Electrical Excitability of Early Neurons in the Human Cerebral Cortex during the Second Trimester of Gestation

Information about development of the human cerebral cortex (proliferation, migration, and differentiation of neurons) is largely based on postmortem histology. Physiological properties of developing human cortical neurons are difficult to access experimentally and therefore remain largely unexplored. Animal studies have shown that information about the arousal of electrical activity in individual cells within fundamental cortical zones (subventricular zone [SVZ], intermediate zone, subplate [SP], and cortical plate [CP]) is necessary for understanding normal brain development. Here we ask where, in what cortical zone, and when, in what zone (SVZ), intermediate zone, subplate [SP], and cortical plate [CP]) is necessary for understanding normal brain development.

Introduction

Prenatal development of the human cerebral cortex involves the proliferation, migration, and differentiation of neurons (Sidman and Rakic 1973; Kriegstein and Parnavelas 2003), navigation and organization of axonal projections (Barone et al. 1995), and construction and maturation of synaptic contacts (Bourgeois et al. 1994). By the end of the eighth gestational week (gw) in the human embryo, the subventricular zone (SVZ) is positioned above the ventricular zone (VZ) and serves as the main proliferative site for the remainder of gestation (40 gw in humans). Cortical cells, generated in the SVZ, migrate through the intermediate zone (IZ) to the cortical plate (CP). In the CP, neurons continue to differentiate and form synaptic connections and neuronal circuits, expanding into layers II–VI of the mature cerebral cortex. Neuronal electrical activity (i.e., the firing of action potentials [APs]) is thought to play an important role in brain development and synaptogenesis. Immature neurons of the central nervous system (CNS) generate spontaneous electrical activity, driving electrically dependent processes, including neuronal survival and differentiation, the growth of afferents, establishment of connections, and synapse stabilization (Katz and Shatz 1996; Moody 1998; Spitzer et al. 2002). Disruptions in this dynamic process are implicated in a number of neurological and psychiatric disorders including mental retardation, autism, schizophrenia, and epilepsy (Maynard et al. 2001; Levitt 2005).

Much of our understanding of cortical development is based on experiments performed in rodents, outlining detailed patterns of neuronal migration from the SVZ to CP and the maturation of sodium and potassium currents once neurons reach the CP (Bahrey and Moody 2003; Picken Bahrey and Moody 2003; Noctor et al. 2004). Although invaluable for providing us with a basic model of neuronal development, rodent studies cannot directly be applied to human cerebrocortical development (Levitt 2003). For example, the human forebrain is more than 100 times larger than that in rodent. Cortical neurogenesis in humans lasts 10 times longer than that reported in rodents (Takahashi et al. 1995; Kornack and Rakic 1998). Prolonged neurogenesis and larger forebrain size in human lead to an overrepresentation of upper cortical layers II/III resulting in more elaborate cortical circuit diagrams (Marin-Padilla 1992). Finally, glutamatergic pyramidal cells and γ-aminobutyric acid-ergic interneurons appear to have different sites of origin and migratory pathways in rodents and primates (Anderson et al. 1999; Letinic et al. 2002). Taken together, these pronounced differences between species make it difficult to directly compare the temporal and functional developmental sequences of neurons and limit our ability to generalize among species.

Studies performed on postmortem tissue reveal the characteristic anatomy and molecular composition of the human CP (Marin-Padilla 1992; Rakic and Zecevic 2003; Zecevic et al. 2005). To understand, however, the physiological aspect, arousal and development of cortical electrical excitability, direct physiological measurements from human cells are essential. Electroencephalographic recordings and functional magnetic resonance imaging do not have the necessary resolution to resolve electrical activity in a single cortical layer (e.g., SVZ or CP), particularly at the level of an individual cell. Because human tissue is not routinely available, direct intracellular electrical recordings have been limited to cultured cells and embryonic stem cells (Kerkovich et al. 1999; Johnson et al. 2007). Here we provide a detailed account of human cortical cells and their physiological properties in an intact acute human brain slice obtained between 16 and 22 gw (second trimester of gestation).

We found substantial differences in the cellular properties (i.e., morphology, molecular composition, and passive and
active membrane properties) between the SVZ, (site of proliferation) and CP (site of neuronal differentiation), capable of generating an AP upon direct current depolarization, can be found within the human CP as early as 16 gw but not in the SVZ. The differentiation process of CP neurons involves a gradual increase in the amplitude of inward sodium current occurring on an individual cell-to-cell basis. Thus, throughout the human CP, we find neighboring cells with remarkably different magnitudes of sodium current and, consequently, with different abilities to generate an abortive or full-size AP. By 19 gw, young cortical neurons acquire axon initial segments (AIS) populated with voltage-gated sodium channels. Based on their physiological (e.g., ability to fire repetitive APs) and molecular properties (correctly aligned AIS with clusters of sodium channels), the most mature neurons of the human fetal cortex reside not in the CP but rather in the upper parts of the subplate (SP), a cortical zone immediately below CP. Portions of this work have previously been presented in abstract form (Moore et al. 2007).

