Voltage-gated Currents, Dye and Electrical Coupling in the Embryonic Mouse Neocortex

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We measured dye coupling, electrical coupling, and voltage-gated currents using whole-cell voltage clamp in slices of mouse sensorimotor cortex at embryonic day 14 (E14). As in rat ventricular zone (VZ), cells of the VZ were extensively dye coupled, often in clusters of >100 cells. In mouse VZ, however, cells were much less electrically coupled, making measurement of voltage-gated currents more accurate. All VZ cells expressed delayed K+ currents (I_k), and 30%, including morphologically identified radial glia, also expressed inward Na+ currents (I_{Na}). This fraction is consistent with I_{Na} expression being an early event following cell cycle exit. Intermediate zone (IZ) cells also expressed I_k and I_{Na}. Na+ current amplitude distributions indicated three populations of IZ cells: those without I_{Na}, those with I_{Na} similar in amplitude to VZ cells, and those with I_{Na} being almost 10 times larger than in VZ cells. Cells of the cortical plate (CP) expressed both I_k and I_{Na}, with I_{Na} being almost 10-fold larger in VZ cells. No cell in any zone expressed detectable hyperpolarization-activated currents. Our data suggest that the distribution and density of I_{Na} may be related to early events of cell cycle exit and migration.

Introduction
The sequence with which functional voltage- and transmitter-gated ionic currents appear during development in many neurons and muscle cells is more complex than would be predicted from a simple linear progression to the mature physiological state. This complexity allows cells to pass through stages of embryogenesis during which the ion channel populations and electrical activity are qualitatively different from those found in the fully developed cell (O'Dowd et al., 1988; Lindsell and Moody, 1995; Greaves et al., 1996). These early stages of development are often characterized by spontaneous action potentials, which are caused by the particular pattern of functional ion channel expression at that time, and which are required for certain aspects of later development (Wong et al., 1993; Gu and Spitzer, 1995; Catalano et al., 1997; Dalman et al., 1998). Although it is clear that spontaneous electrical activity plays important roles in the early development of the mammalian brain (Komuro and Rakic, 1992, 1993, 1998; Herrmann and Shatz, 1995; Catalano et al., 1997; Catalano and Shatz, 1998), how that activity is generated or how it relates to patterns of ion channel development in embryonic neurons is not known.

Most neurons of the mammalian neocortex are generated in a proliferative zone that borders the lateral ventricles (Takahashi et al., 1994). In the mouse, cell division before embryonic day 11 (E11) creates a founder population of ventricular zone (VZ) cells. Recent studies have indicated that much of the resulting neurogenic proliferative population is comprised of radial glia (Parnavelas and Nadarajah, 2001; Noctor et al., 2002). From embryonic day 11 to 17 (E11 to E17), these cells divide 11 times and a steadily increasing fraction of the progeny of these divisions exits the cell cycle and begins terminal differentiation into cortical projection neurons (Takahashi et al., 1995, 1996b; Mione et al., 1997; Tan et al., 1998). These differentiating neurons migrate radially away from the ventricle, cross the intermediate zone (IZ), and populate the cortical plate (CP) where they will form, in an inside-out developmental sequence, the layers of the neocortex (Sidman and Rakic, 1973; Shoukimas and Hinds, 1978; Caviness, 1982). Inhibitory interneurons of the mouse cortex appear to arise in a different manner, migrating tangentially from the VZ of the ganglionic eminence (Anderson et al., 1999; Parnavelas et al., 2000).

Information about the electrophysiological properties of cortical cells during this period of neurogenesis is somewhat incomplete. In the Cajal-Retzius cells of the preplate, fast transient (A-type) K+ and Na+ currents have been reported at prenatal stages (Mienville et al., 1994; Zhou and Hablitz, 1996; Albrieux et al., 1999). Some cells of the embryonic rat VZ express voltage-gated Na+ and K+ currents, Ca2+-activated K+ currents, and GABA_A receptors (Martin-Moutot et al., 1987; Bulan et al., 1994; Mienville et al., 1994; LoTurco et al., 1995; Mienville and Barker, 1997; Hallows and Tempel, 1998). Other evidence indicates that voltage-gated Na+ currents, and perhaps K+ currents as well, are absent in the proliferative populations, including morphologically identified radial glia (Noctor et al., 2002). Rat VZ cells also show extensive dye and electrical coupling (LoTurco and Kriegstein, 1991; Mienville et al., 1994). It is not clear whether the biological significance of this coupling resides in the ability of the cells to exchange developmentally significant metabolites or in their ability to pass electrical signals from cell to cell. It is also not clear whether this extensive electrical coupling may obscure the presence of voltage-gated currents in the proliferative populations when measured in intact brain slices (Noctor et al., 2002).

It became clear from our experiments on asclidian muscle development that detailed maps of the functional expression of ionic currents in developing cells are essential in understanding how ion channel expression at early embryonic stages relates to activity-dependent developmental events (Greaves et al., 1996; Dalman et al., 1998). We have therefore begun such a study in the lateral embryonic mouse sensorimotor cortex and in this paper report results of whole-cell voltage clamp recordings made from brain slices of this region at embryonic day 14 (E14). E14 is near the midpoint of cortical neurogenesis in the mouse. The proliferative zone (ventricular zone; VZ) is at its largest. Cells of the VZ are in cell cycles 8–9 of neurogenesis with a cell cycle time of ∼18 h and the Q, or leaving fraction, is near 0.6 (Takahashi et al., 1995, 1996b; Miyama et al., 1997). This means that on the average, 60% of the progeny of divisions will exit the cell cycle and begin terminal neuronal differentiation. Cells that exit the VZ at this time will primarily populate layer IV of the cortex. Cells in the IZ on this day will populate the deeper layers V and VI (Takahashi et al., 1999).
**Materials and Methods**

