began migration at a similar time interval following cell cycle exit.

As shown in Fig. 2A (also see insets Fig. 3E), $I_{Na}$ amplitude was similar in IZ cells at most of these stages, with no significant differences between any pair of stages except E13 and E14–E16 (E13: $24 ± 7$ pA, $n = 9$; E14: $58 ± 6$, $n = 40$; $P = 0.02$; data included only from cells with measurable $I_{Na}$). This indicates that presumptive neurons acquire functional $I_{Na}$ as a result of individual differentiation and migration, not as a function of overall developmental time. Because capacitance of IZ cells did not vary during this period (Fig. 1E), $I_{Na}$ density

FIG. 1. Membrane properties of intermediate zone (IZ) cells. A: 200-μm-thick slice of the embryonic day 15 lateral neocortex viewed at ×40, showing approximate locations of the ventricular zone (VZ), IZ and cortical plate (CP). TuJ1 is used to stain neurobiotin-filled of a small dye-coupled cluster of IZ cells. C: field of IZ cells under DIC optics, as we used to target IZ cells for recording. D: input resistance of IZ cells as a function of stage (E13: 4,663 ± 785 MΩ; E14: 4,275 ± 500 MΩ; E15: 5,436 ± 2,217 MΩ; E16: 7,350 ± 2,594 MΩ; E17: 4,085 ± 714 MΩ; E18: 5,875 ± 1,455 MΩ). No significant differences were found between the means at any 2 stages. E: IZ cell capacitance as a function of stage (E13: 5 ± 1 pF; E14: 5 ± 0.3 pF; E15: 6 ± 1 pF; E16: 5 ± 0.3 pF; E17: 6 ± 1 pF; E18: 4 ± 0.3 pF). No significant differences were found between any 2 stages.
in IZ cells was also constant during this interval of development. There was, however, some variation in the fraction of IZ cells expressing detectable $I_{\text{Na}}$, decreasing from a high of 100% at E16 to a low of 67% at E18 ($P = 0.021$ by Fisher’s exact test; Fig. 2B).

The other major current in these cells is a delayed outward $K^+$ current, which is present in virtually all cells at all stages ($102/103$), as it is in VZ cells (Picken Bahrey and Moody 2003; M. Albrieux, J. C. Platel, A. Dupuis, M. Villaz, and W. J. Moody, unpublished data). Unlike $I_{\text{Na}}$, the mean amplitude of $I_K$ decreases steadily by about 50% between E13 and E18 ($392 \pm 62$ pA at E13 to $157 \pm 46$ pA at E18, $n = 11$ and 12, respectively; $P = 0.008$; Fig. 3, A and insets in E). Because capacitance does not change during this period (see Fig. 1E), average $I_K$ density declines similarly. In addition to the decrease in amplitude, the activation kinetics of $I_K$ slowed some-

**FIG. 2.** $Na^+$ current in migrating IZ cells. **A:** $I_{\text{Na}}$ amplitude in IZ cells as a function of stage (E13: 24 ± 7 pA; E14: 58 ± 6 pA; E15: 65 ± 13 pA; E16: 70 ± 15 pA; E17: 37 ± 8 pA; E18: 44 ± 14 pA). The only significant difference is between E13 and E14–16. **B:** percentage of IZ cells expressing detectable $I_{\text{Na}}$ as a function of stage. The percentages of cells expressing $I_{\text{Na}}$ varied with a slight but significant decrease at the end of embryonic development (100% at E16; 67% at E18; $P = 0.021$ by Fisher’s exact test).

**FIG. 3.** Delayed K$^+$ current in IZ cells. **A:** the average peak K$^+$ current (measured at $\pm 70$ mV) steadily declines during development in the migrating population (E13: 392 ± 62 pA; E14: 328 ± 25 pA; E15: 253 ± 37 pA; E16: 192 ± 27 pA; E17: 196 ± 40 pA; E18: 157 ± 46 pA). Unlike $I_{\text{Na}}$, $I_K$ decreases steadily by about 50% between E13 and E18 ($392 \pm 62$ pA at E13 to $157 \pm 46$ pA at E18, $n = 11$ and 12, respectively; $P = 0.008$; Fig. 3, A and insets in E). Because capacitance does not change during this period (see Fig. 1E), average $I_K$ density declines similarly. In addition to the decrease in amplitude, the activation kinetics of $I_K$ slowed some-
what between E13 and E17 (E14: 0.9 ± 0.1 ms, n = 41; vs. E17: 5.3 ± 1.4 ms, n = 5; P = 6.9 × 10⁻¹⁰; Fig. 3C). Neither the voltage dependence nor the inactivation of the outward K⁺ current change during this time (Fig. 3E).