**Whole-cell Patch-Clamp Recordings**

All physiological recordings were done on the day of tissue collection. Brain slices, 500 μm thick, encompassing both the CP and SVZ obtained from the medial telencephalic wall of the occipital or frontal lobe, were sectioned on a standard Vibratome (Campden Instruments, Lafayette, IN) in ice-cold artificial cerebral spinal fluid (ACSF) solution. The ACSF contained (in mM) 125 NaCl, 2.3 KCl, 26 NaHCO₃, 2 MgSO₄, 1.26 KH₂PO₄, 2 CaCl₂, and 20 glucose (pH = 7.3, osmolality = 310 mOsm/kg). Slices were incubated at 37 °C for one and a half hour and then stored at room temperature prior to recordings. Patch-clamp recordings were performed as previously described (Mo et al. 2007). Briefly, acute brain slices were transferred to an Olympus BX51WI upright microscope (equipped with infrared camera IR-1000; Dage-MTI, Michigan City, IN) and gravity perfused with aerated (95% O₂/5% CO₂) ACSF at 32 °C. Individual cells in the SVZ, SP, or CP were selected using infrared differential interference contrast video microscopy. Patch pipettes (7–10 Ω) were filled with an intracellular solution containing (in mM) 135 K-gluconate, 10 HEPES, 2 MgCl₂, 3 ATP-Na₂, 0.3 GTP-Na₂, and 10 phosphocreatine Na₂ (pH = 7.3 adjusted with KOH, osmolality = 300 mOsm/kg). For fluorescent identification of cellular morphology, sulforhodamine 101 (50 μM, molecular weight [MW] 606.7) or Lucifer Yellow CH potassium salt (60 μM, MW 521.56; Molecular Probes, Eugene, OR) was added to the patch pipette. Membrane potential values were corrected for liquid junction potentials: −10.4 mV for the gluconate-based electrode solution and −6.8 mV for the cesium-rich solution. Standard whole-cell patch-clamp recordings, both voltage- and current-clamp configuration, were used to determine the basic electrophysiological properties of each individual cell. For analysis of the current activation dynamics, cells were given a series of voltage steps of −90 to +30 mV for 50 ms, from a holding potential of −70 mV. For inactivation, cells were given a series of prepulse potentials, from −90 to +30 mV, duration 50 ms (holding −70 mV), and stepped to a holding potential of 0 mV for 50 ms. Cesium, a potassium channel blocker, was substituted for potassium in our intracellular solution to block K⁺ currents. Intracellular solution with cesium contained (in mM) 70 Cs₂SO₄, 6 EGTA, 0.1 CaCl₂, 10 HEPES, 4 ATP-Mg, 0.3 Na-GTP, and 10 phosphocreatine. In addition, this subset of cells also received a local application of tetrodotoxin (TTX) 50 μM to block sodium currents and were subtracted offline using Clampfit 9.2 (Molecular Devices, Union City, CA). Recordings were performed with Multiclamp 700B and Clampex 9.2 and measured offline. These data were subsequently

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**Figure 1.** Cellular and molecular composition of the human fetal cortex. (A) Hoechst staining of an acute cortical slice taken from the lateral telencephalic wall of the human fetal cortex. Scale bar: 100 μm. (B) Live infrared video microscopy of cortical cells in CP (top), IZ (middle), and SVZ (bottom). Scale bar: 20 μm. (C) Cells in the CP (top) and SVZ (bottom), labeled with GFAP (red) and MAP2 (green). Scale bar: 20 μm.
analyzed using Excel (Microsoft, Seattle, WA) and Origin (OriginLab, Northampton, MA). Current traces were analyzed assuming a Hodgkin-Huxley model. Activation and inactivation current traces were determined based on the peak current: $I = G(V_m - E_q)$. Where $V_m$ is the membrane command potential and $E_q$ is the reversal potential. The normalized conductances ($G/G_{max}$) were fitted with a Boltzmann equation, $y = A_1 - A_2/(1 + e^{(V-V_0)/k}) + A_2$. The time constant of activation ($\tau_{act}$) for potassium current was calculated in the 20-ms time window starting just after the capacitative transient and fitted with a first-order exponential, $R(t) = A_1(1-e^{-(t/\tau)})^2 + C$. 

**Voltage Waveform Analysis**

The first derivative ($dV/dt$) of membrane voltage waveform was used to differentiate between linear (passive) and nonlinear (regenerative) membrane responses. Previously recorded waveforms were passed through a derivative function constructed in Clampfit 9.2 (Molecular Devices) resulting in a second waveform (Fig. 4B). Although the positive component of $dV/dt$ is attributed to the activation of sodium channels, we found a better correlation between the negative component of $dV/dt$ and peak $I_{Na}$ (Supplementary Fig. 5) because even purely passive membrane responses have a measurable positive component in the $dV/dt$ trace (see Fig. 4A, passive). Therefore, in this study, the amplitude of the negative component in the $dV/dt$ trace was used as an objective measure of nonlinearity of the membrane response. We empirically determined that zero or small amplitude of the negative $dV/dt$ component (in the range 0 to $-2 V/s$) signified a passive response, whereas nonlinear membrane responses (spikes) produced large negative deflections in $dV/dt$ in the range of $-2$ to $-18 V/s$ and had a visually obvious deflection in the voltage trace. In this analysis (Fig. 4C,D), we did not include data from the SP at 22 gw, where cells were in a more advanced state of maturation (full-size AP and robust repetitive firing). Sequential voltage- and current-clamp recordings allowed us to perform a correlation analysis between $dV/dt$ of the voltage waveform and the peak sodium current amplitude obtained in the same CP cell ($n = 10^7$).

Besides correlation analysis, we also performed Pearson’s chi-squared test. Data were grouped into 3 bins depending on the waveform amplitude ($dV/dt: 0-2, 2-4, and 4-20 V/s$) as well as 3 bins of peak voltage sodium current ($I_{Na}: 0-250, 250-500, and 500-2000 pA$) Table 2. The chi-squared analysis confirmed a dependency between sodium current amplitude and voltage waveform ($\chi^2 = 158.2$, degrees of freedom = 4, $P < 0.05$).