**Animals**

Timed pregnant C57BL/6 mice were euthanized on gestational day 14 (E14) by CO₂ inhalation, according to NIH and institutional guidelines. Both uterine horns were dissected out and placed in ice cold artificial cerebral spinal fluid (ACSF; mM): 119 NaCl, 2.5 KCl, 1.3 MgCl₂, 2.5 CaCl₂, 1 NaH₂PO₄, 26.2 NaHCO₃, 11 t-glucose; all obtained from Sigma, St Louis, MO) bubbled with carbogen (95% O₂/5% CO₂). To study Ca²⁺ currents we used two variations on this solution: High Ba²⁺ ACSF (mM): 114 NaCl, 2.5 KCl, 1.3 MgCl₂, 5.0 BaCl₂, 1 NaH₂PO₄, 26.2 NaHCO₃, 11 t-glucose), and divalent-free ACSF (mM: 126.6 NaCl, 2.5 KCl, 1 NaH₂PO₄, 26.2 NaHCO₃, 11 t-glucose). Pups were removed from the uterus, staged by visual inspection or crown to rump length, and cerebral cortices were dissected out and placed in ice cold ACSF, keeping intact the section from olfactory lobes through a portion of the brainstem. Brains were then embedded in a 3% solution of Type IX-A Ultra-low temperature gelling agent (Sigma) in ACSF that had cooled to <32°C, and placed on ice until the agarose was gelled. Coronal slices (200 µm) were cut using a vibratome, removed from the agar, and allowed to recover in oxygenated room temperature ACSF for 60–90 min.

**Recordings**

Pipettes were pulled to a resistance of 8–12 MΩ from 50 µl hemacrit glass capillary tubes using a Narishige two-stage puller and were filled with a potassium or a cesium internal solution (mM: 100 potassium glass capillary tubes using a Narishige two-stage puller and were filled with carbogen bubbled ACSF. In some experiments 1 mM octanol (Sigma) was added to the ACSF for 10 min prior to and during recording. In experiments investigating the identity of inward currents, tetrodotoxin (TTX, 1 µM; Alomone Labs, Jerusalem, Israel) was added to the ACSF to block Na⁺ currents.

Individual cell somas were visualized with a Zeiss Axioskop using water immersion 40× and 63× objectives with DIC optics. Cells within 200 µm dorsal to the LGE border were targeted (see Fig. 1A). Within this area, cell layer was determined by noting the physical position of the recording pipette and by the appearance of the surrounding cells. The VZ appeared as a band of closely packed radial cells adjacent to the ventricle, and was at its thickest nearest the LGE. The IZ was a loose arrangement of cells with an overall appearance predominated by tangential processes. The thinner outer rind of the CP was again more closely packed, often with radial processes similar to the VZ. The location of these layers was further confirmed using measurements from a series of slices that were fixed and stained with the TUJ1 antibody, which identifies neural-specific tubulin. At the transitions between VZ/IZ and IZ/CP are the distinct populations of the subventricular zone (SVZ) and the subplate, respectively. We tried to avoid these cell populations by avoiding cells very near the transition zones, but may have included some SVZ cells in the IZ population, and some subplate cells in our CP population.

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 greater than 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 an Axopatch-1D Patch Clamp amplifier. The resulting currents were filtered at 1 kHz, and recorded and analyzed using pCLAMP 8 software. In some cells current clamp record- ings were made to measure excitability and action potential waveform. When both voltage and current clamp recordings were made from the same cell, the order of the two was randomized.

To measure dye coupling, either Neurobiotin (2%; Vector Laborato ries, Burlingame, CA) or Lucifer Yellow (2%; Sigma) was added to the internal solution. Both of these dyes are small enough to pass through open gap junction channels. Using these dyes, however, precluded identification of the recorded cell. To examine morphology of recorded cells, 3000 (4%) or 10 000 (10%) MW biotin dextran (both of which are too large to pass through gap junctions; Molecular Probes, Eugene, OR) was added to the pipette solution. Cells were held for at least 20 min to allow diffusion of the dye into the cell.

**Histology**

Slices were fixed in 4% paraformaldehyde (Sigma) 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; Sigma). 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 diamino-benzidine and glucose oxidase, β-glucose, NH₄Cl, NiCl₂ and dehydrated through an ethanol series (all chemicals from Sigma). Slices were cleared using cedarwood oil or xylene (Sigma) and mounted on slides in DPX (Fluka, Switzerland).

**Analysis Methods**

Input resistance (Rm) was calculated from the average of responses to voltage pulses to ±10 mV and ±20 mV from ~60 mV. No voltage-gated currents were activated within this range. Capacitance was measured with two methods for each cell. First, a triangle wave voltage command was played to the cell and capacitance measurements were calculated from amplitude of the resulting square-wave current (Moody and Bosma, 1985). Second, the area under the capacitive transient was calculated for a ~20 mV pulse from ~60 mV after that trace was leak subtracted. Peak outward current was measured at ~70 mV. Current density was calculated as peak current divided by the capacitance of the cell as measured in response to the triangle wave voltage command. Kinetics of activation and inactivation were determined by fitting exponential curves to the appropriate portions of the current trace. Gaussians were fit with pCLAMP 8 software and statistics (χ² test and Student’s t-test) were done in Microsoft Excel.

**Results**

**Properties of Cells in the E14 VZ**

**Extensive Dye Coupling among Mouse VZ Cells**

In experiments to measure dye coupling, we successfully recovered 11 Neurobiotin fills from the VZ. All were tightly packed clusters including cells with various morphologies. Some contained radial cells with processes spanning to the pial surface, as well as many cells without long processes (Fig. 1B). Other clusters contained a combination of cells with-out processes and cells with processes extending only from the ventricular surface into the outer IZ (Fig. 1C). Most clusters contained too many cells for a direct count of cell number to be accurate. We estimated cluster size by assuming that the cluster had the same dimension in the anterior–posterior dimension (not visible in coronal sections) as in the visible left–right dimension. We calculated volume of each cluster as the square of the left–right dimension multiplied by the ventricular–pial dimension and divided cluster volume by the volume of a single cell. This yielded an average cluster size of 115 ± 44 cells. This number is similar to the value of 15–90 cells reported in embryonic rat VZ (LoTurco et al., 1991).