Closer analysis of Iₖ amplitudes suggests that the decrease in amplitude may represent a loss of cells with large Iₖ amplitudes rather than a shift of the entire population to lower amplitudes. Figure 3, B and D, compares frequency histograms of Iₖ amplitudes at E13–15 and E16–18. At the earlier stages, the distribution shows some indication of two populations, one centered around 150 pA and the other around 350 pA. At the later stages, the entire population is well described by a single distribution centered around the lower value of 150 pA with an almost complete absence of the high Iₖ cells.

**Development of Na⁺ and K⁺ currents in CP and deep layer pyramidal cells**

The IZ is a continuously changing cell population, in which cells present on different embryonic days share a common state of differentiation. The preceding data indicate that this is reflected in relatively static physiological properties. Once these cells take up their positions in the layers of the forming cortical plate, however, this situation changes. As development time progresses, CP cells should continue to differentiate physiologically to gain the ability for robust repetitive firing and to form the variety of firing types characteristic of the mature cortex (Connors and Gutnick 1990; Massengill et al. 1997). To follow the early stages of this process, we recorded from cortical cells at stages between E14 and P17, binning our data as follows: E14 (E13–14), E16 (E15–16), and E18 (E17–18) prenatally; P0 (P0–1), P2 (P2–3), P4 (P4–5), P6 (P6–7), P10 (P10–11), and P12 (P12–17) from postnatal cortex.

The same lateral region of the cortex was targeted at each stage, and cell type was noted, where possible. During embryonic stage recordings, we targeted cells in the mid-CP, with a few in the deeper parts of the developing CP. These cells are primarily cells of the early forming deeper layers but may include cells from the subplate or those of mid-layers still migrating through the deeper layers toward the pia. For postnatal recordings, we almost exclusively targeted pyramidal cells in the deeper layers by noting morphology under DIC optics. This morphology was confirmed in some cases by dye filling with a biotin-conjugated dextran (Fig. 4A).

The input resistance of CP cells dropped dramatically, by about a factor of 30, between E14 and P12 (Fig. 4B). This decrease occurred in two abrupt phases, one between E14 and E16 (E14: 17,065 ± 4,104 MΩ, n = 23; E16: 6,396 ± 1,502 MΩ, n = 20; P = 0.03), and another between P2 and P6 (P2: 4,901 ± 1,403 MΩ, n = 8; P6: 1,662 ± 823 MΩ, n = 18; P = 0.047). Except for the high value at E14, the input resistances of CP cells in the embryonic and perinatal period (E16–P2) are the same as those found in IZ cells (5,145 ± 554 MΩ; Fig. 4B, dashed line). By P12, CP input resistances have decreased by a factor of 10 from this value. This indicates that, for the most part, cells that are migrating through the IZ in the embryonic period maintain their resting membrane resistance as they enter the CP and then subsequently acquire the low resting membrane resistance characteristic of mature cortical neurons over their first 2 weeks of residence in the CP.

To test whether this decrease in input resistance could simply be a function of cell growth, we measured capacitance of CP cells over the same period of development (Fig. 4C). Capacitance showed a substantial increase, beginning at about the time of birth, by a factor of about 6 (E14: 6.7 ± 0.3 pF, n =...
The increase in capacitance is not large enough to account completely for the decrease in input resistance and does not exactly match its time course. Therefore it is likely that the decrease in resistance of CP cells during this period is caused by a combination of growth and the insertion of new resting channels.

Over the same period of development, the amplitude of inward Na⁺ currents in CP cells increases by a factor of 10, from 72 ± 24 pA at E14 (n = 18) to values averaging about 800 pA between P2 and P12 (Figs. 5 and 7). The apparent peak at P10 is not significantly different from values at P6 and P12. This increase is larger than the increase in capacitance over the same period, so Na⁺ current density shows a similar, though somewhat smaller, increase during this period (I_{Na} density is 14 ± 5 pA/pF at E14, n = 16, and 83 ± 30 pA/pF at P6, n = 13; P = 0.016). Although we also measured a slight negative shift in the activation voltage of I_{Na} during this period, it is likely that at later postnatal stages, when Na⁺ currents are quite large, voltage-clamp control is degraded sufficiently to make measurement of this difference unreliable. For the same reason, it is possible that our values for maximal I_{Na} at later stages are underestimates of the actual values.