**Immunohistochemistry**

Following electrophysiological recordings, brain slices were fixed in 4% paraformaldehyde for 30 min, embedded in Tissue-Tec OCT mounting medium, and resectioned on a microtome to 20-μm thick sections ($n = 3$ cases, 19 gw; $n = 1$ case, 22 gw, $n = 2$). Sections were mounted onto gelatin-coated glass coverslips, permeabilized, and blocked with normal goat serum in 3% Triton X-100. The mouse monoclonal PanNav channel and rabbit polyclonal βIV spectrin antibodies have been previously described (Rashid et al. 1999; Ogawa et al. 2006). Antibodies were applied overnight at the following concentrations: glial fibrillary acidic protein (GFAP) polyclonal (Dako, Carpinteria, CA; 1:200), microtubule-associated protein 2 (MAP2) monoclonal (Sigma; 1:40), PanNav monoclonal (1:200), and βIV spectrin polyclonal (1:300). Sections were rinsed 3 x 8 min in phosphate buffer (PB) with normal goat serum, and secondary antibodies were applied at room temperature for 1 h. Alexa Fluor 488 anti-rabbit and 594 anti-mouse–conjugated secondary antibodies were purchased from Invitrogen (Carlsbad, CA). Sections were rinsed 3 x 10 min with PB and coverslipped with glycerol and p-phenylenediamine (Sigma).

**Figure 2.** Morphological and passive physiological properties of SVZ and CP cells. (A) Rhodamine-filled cells in CP (top) and SVZ (bottom). Arrows mark cellular processes. Scale bar: 5 μm. Actual current traces for these 4 cells are displayed in Supplementary Fig. 1. [B] SVZ cells (gray) typically had 1 or 2 long process, whereas CP cells (black) have 2 or more, shorter processes. n.d.a., no data available due to no dye filling or loss of cell prior to sufficient filling time. (C) CP cells, on average, have higher input resistance than SVZ cells. (D) Peak $I_{Na}$ amplitudes in SVZ cells with 1-5 processes. “n” Values indicate number of cells recorded with zero sodium current at each process. For clarity, 0 process ($n = 3$) and 1 process ($n = 4$ at zero $I_{Na}$) were omitted from the graph. Note that SVZ cells with detectable $I_{Na}$ greater than 0, typically had 1 main process. (E) Peak sodium current amplitudes for CP cells with 1-5 primary processes. Note, CP cells endowed with an $I_{Na}$ greater than 0 typically had 2-4 primary processes.
Data Analysis

Summary data are expressed as mean ± standard error of the mean. Statistical analyses were performed between different fetal ages and between anatomical layers using the alpha 2-tailed Student’s t-test and univariate analysis of variance, respectively, with a significance level of *P < 0.05 considered significant. Measurements of segment lengths and density were performed offline from high-resolution microphotographs using AxiosVision LE 4.5 (Zeiss, Thornwood, NY) at ×40 magnification and a visual field of 220 × 160 μm. The term “excitability” was used in the text to indicate intrinsic membrane excitability, as opposed to synaptic excitability which was not investigated.

Results

Electrophysiological Properties of the Developing Human Cerebral Cortex

Brain slices were harvested from the medial telencephalic region at 16, 17, 19 (2), 20 (2), 21, and 22 gw (n = 8) containing dissected portions of the SVZ, IZ, SP, and CP (Fig. 1A). Live infrared imaging of the acute brain slice portrayed some distinct characteristics of the aforementioned anatomical layers (Fig. 1B). Individual SVZ cells with small round cell bodies resided in large cell clusters (Fig. 1B, bottom). The IZ/SP had long radial fibers with translocating cell bodies suggestive of cellular migration from the SVZ to the developing CP (Fig. 1B, middle) (Sisman and Rakic 1973). Within the CP, the cells were densely packed similar to that in the SVZ but more elongated and ovoid in shape (Fig. 1B, top).

At the earliest gestational age examined, 16 gw, young SVZ cells expressed molecular markers for radial glia (RG) and astrocytes (GFAP) and neurons (MAP2, Fig. 1C). In the CP, there were RG shafts and more defined MAP2-positive neurons (Fig. 1C, compare SVZ with CP). This early expression of different molecular markers makes it difficult to establish neuronal identity based solely on immunodetection (Howard et al. 2006). In addition to analysis of molecular markers via immunolabeling, electrical recordings may greatly facilitate the determination of neuronal lineage and the time course of neuronal differentiation (Mo et al. 2007).

Whole-cell patch-clamp recordings were performed with a patch-clamp electrode filled with fluorescent dye, sulforhodamine (50 μM), to visualize individual cellular morphologies (Fig. 2.4). Fluorescently labeled cells in the SVZ typically had 1 or 2 processes (Fig. 2A,B; average = 1.5 ± 0.2, n = 24) extending toward the ventricular and/or pial surface. In the CP, on the other hand, fluorescently labeled cells had 2 or more primary processes (Fig. 2A, arrows; average = 2.3 ± 0.1, n = 65) and more extensive branch points. A small number of rhodamine-filled cells in the IZ had long leading and/or trailing processes (Supplementary Fig. 2A).