**Low Levels of Electrical Coupling among Mouse VZ Cells**

In rat VZ, the unexpectedly low value of input resistance (Rm), its negative correlation with dye-coupled cluster size, and its increase by uncoupling agents indicate extensive electrical coupling (LoTurco et al., 1991). Among cells identified by dye filling in the mouse VZ, we measured an average Rm of 1909 ± 421 MΩ (n = 53). This value is substantially higher than that reported in rat VZ at similar stages (151 ± 64 MΩ), and is
similar to the \( R_{in} \) value of 2600 M\( \Omega \) calculated in rat VZ by extrapolating a plot of \( R_{in} \) versus cluster size to a cluster size of one (LoTurco et al., 1991).

These high \( R_{in} \) values suggest that electrical coupling among VZ cells may be much weaker in mouse than in rat. To test this hypothesis, we blocked gap junctional coupling with octanol (1 mM) in the external solution. Octanol reduced dye-coupled cluster size from 115 ± 44 (\( n = 11 \)) cells to 1.75 ± 0.31 cells (\( n = 8 \); \( P = 0.03 \); see Fig. 1B inset and Fig. 1D), and also increased input resistance, although the increase was not significant (\( R_{in} \): 4120 ± 1856, octanol; \( n = 8 \); versus 1909 ± 421 M\( \Omega \) control; \( n = 53 \); \( P = 0.09 \); Fig. 1D). On further investigation, we found that an identical increase in \( R_{in} \) can be effected simply by not including dye in the recording pipette (without dye: 3645 ± 675 M\( \Omega \), \( n = 103 \); \( P = 0.08 \) when compared with dye fills; \( P = 0.85 \) when compared with octanol; VZ \( R_{in} = 3083 ± 471 \) for total population of cells, with and without dye). Cells along the ventricular margin (M phase) or cells in the middle of the identified VZ (G1, G2, and newly differentiating, migrating neurons) were targeted for this group. These data suggest that the presence of dyes such as Neurobiotin or Lucifer Yellow may artifactually reduce input resistance in these cells by increasing gap junctional conductance. Whatever the mechanism, caution is needed in comparing parameters such as input resistance under different conditions, if the presence of dye in the recording pipette is an additional variable in the experiments.

To ensure that subtle differences in the methods between our experiments on mouse and those of others on rat were not responsible for the difference in electrical coupling, we repeated our \( R_{in} \) measurements on E15 rat VZ (developmentally similar to E14 mouse), and obtained the low input resistance values reported by others (404 ± 106 M\( \Omega \), \( n = 7 \)).

The above results indicate that, although VZ cells of both rat and mouse are extensively dye-coupled, those in mouse are much less electrically coupled. Using the most conservative estimate of coupling, we can assume a single cell \( R_{in} \) of 4 G\( \Omega \) (the value in octanol) and can use the mean value of \( R_{in} \) measured in the VZ of ~2 G\( \Omega \), ignoring the lack of statistical significance of this difference. These values predict that, as an upper limit, 50% of the current during voltage-clamp steps near the resting potential...
leaves the recorded cell across gap junctions into the rest of the dye-coupled cell cluster. A similar estimate made from the data of LoTurco and Kriegstein (LoTurco and Kriegstein, 1991) indicates that ∼95% of injected current leaves the recorded cell in rat VZ. Even if this upper limit were true, however, the fraction of current leaving the recorded cell would be greatly reduced during the flow of voltage-gated currents. At maximal outward K⁺ current, for example, the resistance of the recorded cell falls by ∼10-fold (see below), whereas the cells of the cluster remain at high resistance because they are not depolarized sufficiently to activate the currents. At this point, >90% of the current passed during the voltage-clamp step will flow across the membrane of the recorded cell, providing sufficiently accurate voltage clamp control. Thus voltage-clamp studies can be done on mouse VZ cells without recourse to uncoupling methods (Mienville et al., 1994). This conclusion is confirmed by our observations that uncoupling cells with octanol does not affect the properties of voltage-gated currents in VZ cells (see below).

Although octanol may have effects other than blocking gap junctional communication, a lack of specificity does not affect the interpretation of our results. Our observations indicate that octanol does not directly affect voltage-gated Na⁺ or K⁺ currents in these cells (see below) and it does reduce dye coupled cluster size. This allows us to compare input resistance as a function of cluster size directly between mouse and rat. In rat, a cluster size of one or two cells has an input resistance ∼20 times higher than a cluster size of 90 (LoTurco and Kriegstein, 1991). In our mouse data, a similar reduction in cluster size produced by octanol increases input resistance by at most a factor of 2, although even that average change is not statistically significant.

**Electrical Properties of E14 VZ Cells**

We recorded from 156 cells in the VZ at E14 (Fig. 2C), and confirmed the location of 53 of these by including 3000 MW dextran-conjugated biotin in the pipette (Fig. 5A,B). Almost all (150/156; 96%) VZ cells showed delayed outward K⁺ currents (Fig. 2A). Replacing pipette K⁺ with Cs⁺ blocked these outward currents. The average amplitude of the outward K⁺ current was 301 ± 16 pA (n = 148), measured at +70 mV (density: 69 ± 5 pA/pF; n = 116). These currents first activated at ∼100 ms, with a time constant of activation at +70 mV of ∼1 ms. An averaged current–voltage relation is shown in Figure 2B. The outward currents in most cells showed some inactivation, with current decreasing to 76 ± 1% (n = 137) of its peak value at 80 ms after onset of the pulse (Fig. 2A). These outward currents were not affected by octanol (octanol: 239 ± 49 pA, n = 8 versus control: 301 ± 16 pA, n = 148, P = 0.47) providing further evidence for the lack of substantial electrical coupling among mouse VZ cells. We examined 14 of these cells for fast transient (A-type) K⁺ currents using –80 mV holding potentials, and found no evidence of A-currents in any cell. None of the VZ cells (in fact, no cell in all of our recordings at E14) showed any currents activated by hyperpolarization in the range of –70 mV to –130 mV (Fig. 2D).