Delayed K⁺ currents showed a similar pattern of increase in CP cells from E14 through P12. At E14, I_{K} amplitude (measured at −70 mV) was 327 ± 41 pA (n = 23), and by P12 had increased by about a factor of four to 1,198 ± 526 pA (n = 9; P = 0.012 compared with E14; Figs. 6A and 7). Unlike the case for I_{Na}, however, the I_{K} increase was approximately the same as the increase in capacitance, so that I_{K} density showed no significant increase through the period from E14 to P12.
The large increases in $I_{\text{Na}}$ amplitude that occur perinatally (see Figs. 5 and 7) change this situation considerably. By P0, 83% of cells at any embryonic stage showed any spontaneous action potentials. By P10, none were capable of generating an active response following the termination of a short stimulus, a fraction only half as large as the fraction of cells with detectable Na$^+$ currents measured in voltage clamp (65%). This discrepancy is probably due to the long time constant of the cells at this stage shunting the relatively rapid Na$^+$ current. No cell at E14 showed spontaneous action potentials in the absence of stimuli. By the later embryonic stages, a much larger fraction of cells responded to stimuli with active responses. At E17–18, 80% (4/5) of cells were capable of generating an active response following the termination of a brief stimulus. These were broad responses with a high threshold ($-21 \pm 3$ mV, $n = 7$; Fig. 9, B and C). Despite this change, however, no cells at any embryonic stage showed any spontaneous action potential activity in the absence of stimuli (resting potential was fairly depolarized: $-40 \pm 2$ mV, $n = 34$), and none were capable of firing repetitive action potentials during a 150-ms stimulus (Figs. 8, E17, and 9D).

The large increases in $I_{\text{Na}}$ amplitude that occur perinatally (see Figs. 5 and 7) change this situation considerably. By P0, 83% of cells generated an action potential with a slightly shorter duration.
following the termination of a short pulse, and two of eight cells tested were capable of firing repetitively during a 150-ms depolarizing stimulus. These same two cells also showed some spontaneous action potentials, apparently resulting from postsynaptic potential (psp) like input on a resting potential of $-54 \pm 5$ mV ($n = 14$; Figs. 8, P0, and 9D). Cells at P2 appeared to be similar to P0 neurons in firing ability with 57% (4/7) generating action potentials after a short pulse and 38% (3/8) both firing repetitively during a 150-ms pulse and generating spontaneous spikes.

Although the percentage of cells generating an action potential after a short stimulus did not change from earlier postnatal stages (66%), at P6, we saw an increase in the fraction of cells generating repetitive action potentials during long pulses (83%). These action potentials were even shorter in duration than those at early postnatal stages (Fig. 9C). Only 25% of cells generated spontaneous action potentials, however.

At the latest stages, P10–P12, 100% of recorded cells generated action potentials after a short stimulus at a threshold that had dropped more than 10 mV, to $-34 \pm 3$ mV ($n = 12$; Fig. 9B). Almost 70% of recorded pyramidal neurons were capable of repetitive firing during a long stimulus, and 65% fired spontaneously, often in response to psp-like depolarizations on an already depolarized resting potential (see Figs. 8, P10, and 9B).

**DISCUSSION**

To begin to understand how the patterns with which voltage-gated ion channels develop in the mammalian neocortex, we have measured Na$^+$, delayed K$^+$, and inwardly rectifying currents in neurons of the mouse sensorimotor cortex between E13 and P17. We concentrated on migratory presumptive neurons of the IZ from E13 to E18, and differentiating neurons of the cortical plate and deep cortical layers from E14 to P17. Our data indicate that all three types of currents, and cell input resistance, develop in different patterns during this interval.