Along with morphological characterizations, we examined potential differences in the passive physiological properties among SVZ and CP cells. We plotted the effective input resistance and the number of primary neurites for each individual cell, when available (Fig. 2B,C). In 132 of 221 recordings, the cells were either lost before the adequate filling of the dendritic tree was accomplished or dye was not included in the recording pipette (Fig. 2B, no data available). Compared with the CP, SVZ cells had a smaller input resistance (SVZ, 2.26 ± 0.21 GΩ; CP, 3.05 ± 0.18 GΩ, Fig. 2C) and fewer neurites (Fig. 2B). Previous studies have suggested that including dye in the recording pipette can cause a decrease in coupling and change input resistance values (Ara et al. 2002). We, thus, compared the input resistances of SVZ cells recorded both with and without dye and found no statistical difference (2.13 ± 0.63 GΩ [dye, n = 24] vs. 2.34 ± 0.27 GΩ [no dye, n = 29]; P = 0.63). Therefore, the presence of sulforhodamine in the recording pipette did not alter the cellular input resistance. Next we used a fluorescent dye with the potential to cross through gap junctions (Lucifer Yellow, MW 521). In both SVZ (n = 10) and CP cells (n = 22) injected with Lucifer Yellow, we found no evidence of dye coupling among neighboring cells. Because gap junctions are sensitive to metabolites such as pH and dye transfer requires an extended amount of time, which we were not always able to achieve, this result is not sufficient to argue that gap junctions are not present in the human SVZ as they have been reported in other species (Bittman et al. 1997; Bahrey and Moody 2003). In addition to input resistance, we also evaluated the resting membrane potential and membrane capacitance of SVZ and CP cells and observed no significant differences among the resting membrane potential or the membrane capacitance in either location (SVZ, −54 ± 2.2 mV, and CP, −49.8 ± 1.3 mV, P = 0.078; SVZ, 17.7 ± 1.33, and CP, 15.6 ± 0.88 pF, P = 0.21, respectively, Table 1).

### Table 1

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<th>V_m (mV)</th>
<th>C (pF)</th>
<th>R_m (GΩ)</th>
<th>Peak I_h (pA)</th>
<th>Peak I_Na (pA)</th>
<th>No. with I_m</th>
<th>Total</th>
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<td></td>
<td>17</td>
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<td>CP gw</td>
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<td>1.52</td>
<td>−41.9</td>
<td>376.4 ± 28.7</td>
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<td>18.5</td>
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<td>−607.9</td>
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<td>14.6</td>
<td>−651.8</td>
<td>576.7 ± 114.1</td>
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Note: I_m, potassium current; I_h, sodium current; R_m, input resistance; V_m, resting membrane potential. Values are expressed as mean ± standard error of the mean with P values from independent Student’s t-test in parentheses.
Voltage-gated Sodium and Potassium Currents in the Human SVZ and CP

Voltage-clamp analysis revealed a wide range of sodium current amplitudes present in human fetal cortical cells (Fig. 3A, zero, small, medium, and large $I_{Na}$). Although all the recorded cells ($n = 221$) expressed some level of potassium current, the transient TTX-sensitive inward components (Fig. 3A, arrows) were present in only 20% ($n = 12/60$) of cells examined in SVZ and 71% ($n = 114/161$) of recorded CP cells (Fig. 3B,C). Cells with medium (200–500 pA) and large (500–2000 pA) peak $I_{Na}$ were only found in the CP, resulting in an average peak $I_{Na}$ significantly ($P < 0.05$) greater than in SVZ (Table 1 and Supplementary Fig. 1, top row). In contrast to SVZ, there was a gradual increase in $I_{Na}$ peak amplitude in correspondence with an increase in process number in the CP (from 1 to 5, Fig. 4A, right, and Supplementary Fig. 1, top row).

We next examined whether $I_{Na}$ density was increasing over different gestational weeks. The average INA density in the CP more than doubled from 16 gw to 22 gw (16 gw = $46.6 \pm 8.6$ pA/pF, $n = 34$; compared with 22 gw = $96.8 \pm 20$ pA/pF, $n = 13$; Fig. 3F). There was a statistically significant difference in $I_{Na}$ density between SVZ and CP at each gw (Fig. 3F, asterisks). The average $I_{Na}$ density in the SVZ, however, remained unchanged from 16 to 21 gw, and interestingly, by 22 gw, we did not find any cells in the SVZ with detectable $I_{Na}$. Across all gestational ages examined, we found a continuum of INa rather than distinct populations (Supplementary Fig. 4).

Potassium current ($I_{K}$) density in the SVZ was also relatively uniform across gw examined (Fig. 3F, gray). In the CP, on the other hand, $I_{K}$ density steadily increased with age (Fig. 3F, black). By 22 gw in the CP, there was a statistically significant increase in $I_{K}$ density, compared with 16 gw (Fig. 3F, star). Among the 2 cortical zones (SVZ and CP), a significant difference in $I_{K}$ density was not observed until 21 and 22 gw (Fig. 3F, asterisks).

We analyzed the relationship between the number of processes with input resistance, $I_{K}$, and $I_{Na}$ peak amplitudes (SVZ, $n = 24$; CP, $n = 60$). In both the CP and SVZ, we found no difference among input resistance and $I_{K}$ levels in regard to the number of processes. We did, however, notice that only cells with one main process in the SVZ had $I_{Na}$ (Fig. 2D, left, and Supplementary Fig. 1, bottom row). In contrast to SVZ, there was a gradual increase in $I_{Na}$ peak amplitude in correspondence with an increase in process number in the CP (from 1 to 5, Fig. 2D, right, and Supplementary Fig. 1, top row).