Approximately 30% (45/156) of the VZ cells generated inward

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**Figure 2.** K⁺ and Na⁺ currents are activated by depolarizing pulses in cells of the E14 VZ. (A) VZ cells were voltage clamped at –60 mV and maximal current was measured during each of a series of 80 ms voltage pulses ranging from –80 to +70 mV in increments of 10 mV. Shown here are currents elicited by –30, –10, 0, +20, +70 mV pulses from a representative cell. Delayed rectifier type K⁺ currents were nearly always present (150/156), activating around –10 mV and exhibiting moderate inactivation. (B) An averaged I–V curve of normalized K⁺ current from 35 cells. Means ± SEMs are shown. (C) Cells in the VZ were targeted under DIC optics at 63× magnification. The ventricular surface is to the right, and the VZ comprises the center third of the photo with tightly packed, radially oriented cells. (D) Cells were also held at –60 mV and given a series of 80 ms pulses in 10 mV increments from –40 to –130 mV (shown are: –40, –60, –80, –100, –120 mV). No currents were activated upon hyperpolarization in any VZ cell.
Na\(^+\) currents with depolarization (Fig. 3A). In cells with Na\(^+\) currents, the average amplitude was 22 ± 3 pA (n = 45) and the current first activated at about −20 mV. These numbers are derived from cells recorded with K\(^+\) in the internal solution, which could have caused us to underestimate the number of cells with detectable Na\(^+\) currents because of interference by outward K\(^+\) currents. To estimate the effect of this interference, we replaced pipette K\(^+\) with Cs\(^+\) in several experiments (Fig. 3B).

With intracellular Cs\(^+\), we detected inward Na\(^+\) currents in five out of nine cells, with a mean amplitude (9 ± 5 pA) which was significantly smaller than with intracellular K\(^+\). This suggests that internal Cs\(^+\) is exerting a small blocking effect on the Na\(^+\) currents in these cells, which offsets the removal of interfering outward K\(^+\) currents. To estimate the effect of this interference, we replaced pipette K\(^+\) with Cs\(^+\) in several experiments (Fig. 3B).

In recordings with both Na\(^+\) and K\(^+\) currents blocked (5 mM Ba\(^{2+}\) ACSF, internal Cs\(^+\), and 1 µM TTX), we detected small inward currents in four out of nine cells, presumably resulting from Ba\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels. In two of the cells the Ca\(^{2+}\) current was sustained, in one it was transient, and in one it was a combination of both (Fig. 4A). In a further experiment we perfused the slice with divalent free ACSF and recorded large sustained or slowly inactivating inward currents in response to depolarizing voltage pulses with Cs\(^+\) pipette solution in two out of three cells recorded (Fig. 4B). These currents are larger than any inward Na\(^+\) current we saw in any cell with normal ACSF. Some Ca\(^{2+}\) channels become highly permeable to Na\(^+\) ions when external divalent ion concentration is very low (Kostyuk and Krishtal, 1977), therefore these inward currents are likely flowing through Ca\(^{2+}\) channels in E14 VZ cells.

We also investigated the properties of VZ cells under current clamp. The average resting potential was −61 ± 4 mV (n = 16). On injection of long (140 ms) depolarizing current steps, 56% (9/16) of the cells generated a single small active response (Fig. 3C; defined as a positive value of d\(^2\)V/dt\(^2\) during the rising phase). One of eight of the cells tested was also able to generate a small active response following the termination of brief depolarizing current pulses (Fig. 3D). These responses did not overshoot 0 mV, and showed slow repolarizations that approximated the passive decay properties of the membrane (compare Fig. 3C,D). This probably occurs because the peak of the response is negative to potentials that activated significant outward K\(^+\) currents (see Fig. 2B).

Including 3000 MW dextran (which does not cross gap junctions) in the pipette during recording of 53 cells in the VZ also allowed us to investigate correlations between cell morphology and physiology. Because we know that at least two cell populations exist simultaneously in the mouse VZ at E14, precursors and newly postmitotic, migrating neurons, we investigated any morphological or physiological characteristics that would denote separate cell populations. Morphologically, these cells fell into two loose groups: radial cells with somata in the VZ and one long process that extends radially to at least the outer IZ (the classical morphological definition of a radial glial cell), which we will refer to inclusively as radial glial type (Fig. 5B); and those cells with somata within the VZ and short processes confined to the VZ, which we will refer to as non-radial glial type cells (Fig. 5A). The radial glial types may include cycling precursors, neurons that have left the cell cycle and are beginning to translocate their soma up their radial process (Nadarajah et al., 2001; Nadarajah and Parnavelas, 2002), or other populations. Nine of these radial cells had processes extending fully to the pial surface, nine had processes extending to or into the CP, and three had processes that terminated within the outer IZ. Of these, 10 also had short processes that visibly extended to the ventricular surface, a characteristic of cycling...
precursor cells. The non-radial glial types may include cycling precursors, neurons that have exited the cell cycle but have not yet left the VZ, or other populations migrating through the VZ. Sixteen of these cells had no filled processes, 11 had one visible radial process, six were obviously bipolar, one had three processes (one toward the ventricle and two diagonally toward the radial process, six were obviously bipolar, one had three processes, and two had multiple tiny processes.