The IZ represents a continuously changing population of cells that have exited the cell cycle in the VZ recently and that are migrating to take up their positions in the CP. Therefore IZ cells at E14 and E18, for example, should be similar in developmental state but different in both chronological age and in the cortical layer they are destined to occupy. We found that IZ cells were almost identical in their electrophysiological properties over the interval E13–E18. Cell input resistance, capacitance, and $I_{Na}$-amplitudes did not vary significantly with stage. No IZ cells expressed any hyperpolarization-activated currents. The only change we observed was a steady decrease in $I_K$ amplitude of about 50% between E13 and E18. This could arise in two ways. First, a slight decrease in $I_K$ might occur as cells migrate out of the VZ into the IZ. This could be true only at later stages; however, because we have measured $I_K$ in the VZ at E12, E13, and E14, and mean amplitudes are not distinguishable from $I_K$ amplitudes in E13–E15 IZ. A progressively greater loss of $I_K$ as cells exit the VZ at later and later stages, however, could indicate a layer-specific level of $I_K$ expression, with cells fated to more superficial layers having smaller $I_K$ amplitudes at least during migration. Another pos-
sible explanation is that the presumptive inhibitory interneurons that migrate from the ganglionic eminences into the neocortical IZ (Anderson et al. 2001; Marin and Rubenstein 2001; Parmavelas et al. 2000) express different levels of $I_K$ than do cells that arise locally in the neocortical VZ. This explanation is consistent with our finding of two populations of cells in the IZ defined by $I_K$ amplitude. The first population, with $I_K$ of around 150 pA, persists from E13 to E18 and may represent the population of radially migrating neurons from the VZ. The second population, defined by their larger $I_K$ (300–800 pA), is present from E13 to E15, during the days of greatest migration of subpallial cells destined for the developing cortex (Marin and Rubenstein 2001), but disappears at later stages (see Fig. 3, B and D). If this second population represents the GABAergic interneurons migrating from the ganglionic eminences, their early presence and later loss could easily explain the decline we see in $I_K$ with developmental stage. Previous findings by Hamill et al. (1991), showing that pyramidal and nonpyramidal neurons in embryonic rat cortex express identical Na currents, is consistent with our potential.

The CP presents a different population of cells than the IZ, being composed primarily of postmigratory neurons undergoing terminal neuronal differentiation. We concentrated on the deep layers of the CP and postnatal cortex that are formed earlier in development than the more superficial layers, allowing us to study a longer interval of development in a single population. The population is not entirely postmigratory, however, as migratory neurons destined for the more superficial layers transiently pass through those deeper layers.

We found a dramatic decrease—between 10- and 30-fold—in input resistance over the interval E14 to P12 in CP cells. This decrease occurred in two stages, one between E14 and E16, the other between P2 and P4. A portion of the drop in input resistance may be due simply to cell growth because capacitance decreases over the same period, although by a much smaller amount. It is possible, however, that our measurement of the increase in capacitance underestimates membrane growth relative to input resistance measurements because capacitance is measured at considerably higher frequencies and thus may measure a smaller fraction of actual surface area in cell processes that are extending during this period. If we assume that the increase in surface area does, in fact, account only for a portion of the input resistance change, then the appearance of a new resting conductance must be responsible for the remainder of this change. A hyperpolarization-activated current (presumably $I_h$) appears postnatally in CP cells (Fig. 6B). Although little of this current is activated at the resting potential, the time course of its appearance is similar to the drop in input resistance, and the appearance of even a small resting conductance may have a large effect on cells that previously had a very low resting conductance. We do also see a positive shift in resting potential during the second postnatal week (see Fig. 9B), after a significant number of cells already express $I_h$, so it is possible that even a small contribution of $I_h$, with its positive reversal potential, to an initially small resting conductance could cause both the decrease in input resistance and the positive shift in resting potential.

The second major change we see in CP cells is the very large increase in amplitude of $I_{Na_h}$ beginning at late embryonic stages (Figs. 5 and 7). $I_{Na_h}$ increases by more than a factor of 10, much greater than the membrane area increase during the same period. It is not clear whether this represents the appearance of a new Na channel subtype or an increase in density of the subtype already present (Gong et al. 1999; Yarowsky et al. 1991; Zhang et al. 2001). Also beginning in the late embryonic period, there is an increase in amplitude of the delayed outward $K^+$ current, although this change is much smaller and can be accounted for simply by the increase in cell surface area. Finally, we see the appearance of a hyperpolarization-activated (inwardly rectifying) current in CP cells. This current appears relatively late in comparison to the increases in $I_{Na_h}$ and $I_K$. Between E14 and P4, only very few cells express this current. Between P4 and P6, there is a large increase in the fraction of cells expressing this current so that from P6 to P17, it can be detected in more than 80% of cells. This inward current activates slowly on hyperpolarization, with very little current passing positive to $-70 \text{ mV}$ (although there was measurable current at voltages as positive as $-50 \text{ mV}$ in a few cells) and a voltage-dependent time constant of about 330 ms at $-90 \text{ mV}$ and 180 ms at $-120 \text{ mV}$. Although we cannot definitively identify this current as $I_h$, the kinetics and voltage dependence are very similar to those published previously to describe $I_h$ (DiFrancesco et al. 1986; McCormick and Pape 1990; Spain et al. 1987), and block by Cs+ is a further indication that this current is $I_h$.