Sodium APs in the Human Fetal Cortex
To test whether fetal neurons in the human cerebral cortex can generate APs, we injected SVZ and CP cells with gradually increasing levels of depolarizing current at both their natural resting membrane potentials and a holding potential of −60 mV. All cells in the SVZ ($n = 42/42$), as well as the majority of CP cells ($n = 74/107$), produced only passive membrane responses (Fig. 4A, passive). However, approximately one-third of CP cells ($n = 33/107$) produced a nonlinear transient in the membrane potential waveform, characterized as either an abortive ($n = 17/107$, Fig. 4A, abortive) or full-size AP ($n = 16/107$, Fig. 4A, full). Additionally, a small population of CP cells by 20 and 22 gw ($n = 8/56$) produced repetitive AP firing (Fig. 4A, repetitive) as indicative of progressive neuronal maturation (McCormick and Prince 1987; Spitzer et al. 2002). Cells capable of firing abortive and full-size APs had a multipolar morphology containing more primary processes and branch points than cells with a purely passive membrane response (as previously described in Fig. 2), again suggesting a strong correlation between morphological maturation and the ability to fire APs. Within the IZ, cells with 2 long radially oriented processes more closely resembled cells in the SVZ with no AP firing (Supplementary Fig. 2A), and a subset

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**Table 2**

<table>
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<th># of cells</th>
<th>dV/dt range (V/s)</th>
<th>Median (V/s)</th>
<th>Bins (V/s)</th>
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<td>Passive</td>
<td>74</td>
<td>0.03–1.862</td>
<td>0.43</td>
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<tr>
<td>Abortive</td>
<td>17</td>
<td>0.214–4.913</td>
<td>2.40</td>
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<tr>
<td>Full</td>
<td>16</td>
<td>3.72–18.34</td>
<td>9.34</td>
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Note: All values for dV/dt are negative—minuses are omitted for clarity.
Figure 4. CP cells fire sodium APs. (A) Voltage waveforms in the human cerebral cortex during the second trimester of gestation. Labels indicate cortical zones where selected voltage waveforms (passive, abortive, full-size, or repetitive AP) were observed. Note that all cells in SVZ showed passive responses upon current depolarization. (B) First derivative (dV/dt) of the voltage waveforms. Cells with a nonlinear voltage waveform (active membrane response, spike) showed a large negative component in dV/dt (arrow), more negative than −2 V/s. Cells with a passive membrane response had very small or zero negative component. (C) Amplitude of the negative component of dV/dt is plotted versus the peak sodium current (INa) obtained in the same CP neuron (n = 107), on a semilogarithmic scale. Horizontal line indicates cutoff (−2 V/s) for passive response (Table 2). (D) Same data as in (C) plotted on a linear scale. Horizontal dashed line depicts the cutoff amplitude of INa (−222 pA). Cells exhibiting an INa amplitude greater than −222 pA have a nonlinear membrane response to direct depolarization (abortive or full-size AP) and, therefore, can be identified as neuronal progeny. All data in (C) and (D) are negative values; minuses before labels for x- and y-axes are omitted for clarity.

of cells with 3 or more extending processes were able to fire a single abortive spike upon current stimulation. In one subject (22 gw), we recorded from cells in the SP. All SP cells examined (n = 5), identified by their large cell bodies and number of process extensions (Supplementary Fig. 2B, a), had large INa peak amplitude (range 513-1377 pA) and generated repetitive AP firing (Supplementary Fig. 2B, b, c) following direct current stimulation.

Although it is well understood that AP firing is highly dependent on levels of INa, exactly how much INa is required for a developing human neuron to fire a regenerative spike remains unknown. To analyze the relationship between membrane voltage response (AP firing) and peak INa, each cell was recorded in both current- and voltage-clamp configurations. This method yielded both voltage traces and current traces (Figs 4A and 3A) obtained from the same cell (n = 107). Voltage waveforms (Fig. 4A) were separated into 3 bins, passive, abortive, and active (Table 2), based on a set of criteria described in Materials and Methods. Amplitude of the dV/dt negative component in the range 0 to −2 V/s signified a passive response (Fig. 4B, right), whereas nonlinear membrane responses (spikes) produced large negative deflections in dV/dt (Fig. 4B, left, arrow) in the range of −2 to −18 V/s. This analysis only included the data set 16–21 gw from cells recorded in the CP (n = 107). CP cells with passive responses (0 to −2 V/s) had an average INa of only −69 ± 10.2 pA (n = 74). Cells showing abortive APs (Fig. 4A, abortive; range, −2 to −4 V/s) had an average INa of −350 ± 52.4 pA (n = 17). Cells capable of generating a full AP (Fig. 4A, full) had a dV/dt range between −4 and −18 V/s and an average INa of −929 ± 116.9 pA (n = 16). Both the chi-squared test (Materials and Methods) and linear fit of the paired data revealed a strong dependence of dV/dt on the peak INa amplitude (R² = 0.84, n = 107).

The rapid change in slope of the trend line occurring around 2 V/s (Fig. 4C) indicated that human cells capable of firing an abortive or full-size AP fell above this value and had an INa amplitude around 230 pA or greater. Plotting the data on a linear scale (Fig. 4D) allowed us to determine a precise INa value (222 pA) at the cutoff between passive and active (abortive AP) voltage waveforms. Therefore, cells exhibiting peak INa equal or greater than 222 pA will invariably generate an AP upon adequate depolarization. Although it is no surprise that AP firing is related to INa amplitude, it is the first time the relationship has been analyzed on an individual cell basis and in light of human cortical cell maturation.