Almost all cells in both groups exhibit delayed outward K+ currents with similar amplitudes (non-radial glial 291 ± 38 pA, n = 27; radial glial 258 ± 39 pA, n = 12; P = 0.6) and inactivation (77 ± 3% of maximal current remains at 75–80 ms in both populations; Fig. 5C,D). Outward currents are not affected by octanol (octanol: 239 ± 49 pA, n = 8; versus non-radial glial, P = 0.61; or versus radial glial, P = 0.92). Only activation kinetics of outward currents differed significantly between these morphologically defined groups (tau for non-radial glial: 0.92 ± 0.07 ms; n = 23; versus radial glial: 1.64 ± 0.35 ms; n = 11; P = 0.009).

Approximately half (13/27) of the non-radial glial types showed inward Na+ currents with depolarization whereas only 3/12 of radial glial types did (Fig. 5E,F). We also measured Na+ currents in 18 cells using Cs+ in the pipette solution and found no difference between the groups (five out of nine non-radial glial types and five out of nine radial glial types expressed Na+ currents). In cells with Na+ currents, the average amplitude with K+ in the pipette was 18 ± 3.2 pA (n = 13) for non-radial glial and 29 ± 11 pA (n = 3) for radial glial types, and the current first activated at about –20 mV. Neither amplitudes nor kinetics differed significantly between the populations. With Cs+ in the pipette the amplitudes for both groups (9 ± 5 pA, n = 5 for non-radial glial and 7 ± 9 pA, n = 5 for radial glial types), although not different from each other, were significantly smaller than with K+ in the pipette solution. Na+ currents in both populations were blocked by 1 µM TTX.

Because we detected Na+ currents in VZ cells of so many different morphologies and because of the functional overlap in VZ cell types such as neuronal precursors and radial glia (Malatesta et al., 2000; Noctor et al., 2001, 2002), we chose to look for physiologically distinct categories within our sample of recordings based on histograms of Na+ and K+ current amplitudes. A histogram of peak Na+ current amplitudes that included cells with no detectable Na+ current was only poorly fit by a Gaussian distribution (χ2 = 4.8 × 10–7). However, when only cells with detectable Na+ current were included, a single-mode Gaussian distribution with a peak at 11 pA fit the data well (Fig. 6A; χ2 = 0.98). This distribution predicted 12 cells with no Na+ current when in fact there were 111. This indicates that ~90% of the VZ cells that show no detectable Na+ current belong to a separate population. Histograms of K+ current amplitude in VZ cells were well fit with a bimodal Gaussian distribution (Fig. 6B; χ2 = 0.99), but less well with a single Gaussian (χ2 = 0.83), suggesting that there are two populations of VZ cells distinguishable by K+ current amplitude. These two K+ current populations were not, however, related to the Na+ current distribution: cells with and without detectable Na+ current did not differ in their mean K+ current amplitudes (without INa, 282 ± 22 pA K+ current, n = 88; with INa, 270 ± 23 pA K+ current, n = 41; P = 0.74).

Properties of Cells in the E14 IZ

Dye Coupling

We recorded from 62 cells in the IZ at E14, and recovered 12 successful fills (Fig. 7A, B). Nine of these were Neurobiotin fills but only five revealed any dye coupling (i.e. cluster size > 1). That coupling was much less extensive than that found in the VZ, with an average cluster size of 3 ± 1 (average includes all clusters, including clusters of one). Filled processes were oriented both

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**Figure 4.** Evidence of Ca2+ currents in E14 VZ. (A) Depolarizing pulses as in Figure 2A were delivered to E14 VZ with Cs+ pipette solution and both 5 mM Ba2+ and 1 µM TTX in the perfusing ACSF. This protocol elicited small transient or sustained Ca2+ or TTX-resistant Na+ inward currents in a subset of cells. (B) Inward currents, likely carried by Na+ ions through Ca2+ channels, with similar kinetics to those in (A) but with much larger amplitudes than any other inward current we saw were elicited by the same voltage protocols with Cs+ pipette solution and 0 Ca2+ – 0 Mg2+ ACSF.
Figure 6. Histograms of Na⁺ and K⁺ currents in the E14 VZ. (A) This histogram shows all Na⁺ currents recorded in VZ cells, with a bin size of 10 pA. The heavy solid line shows a single Gaussian function fit to all non-zero Na⁺ current data. This Gaussian, if extended to 0 pA of current, predicts 12 cells with no measurable current, significantly fewer than the 111 cells without measurable inward current in our sample. (B) A histogram of all K⁺ currents recorded in VZ cells, with a bin size of 50 pA. This histogram is fit with a bimodal Gaussian function (heavy solid line), indicating two separate populations of cells, with population peaks near 200 pA and 600 pA, based on K⁺ current alone.
tangentially and radially, with coupled cells having similarly oriented processes that were confined to the IZ in our sample (Fig. 7A). Clusters were less tightly packed than in the VZ and at least some cells were clearly coupled via their processes.

**Electrical Properties of Cells in the E14 IZ**

Input resistances of all cells recorded in the IZ were not significantly different from those in the VZ [IZ: 4492 ± 539 MΩ (n = 62); VZ: 3083 ± 471 (n = 156; P = 0.09)]. The fact that $R_{in}$ values for IZ and VZ cells are similar despite the large difference in dye-coupled cluster size argues further for the lack of significant electrical coupling among VZ cells.

All IZ cells showed delayed outward $K^+$ currents of the same average amplitude as those in the VZ (IZ: 336 ± 25, n = 62 versus VZ: 501 ± 16, n = 148; P = 0.25; Fig. 7C). The I–V relation of the $K^+$ currents in IZ cells appeared shifted ~20 mV to the left compared with the VZ (see Figs 2B versus 7D). The outward currents in IZ cells also showed some inactivation, with current decreasing to 81 ± 2% of its peak value at 75–80 ms after onset of the pulse, significantly less inactivation than is seen in VZ $K^+$ currents (74 ± 3%, P = 0.01). No hyperpolarization-activated currents were seen in any IZ cell.