These complex changes in channel expression in developing CP cells have profound effects on the firing properties of the cells. In embryonic stages, CP cells show relatively low levels of excitability. When active responses can be elicited (27% of embryonic CP cells overall), they tend to be small and long in duration (Figs. 8, E14 and E17, and 9C). The peaks of these responses are not sufficiently depolarized to activate a substantial fraction of the delayed K+ current, and combined with the long time constant of embryonic cells (due to their high-input resistances), this results in a prolongation of the falling phase of the active response so that it follows the membrane time constant (see Fig. 8, E14-middle). None of our embryonic CP cells fired repetitively during long depolarizing stimuli. This does not, however, mean that embryonic CP cells do not fire repetitively in vivo. It is quite possible that these cells are subject to complex waveforms of depolarizing stimuli normally and that some of these can evoke different firing patterns than we see in response to simple square-wave depolarizations.

This situation changes after the perinatal period. The combination of increasing Na and K current, decreased input resistance leading to faster time constants, and eventually, a more depolarized resting potential at the end of the second postnatal week results in the appearance of more rapidly rising and falling action potentials and repetitive firing ability. $I_h$ may also impart a slow depolarization encouraging repetitive firing at these later stages (Bender et al. 2001; Luthi and McCormick 1998; Moosman et al. 1999). Consistent with this idea, we found that 78% of cells expressing $I_h$ generate evoked or spontaneous AP’s compared with only 37% of cells not expressing $I_h$ ($P = 0.0078$ Fisher’s exact test). An even greater difference exists between the percentage of cells with $I_h$ that generate either evoked or spontaneous repetitive activity (72%) and those without $I_h$ (7%; $P = 3.5 \times 10^{-6}$, Fisher’s exact test). In addition, current-clamp recordings made in the absence of stimuli indicate that a steadily increasing fraction of postnatal
cells are normally firing repetitively (see Fig. 8, P2 and P10). This latter conclusion, however, must be made with caution. Although the ability to fire repetitively clearly develops during this early postnatal period, we cannot be certain that the spontaneous activity we observe is not secondary to depolarization induced by leakage currents through the seal resistance. Although this leak should be less important in the lower resistance postnatal cells than in the high resistance embryonic cells, it still may interact with the negative threshold to induce activity in some cases. We believe that at least some spontaneous activity is occurring normally, though, because we have observed activity on hyperpolarized baselines and because we have observed spontaneous transient increases in intracellular Ca\(^{2+}\) using optical methods in these cells in the early postnatal period (unpublished observations).

Many of the developmental changes we have observed are summarized in Fig. 10. Here, we take our data (including that of Picken Bahrey and Moody 2003) in VZ, IZ, and CP cells between E14 and P17 to recreate a likely picture of the development of ionic currents in a single cell as it divides in the VZ, exits the cell cycle there at E14, migrates through the IZ, arrives in the CP on E16, and then undergoes later neuronal differentiation. As it proliferates, the presumptive neuron has very simple electrical properties: a high-input resistance and outward K\(^{+}\) current. On cell cycle exit, a second conductance—a small Na\(^{+}\) current—appears and begins to increase slowly as the cell migrates. This state continues until the differentiating neuron arrives at the cortical plate. At this point, several things happen. First, the cell begins to grow rapidly, causing increases in Na\(^{+}\) and K\(^{+}\) current and a decrease in R\(_{in}\). Na\(^{+}\) current and R\(_{in}\) changes are larger than can be explained by cell growth, however. Lagging slightly behind the increase in I\(_{Na}\) and I\(_{K}\), is the initial expression and increase of a third conductance, the inwardly rectifying cation current I\(_{Kr}\), possibly contributing to the decrease in R\(_{in}\). As the complexity of this cell increases, with expression of I\(_{Kr}\) and increases in Na\(^{+}\) and K\(^{+}\) conductances, the cell begins to mature electrically, gaining the ability to fire repetitively.

The changing patterns of ion channel expression we observe put some constraints on the likely generation of spontaneous activity during early cortical development. If cortical neurons generate repetitive bursts of action potentials that serve important developmental roles, then they should be most likely to do so starting in the very early postnatal period, rather than embryonically. This kind of activity has in fact been observed in the early postnatal rat neocortex (Garaschuk et al. 2000). Although the exact time of onset of such activity has not yet been determined, it is likely that the onset is limited by the ability of the individual neurons to fire repetitively. The termination of such activity, however, is probably determined by the nature of inputs to the cell or by the changing depolarizing drive supplied by conductances active at the resting potential because the intrinsic ability to fire repetitively lasts throughout adulthood. Whatever exact role that intrinsic channel expression in individual neurons plays in timing spontaneous activity, it is clear that the changing patterns of channel expression must be taken into account in describing how developing neurons generate such activity.

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