Kinetics of Sodium and Potassium Currents in Human Fetal Cortical Neuron

To determine the properties of the voltage-gated INa in young neurons of the human CP, we recorded a subset of these
neurons with cesium, a potassium channel blocker, in the intracellular recording pipette (n = 6). Subsequently, recordings were repeated during a local application of TTX (50 μM, intrapipette concentration) and subtracted offline to reveal an isolated I\textsubscript{Na} (Fig. 5A). To determine the activation of voltage-gated I\textsubscript{Na} in young human CP neurons, a series of voltage steps, -90 to +30 mV in 10 mV steps from a holding potential of -70 mV, were applied to each cell (n = 6). The I\textsubscript{Na} obtained from immature human CP neurons demonstrates activation properties consistent with Hodgkin-Huxley type activation (Hille 2001). The half-activation voltage (V\textsubscript{1/2 Act}) of fetal CP neurons was -38 ± 0.31 mV (Fig. 5B), similar to that observed among young mouse cortical neurons (Huguenard et al. 1988).

The inactivation kinetics of I\textsubscript{Na} in fetal cortical neurons was determined by a series of prepulse potentials and stepped to a holding potential of 0 mV (Materials and Methods). The I\textsubscript{Na} inactivation curve was also plotted and fitted with a Boltzmann equation (Fig. 5B). The half-inactivating voltage (V\textsubscript{1/2 Inact}) was -42 ± 0.29 mV (n = 6).

Strong outward I\textsubscript{K} was regularly detected in cells belonging to both CP and SVZ. A small fraction, 6% in CP and 8% in SVZ, exhibited a rapidly inactivating I\textsubscript{K} (Fig. 5C, inactivating). The greater majority of cells in both CP and SVZ exhibited a slow inactivating (25% in CP and 21% in SVZ) or noninactivating I\textsubscript{K} (Fig. 5C, noninactivating, 69% and 71%, respectively). It is among these noninactivating I\textsubscript{K} which we chose to analyze in greater detail. We asked whether the arousal of excitability in CP cells with I\textsubscript{Na} greater than 500 pA (n = 6) was accompanied by voltage-gated I\textsubscript{K} with different kinetics than those found in the immature cells of the SVZ with no I\textsubscript{Na} (n = 6). In the CP, the V\textsubscript{1/2 Act} for I\textsubscript{K} was +16.7 ± 0.89 mV (Fig. 5D, circles). In the SVZ, V\textsubscript{1/2 Act} was -6.1 ± 1.03 mV (Fig. 5D, squares). To further characterize the differences in I\textsubscript{K}, we examined the time constant of activation (τ\textsubscript{Act}). Between the examined cells in the SVZ displaying no I\textsubscript{Na} and CP cells with I\textsubscript{Na} there was a voltage-dependent shift in the peak τ\textsubscript{Act} from -20 mV (SVZ) to -0.4 mV (CP) (Supplementary Fig. 6). The voltage-dependent shift in both the V\textsubscript{1/2 Act} and peak τ\textsubscript{Act} suggests that differentiating neurons in the human CP have acquired a different subset of voltage-gated potassium channel isoforms than undifferentiated cells of SVZ. Alternatively, the channels may be the same, but their regulation (e.g., state of phosphorylation or changes in beta subunit composition) may be different in the 2 developing cortical regions.

**AIS in the Human Fetal Cortex**

To identify the presence of AIS in the human fetal cortex, we used antibodies against βIV spectrin, a protein localized to AIS and nodes of Ranvier (Berghs et al. 2000). Within SVZ, we did not observe any βIV spectrin–positive AIS (Fig. 6C). At 19 gw, the number of βIV spectrin–positive AIS in the SP was small, typically no more than 2 per visual field (220 × 160 μm; Fig. 6B). The number of AIS in human CP, on the other hand, was on average 6.8 ± 0.5 AIS per visual field (n = 3, 19 gw; N = 45 fields), and the average length of human AIS was 16.2 ± 0.8 μm (Fig. 6A). Still, these numbers are in stark contrast to mature AIS in the human cortex (Inda et al. 2006) and our own measurements in the mature rat cortex (postnatal day 36). Under identical tissue processing conditions, we found the density of AIS to be more than 5-fold higher in rat cortex (30.3 ± 1.7 per visual field, n = 4 brain sections, N = 15 visual fields, P < 0.05). Our measurements also show that the average AIS length in the postnatal day 36 rat is nearly 3 times greater 49 ± 0.6 μm (Supplementary Fig. 7) than in the human fetus 16.2 ± 0.8 μm (Fig. 6).

βIV spectrin plays a key role in the localization of sodium channels to the AIS (Hedstrom et al. 2007). We used PanNav, an antibody which labels all sodium channel isoforms (Rasband et al. 1999), to address sodium channel clustering at AIS. In the human fetal cortex, we found some βIV spectrin–positive segments in the CP that did not colocalize with PanNav (Fig. 6B). However,
a significant fraction of βIV spectrin-positive AIS in the CP and SP were positive for PanNav (Fig. 6D, arrows). Taken together, these data suggest that AIS are already present in young CP and SP neurons during the second trimester of gestation.

Discussion

Electrical activity of early cortical neurons in utero is thought to guide the invasion of afferents and the establishment of proper neuronal circuits (Katz and Shatz 1996). Although the physiological properties of developing cortical neurons have been largely studied in animal (Huguenard et al. 1988; Picken Bahrey and Moody 2003; Noctor et al. 2004), the direct profiles of immature neurons from human fetal brain are scarce. Here we utilized brain tissue obtained postmortem from human fetuses in the second trimester of gestation (16-22 gw). In the present study, whole-cell electrical recordings were made from individual cells with preserved morphologies and intact surrounding tissue (acute brain slice preparation). We sampled electrophysiological properties of 221 cells located in SVZ, IZ, SP, and CP. The major findings of the present study are summarized in Figure 7.