The most obvious differences between IZ cells and VZ cells were the larger percentage of cells expressing inward $Na^+$ current and its increased amplitude (Fig. 7E). While only 30% (45/156) of VZ cells express inward current, 68% (42/62) of IZ cells have measurable $Na^+$ current. The mean amplitude of the $Na^+$ current in IZ cells was significantly larger (IZ: 55 ± 6 pA, n = 42; versus VZ: 22 ± 3 pA, n = 45, P = 5 × 10−6). Current densities were also significantly different (11 ± 2 pA/pF, n = 36 in the IZ versus 5 ± 1 pA/pF, n = 39 in the VZ; P = 0.004) indicating that increases in $Na^+$ current are not due to simple increases in cell membrane area. Voltage dependence and inactivation of IZ and VZ $Na^+$ currents were not significantly different.

Under current clamp, the average resting potential of migrat-
ing cells in the IZ was slightly more positive than that in the VZ, \(-52 \pm 2\) mV (n = 25, P = 0.06). IZ cells were somewhat more excitable under current clamp than VZ cells, probably reflecting their larger Na\(^+\) currents. In response to long injections of depolarizing current, 84\% (16/19) of IZ cells generated an active response (threshold: \(-8 \pm 2\) mV; amplitude: \(10 \pm 1\) mV), compared with 56\% (9/16) of VZ cells. Seventy-five per cent of IZ cells (12/16) were also capable of generating small active responses following brief depolarizing current pulses (Fig. 7F), compared with only 12\% (1/8) of VZ cells. These were different from responses in VZ cells in that at least some showed a positive dV/dt following the termination of the current pulse. Like VZ cell responses, the falling phase was prolonged.

As in the VZ, histograms of Na\(^+\) current distribution in the IZ indicated more than one population of cells defined by Na\(^+\) current amplitude. Part of the Na\(^+\) current distribution in IZ was almost identical to that in the VZ. There was one population described by a single Gaussian distribution of Na\(^+\) current amplitudes centered around a peak of \(-13\) pA (Fig. 8A; similar to the peak at 11 pA seen in VZ). As in VZ, this distribution predicted a number of IZ cells lacking Na\(^+\) current (four cells) much smaller than the actual number of 20, suggesting that the majority of cells with no detectable Na\(^+\) current form a distinct population.

Unlike in the VZ, however, the distribution of Na\(^+\) current in IZ with detectable Na\(^+\) current showed a second, broad peak centered at \(-77\) pA (\(\chi^2 = 0.94\) for bimodal fit; \(\chi^2 = 0.52\) for best single Gaussian fit). To test whether these cell populations defined by Na\(^+\) current amplitude might be related to the presence or absence of dye coupling, we filled and recorded cells with Neurobiotin. In these cells, those that were not dye coupled to other IZ cells had significantly smaller Na\(^+\) currents than those that were part of a dye coupled cluster (\(I_{Na} = 3 \pm 3\) pA where cluster size = 1; \(I_{Na} = 54 \pm 13\) pA where cluster size > 1, n = 4 for each, P = 0.008).

The distribution of K\(^+\) current in IZ cells was also bimodal, being fit by two Gaussians centered around peaks of 150 pA and 350 pA (Fig. 8B; \(\chi^2 = 0.89\) for bimodal fit; \(\chi^2 = 0.46\) for best single mode fit). At the extremes of the K\(^+\) current distribution, cells with the smallest K\(^+\) currents also had small Na\(^+\) currents, and cells with the largest K\(^+\) currents had large Na\(^+\) currents (population with \(I_{K} \leq 150\) pA: \(I_{Na} = 5 \pm 3\) pA, n = 12; population with \(I_{K} \geq 350\) pA: \(I_{Na} = 65 \pm 12\), n = 16; P = 0.0003). Between the extreme K\(^+\) current values, however, individual cells did not show a strong correlation between K\(^+\) current and Na\(^+\) current amplitudes (Fig. 8C).

Properties of Cells in the E14 CP

We recorded from 24 CP neurons using K\(^+\) pipette solution (Fig. 9C). The three successful dye fills exhibited minimal dye coupling, similar to that in the IZ, with cluster sizes of 1, 1, and 6 (Fig. 9A). Input resistances of CP cells were very high (mean > 10 GΩ) and difficult to measure accurately as recorded response and noise levels were comparable in the absence of activation of voltage-gated channels. All CP cells also showed delayed outward K\(^+\) currents, with a mean amplitude of 330 ± 40 pA (n = 23) and density of 14 ± 5 pA/pF (n = 16). Neither amplitude nor density was significantly different from VZ or IZ values (Fig. 9C,D). Voltage dependence was similar to that in IZ cells, although inactivation was significantly reduced relative to both VZ and IZ levels (CP: 91 ± 2\% of current remaining after 75–80 ms, n = 22 versus VZ: 76 ± 1\%, n = 137, P = 0.03; or versus IZ: 81 ± 2\%, n = 62, P = 0.0009). No inwardly rectifying currents were seen in CP cells.

Na\(^+\) currents were much larger in CP cells than in VZ cells. With K\(^+\) in the pipette, 67\% (16/24) of the CP cells showed inward Na\(^+\) currents, with a mean amplitude of 83 ± 25 (n = 16) and density of 13.8 ± pA/pF (n = 16) (Fig. 9C,E). The percentage of cells expressing Na\(^+\) current is similar to that found in IZ (68\%) and more than twofold greater than that in the VZ (30\%). Inward current amplitude and density are also significantly larger than those found in the VZ (VZ amplitude 22 ± 3 pA, n = 45, P = 0.0003; VZ density 4.9 ± 0.7 pA, n = 36, P = 0.01) but similar to inward current amplitude in IZ cells (IZ amplitude 55 ± 6 pA, n = 42; P = 0.14; IZ density 11.1 ± 1.9 pA/pF, n = 39; P = 0.52). All means are from cells with measurable Na\(^+\) current only. The fraction of cells expressing detectable Na\(^+\) current is different between VZ and either IZ or CP samples, but not different between our IZ and CP samples (Fisher exact test).