Human SVZ

The SVZ of the human cerebral cortex is a site of active cellular proliferation, producing young cortical neurons destined for the CP (Sidman and Rakic 1982). A number of other cells are also located in the SVZ at this time, including RG, neuroepithelial cells (NEs), dividing oligodendrocyte progenitor cells, and postmitotic neurons (Morest and Silver 2003; Howard et al. 2006). At least 3 different cell types, NE, RG, and postmitotic neurons, exhibit a similar morphology: a cell soma in VZ or SVZ, a short process extending to the ventricular surface, and an elongated process directed toward the cerebral wall (Rakic 1972; Kriegstein and Gotz 2003; Rakic and Zecevic 2003). In fact, the majority of our labeled SVZ cells had 1 or 2 primary processes (n = 20/25) making it difficult to assign cellular identity based on morphology alone. Cell input resistance, membrane capacitance, and sodium current (I_{Na}) did not vary significantly with age, thus suggesting that over the period examined (16-22 gw) SVZ cells are electrically unexcitable and almost identical in their electrophysiological properties (Fig. 7).

Human CP

Neuronal precursors exiting the cell cycle in the SVZ migrate through the IZ to their destined location in the CP, where they undergo terminal neuronal differentiation (Komuro and Rakic 1998). A significant increase in I_{Na} density in the CP versus the SVZ (Fig. 3D-F) are consistent with findings obtained in rodent (Luhmann et al. 1999; Bahrey and Moody 2003) and illustrate neuronal maturation evident by the ability to generate a sodium AP. In the human cortex (present study), single AP firing was present as early as 16 gw, but repetitive AP firing was not detected before 20 gw. Previous animal studies report that repetitive APs are absent until postnatal day 0 (McCormick and Prince 1987; Luhmann et al. 2000; Picken Bahrey and Moody 2003). Thus, the properties of voltage-gated membrane currents in human CP cells (16-22 gw) seem to correspond best to recordings performed in mouse slices between embryonic day 14 (E14) and postnatal day 0 (Bahrey and Moody 2003; Picken Bahrey and Moody 2003), with cells having a more positive resting membrane potential and requiring a higher threshold of firing.

Asynchronous Emergence of Intrinsic Excitability in the Human CP

The composition of migratory and postmigratory neurons throughout the CP is likely to produce a mixture of electrophysiological
properties. At any given experimental time point, we found a wide range of voltage-gated $I_{\text{Na}}$ peak amplitudes and voltage membrane responses in the CP (Figs 3 and 4). Interestingly, the neuronal ability to generate an AP did not arise instantaneously following migration, nor did it emerge synchronously among neighboring neurons. Rather, electrically competent neurons are sparse and appear to neighbor other neurons at different levels of maturation (patchy cortex, Fig. 7, CP). These results may be typically lost in rodent or other nonhuman mammals due to significantly shorter time periods of differentiation. Although previously suggested that early cortical neurons may mature on an individual basis and appear not to be directly influenced by maturation or lack of maturation around neurons may mature on an individual basis and appear not to be directly influenced by maturation or lack of maturation around them (Picken Bahrey and Moody 2003), we now provide the first direct evidence for spontaneous activity among CP cells, perhaps, due to a relatively high-voltage threshold (Fig. 4A) and low $I_{\text{Na}}$ density (Fig. 4E). In rodent, spontaneous activity does not appear until postnatal day 0 when cells are capable of firing repetitive APs (see above), in which the cells examined in the human CP (present study) were often unable to produce.

**Spontaneous Activity**

The complex patterns of ion channel development play a key role in the onset of spontaneous activity forming functional connections in the cerebral cortex (Moody and Bosma 2005). In human CP, the $I_{\text{Na}}$ activation and inactivation curves (Fig. 5B) show considerable overlap, also known as “window current” (Attwell et al. 1979). Within the voltage range of −50 to −30 mV, both activation and inactivation parameters have nonzero values allowing for a flow of steady current. Because the window current lies within the measured resting membrane potential (−50 mV), it is plausible for spontaneous activity to exist. However, we did not find any experimental evidence for spontaneous activity among CP cells, perhaps, due to a relatively high-voltage threshold (Fig. 4A) and low $I_{\text{Na}}$ density (Fig. 4E). In rodent, spontaneous activity does not appear until postnatal day 0 when cells are capable of firing repetitive APs (see above), in which the cells examined in the human CP (present study) were often unable to produce.

**K+ Current in the Human CP**

Rapidly inactivating (A-type) $I_{\text{K}}$ is a critical component of the ensemble of voltage-gated ionic currents that determine somatic and dendritic signal integration in pyramidal cells of the adult cerebral cortex (Johnston et al. 2000; Korngreen and Sakmann 2000). Because A-type current imposes strong control of neuronal excitability, one may expect that only neurons in an advanced differentiated state would have it. Our experiments do not support such a view. In the present study, the rapidly inactivating $I_{\text{K}}$ was found in cells that generated full-size APs, as well as in those with zero $I_{\text{Na}}$. Furthermore, rapidly inactivating $I_{\text{K}}$ was found in all cortical zones, including the proliferative zone (SVZ). Interestingly, animal models of cortical development also show the presence of A-type $I_{\text{K}}$ in embryonic neural progenitor cells prior to differentiation (derived from SVZ), as early as E15 (Smith et al. 2008).

Noninactivating $I_{\text{K}}$ (putative delayed rectifier $I_{\text{K,DR}}$) were the main $I_{\text{K}}$ recorded in human SVZ and CP. It is not clear why these CP neurons are endowed with $I_{\text{K,DR}}$ that activates at a more depolarized potential (+17 mV), whereas the $I_{\text{K}}$ current in nonexcitable SVZ cells activates at a less positive potential (−6 mV). One possibility is that high-voltage threshold of the $I_{\text{K,DR}}$ in CP favors AP generation at this developmental stage (second trimester of gestation), when $I_{\text{Na}}$ is still relatively weak. As a consequence of high-voltage threshold for $I_{\text{K,DR}}$, during the upstroke of the neuronal AP, the only significant current flowing is the $I_{\text{Na}}$.