Current clamp recording revealed that the resting potential for CP cells was –43 ± 4 mV (n = 13), significantly more positive than rest in both the VZ (P = 0.004) and the IZ (P = 0.05).

**Figure 8.** Histograms of Na\(^+\) and K\(^+\) currents in the E14 IZ. (A) This histogram shows all Na\(^+\) currents recorded in IZ cells, with a bin size of 10 pA. The heavy solid line shows a bimodal Gaussian function fit to all non-zero Na\(^+\) current data. This Gaussian shows population peaks near 13 pA and 80 pA. If extended to 0 pA of current, it predicts only four cells with no measurable current, significantly fewer than the 20 cells without measurable inward current in our sample. (B) A histogram of all K\(^+\) currents recorded in VZ cells, with a bin size of 50 pA. This histogram is fit with a bimodal Gaussian function (heavy solid line), indicating two separate populations of cells, with population peaks near 150 pA and 350 pA, based on K\(^+\) current alone. (C) A scatter plot of Na\(^+\) current versus K\(^+\) current shows a correlation between the two at the extremes of K\(^+\) current values. Cells with \(I_{K} \leq 150\) pA have small Na\(^+\) current and cells with \(I_{K} \geq 350\) pA also express a large Na\(^+\) current. A regression line (solid line) and 95% confidence intervals (dashed lines) are shown. Dotted lines mark 150 pA and 350 pA K\(^+\) current, denoting the population peaks of the Gaussian fit.
Eighty-eight per cent (15/17) of CP cells recorded were capable of generating an active response when stimulated with long depolarizing currents. Thirteen of these cells were given brief depolarizing current injections, with 77% generating active responses (Fig. 9F).

Discussion

In this paper, we report the results of voltage clamp measurements on cells of the embryonic mouse neocortex at E14. We chose to begin our study of the electrophysiological properties of developing cortical neurons at this stage, which is the approximate midpoint of neurogenesis in the mouse, because of the variety of cell types and stages of neurogenesis represented in a single brain slice.

We found that cells of the mouse VZ are extensively dye coupled, as they are in rat VZ (LoTurco and Kriegstein, 1991), with an average cluster size measured with Neurobiotin of >100 cells. Unlike in rat, however, mouse VZ cells are not tightly electrically coupled. Although input resistance increased with cell maturity, the average input resistance of even mouse VZ cells was 10-20-fold higher than in rat, was unrelated to dye-coupled cluster size, and was not significantly affected by eliminating dye coupling with octanol. These low levels of

Figure 9. Outward and inward currents in E14 CP cells. (A, B) Cortical plate neurons were identified by cell fills (Neurobiotin) (A) or under DIC optics (B) as a tight arrangement of radially oriented cells adjacent to the pial surface. In (B) the pial surface is at the right edge of the photograph. (C) CP neurons were voltage clamped as in Figure 2B. Outward K+ currents partially inactivate, and a small amount of an A-type current is seen in this recording. Inward currents in the same recording with K+ pipette solution were significantly larger than those in both the IZ and VZ (shown are: –20, 0, 20, 70 mV). (D) Currents were moderately inactivating and activated near –20 mV. Error bars show SEMs. (E) Large Na+ currents are also present with Cs+ in the pipette (shown are: –60, –10, 0, +10). (F) Active responses were elicited in response to brief depolarizing pulses in current clamp.

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electrical coupling allow us to voltage clamp mouse VZ cells without resorting to uncoupling methods. We confirmed this by showing that uncoupling by octanol did not affect the properties of voltage-gated Na⁺ and K⁺ currents. The low average levels of electrical coupling we have observed do not rule out the possibility that specific subsets of VZ cells show stronger electrical coupling. Indeed, experiments in mouse VZ show some specificity in dye coupling by cell type or cell cycle stage (Bittman et al., 1997), so it would not be surprising if the degree of electrical coupling also varied. It is perhaps most useful to think of the frequency domain of coupling. Mouse and rat VZ cells probably exchange small metabolites equally efficiently over the time course of minutes, the frequency domain in which dye coupling is measured and in which exchange of developmentally significant molecules most likely occurs. At high frequencies, the domain at which electrical coupling is commonly measured (1–10 Hz), mouse VZ cells are much less tightly coupled. Even modest amounts of current spread, however, can be highly significant for such functions as synchronizing independently active cells (Moser, 1998; Nolan et al., 1999). Therefore, any major developmental roles of coupling among VZ cells that would be common to both rat and mouse must either involve low-frequency exchange of metabolites or the more subtle effects that can be served even by low levels of high-frequency electrical coupling. Our dye studies do support the expected decrease in coupling as neuronal precursors move into the IZ. This implies that widespread exchange of metabolites within the VZ is generally important in the control of neurogenesis, whereas only much more restricted subsets of co-migrating neurons engage in such exchange during migration.

The high level of electrical coupling in rat is likely to make detection and analysis of small voltage-gated currents difficult without the use of mechanical or pharmacological methods of uncoupling. This may explain why in situ whole-cell clamp in rat VZ detects mainly linear I–V relations (Noctor et al., 2002) whereas in either dissociated cultures from the same preparation or in slices subjected to uncoupling procedures, voltage-gated currents are more commonly seen (Wiedehage et al., 1992; Mienville et al., 1994; Mienville and Barker, 1997). Indeed, with the much lower interference from electrical coupling in mouse, we could show that virtually all cells in all zones expressed delayed outward K⁺ currents. K⁺ current densities did not differ between cells of the VZ, IZ and CP. This indicates that K⁺ currents are not strongly developmentally regulated as cells exit the cell cycle and migrate. It is possible that outward K⁺ currents play a role in the control of cell division, as they appear to do in several other cell types (Amigorena et al., 1990; Vaur et al., 1998). Our large sample of VZ cells (156) allows us to be reasonably certain that all VZ populations (mitotic cells, postmitotic presumptive neurons, radial glia) express similar functional delayed K⁺ currents. To confirm this we recorded K⁺ currents in VZ cells from the E12 cortex, when only a small fraction of VZ cells are exiting the cell cycle, and found K⁺ currents at the same amplitude as at E14 (317 ± 135 pA, n = 36; M. Albrieux and W.J. Moody, unpublished). Within the E14 VZ there were subtle differences in K⁺ currents in different cell types. In cells identified with long processes, K⁺ currents activated slightly more slowly than in other cell types. This is consistent with the fact that at least one K⁺ channel subtype, Kv1.1, is expressed only in cells with the radial glial phenotype at this stage (Hallows and Tempel, 1998).

A significant finding in our voltage clamp experiments was the distribution of inward Na⁺ currents in the VZ. Na⁺ currents were detected in 30% of the total population and approximately half (48%) of the non-radial glial population. Statistical analysis indicated that the fractions of cells expressing and not expressing Na⁺ currents were distinct populations. These fractions are much larger than the population of cells marked by antibodies directed against neural-specific tubulin, thought to be an early marker of cell-cycle exit and the start of neural differentiation (Menezes and Luskin, 1994). This fraction is consistent with the idea that Na⁺ current expression is a very early event after cell cycle exit. In the dorsolateral cortex at E14, the cell cycle time is ~18 h and the fraction of progeny of divisions that exit the cell cycle is ~0.6 (Takahashi et al., 1995, 1996b; Miyama et al., 1997). The fastest migrating cells begin to exit the VZ in ~5 h, and for a given synchronous cohort of progeny, almost all have exited the VZ after 15 h (~80% of the cell cycle time) (Takahashi et al., 1993, 1996a). These numbers suggest that the VZ should be slightly past a steady state at this stage with cell loss by migration somewhat exceeding cell production. This is borne out by direct measurements of VZ size (Takahashi et al., 1996b). Thus we would predict that if Na⁺ current expression followed closely on cell cycle exit then an upper estimate of the fraction of VZ cells with Na⁺ currents should be near 50%, larger than the fraction of total cells with measurable Na⁺ current, but nearly identical to the percentage of cells recorded in the absence of outward K⁺ which expressed Na⁺ current. The hypothesis that Na⁺ current is expressed upon exit from the cell cycle is consistent with reports that toxin-sensitive 22 Na⁺ influx is first detected in mouse brain cells at E12, when the fraction of progeny of divisions that exit the cell cycle first becomes significant (Couraud et al., 1986; Takahashi et al., 1996b). As a further test of this idea, we made voltage clamp recordings from 35 cells in the proliferative population at E9, a stage at which no cell cycle exit is occurring, and from 12 cells in the VZ at E16/17, when most of the progeny leave the cell cycle. We found that no cells had detectable Na⁺ currents at E9 (M. Albrieux and W.J. Moody, unpublished), but that 50% (6/12) of cells expressed measurable Na⁺ currents at E16/17 (H.L. Picken Bahrey and W.J. Moody, unpublished). Also consistent with this idea is the increase in the fraction of cells that express Na⁺ current as well as its increase in density as cells enter the IZ.

The distribution of Na⁺ current among IZ cells suggested three distinct populations: cells lacking Na⁺ current, cells with Na⁺ current amplitudes similar to those in VZ cells (mean near 12 pA), and cells with large Na⁺ current (mean near 80 pA). Although our sample of dye-filled IZ cells is small, our data do suggest that in the IZ, larger Na⁺ currents occur in cells that are dye-coupled to their neighbors. The significance of these populations is unclear, although they may relate to the three types of cells represented in our recordings: migrating cells that originated locally in the neocortical VZ, migrating cells that originated in the ganglionic eminence, and SVZ cells that we may have sampled near the IZ boundary. Different physiological properties in those populations would be of interest in light of the different cell types they give rise to.

Despite the early appearance of Na⁺ currents in development and their increase in density during migration into the CP, cells of all zones at E14 showed only very limited ability to generate active membrane responses. We were unable to elicit repetitive firing in any cell with long current pulses. It is of course possible that the true active responses of CP cells were degraded by the leak resistance of the whole cell recording. This seems unlikely, however, considering that the measured input resistance of CP cells was so high in our recordings. (The response shown in Fig. 9F was from...
a cell with an input resistance of 4.2 GΩ.) In addition, we have recorded numerous examples of repetitive action potentials from cells of the early postnatal cortex. More likely the Na⁺ currents serve to amplify the depolarizations from other sources that we see during current clamp recordings of cells in all layers, or they render the cells responsive to certain frequencies or patterns of inputs that are more complex than our simple square pulse stimuli. We are currently investigating these possibilities.

Many of our findings are consistent with the current picture of neurogenesis in the neocortex. In addition to the large spatial restriction in coupling at the onset of migration, we also see a significant upregulation of inward Na⁺ currents, without concomitant changes in outward K⁺ currents. This would enhance the ability of cells to respond actively to inputs, which in turn would enhance voltage-gated Ca²⁺ influx. This is likely to be important in neuronal migration and differentiation (Komuro and Rakic, 1998). Our finding that cells of radial glial morphology express voltage-gated Na⁺ and K⁺ currents with the same frequency and properties as other VZ cells supports the idea that cells previously thought to be serving radial glial functions may be a major neuronal precursor population as well (Malatesta et al., 2000; Noctor et al., 2001, 2002). In fact, cell identity and stage of differentiation may be better indicated by the presence or absence of Na⁺ current expression than by many other criteria. Molecular identification of the Na⁺ current subtypes expressed by VZ cells and elucidation of factors that regulate their expression is likely to clarify this issue.

Notes
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