**IZ and SP**

Based on neuroanatomical studies, the space between the CP and SVZ contains both the IZ and the SP layers (Bystron et al. 2008). The IZ represents a continuously changing population of cells that have exited the cell cycle in the SVZ and are migrating to take up residence in the CP. Whereas the SP is a transient population of neurons, forming one of the first functional cortical circuits in the brain (Kostovic and Rakic 1990; Hangar et al. 2002). Experiments performed in animal models of cortical development show that electrical activity of SP cells is essential for proper growth of afferents and stabilization of newly formed synaptic contacts (Allendoerfer and Shatz 1994). Abnormal development of SP neurons is implicated in neuropsychiatric disorders (Kanold 2004).

Most of the human IZ cells had 2 visible long processes with a more depolarized potential (+17 mV), whereas the $I_{\text{K}}$ current in nonexcitable SVZ cells activates at a less positive potential (−6 mV). One possibility is that high-voltage threshold of the $I_{\text{K,DR}}$ in CP favors AP generation at this developmental stage (second trimester of gestation), when $I_{\text{Na}}$ is still relatively weak. As a consequence of high-voltage threshold for $I_{\text{K,DR}}$, during the upstroke of the neuronal AP, the only significant current flowing is the $I_{\text{Na}}$. The complex patterns of ion channel development play a key role in the onset of spontaneous activity forming functional connections in the cerebral cortex (Moody and Bosma 2005). In human CP, the $I_{\text{Na}}$ activation and inactivation curves (Fig. 5B) show considerable overlap, also known as “window current” (Attwell et al. 1979). Within the voltage range of −50 to −30 mV, both activation and inactivation parameters have nonzero values allowing for a flow of steady current. Because the window current lies within the measured resting membrane potential (−50 mV), it is plausible for spontaneous activity to exist. However, we did not find any experimental evidence for spontaneous activity among CP cells, perhaps, due to a relatively high-voltage threshold (Fig. 4A) and low $I_{\text{Na}}$ density (Fig. 4E). In rodent, spontaneous activity does not appear until postnatal day 0 when cells are capable of firing repetitive APs (see above), in which the cells examined in the human CP (present study) were often unable to produce.

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recorded SVZ, and majority of the CP cells, these are most likely neurons migrating to the CP. However, a small percentage of these cells mainly located in the upper part of the IZ were actually able to fire a regenerative sodium spike upon direct current stimulation (Supplementary Fig. 2A), which is suggestive of a more mature neuronal progeny than in SVZ. Unfortunately, our measurements could not determine whether this population of young neurons reflects those migrating from SVZ (putative pyramidal cells) or interneurons migrating from the ganglionic eminence through IZ (Marin and Rubenstein 2001; Ang et al. 2003).

Closer to the CP, in the presumed SP, we found large cells with multiple primary branches. These cells were positively identified as neurons in the advanced stage of differentiation by a large $b_{\text{na}}$, greater than 500 pA, and by their ability to generate vigorous spike firing (Fig. 7, SP, repetitive AP). In addition, within the SP, we found radially oriented AIS (βIV spectrin-positive segments) populated with voltage-gated sodium channels (Fig. 6B). Taken together, the present data indicate that neurons found in the human fetal SP show the greatest level of both molecular and physiological maturation (Fig. 7, SP) and, therefore, are likely to form the first functional circuits (Bayatti et al. 2008).

The AIS
Here we show that βIV spectrin-positive AIS protrude from cell bodies in the human CP and occasionally in the IZ as early as 19 gw (Fig. 6A,B). A substantial fraction of fetal human AIS are populated with voltage-gated sodium channels (Fig. 6D). It should be noted that AIS described in the present study are significantly shorter than those in the mature human cortex (Inda et al. 2006), suggesting that, at this age, AIS are still in very early stages of structural and functional maturation. Nevertheless, the formation of AIS in the human CP coincides with the cell’s ability to generate a sodium AP. Besides the well-established role in synaptic integration, reflected in the precise timing and localization of the AP initiation process (Colbert and Johnston 1996; Kole et al. 2007), AIS may play a critical role in the very emergence of cortical electrical activity even prior to formation of synaptic connections (Bourgeois and Rakic 1993; Zecevic et al. 2005; Kostovic and Jovanov-Milosevic 2006).

Conclusions
The period after 22 gw is the most significant time for laminar and cytological differentiation of the CP (Chan 2002). Here we performed whole-cell recordings from human CP, SP, IZ, and SVZ between 16 and 22 gw. We found that cells located in human SVZ cannot produce a regenerative voltage response (Fig. 7, passive), despite the fact that 50% of SVZ cells express neuronal markers (Zecevic et al. 2005). As early as 16 gw, a small number of young, electrically excitable neurons emerge in the CP and are capable of producing an abortive or full-size AP upon direct current stimulation (Fig. 7, top insets). These early CP neurons form AIS copopulated with voltage-gated sodium channels (Fig. 7). The most electrically competent human neurons at this stage of fetal development are undoubtedly cells located just bellow the dense cellular layer of the CP, in the region referred to as SP. In the human SP, young neurons express the largest sodium currents and respond to direct current stimulation with repetitive firing of actions potentials (Fig. 7, repetitive AP).

Supplementary Material
Supplementary Figures 1–7 can be found at: http://www.cercor.oxfordjournals.org/.

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