Postnatal Development of Ionic Currents in Rat Hippocampal Astrocytes In Situ

ANGÉLIQUE BORDEY AND HARALD SONTHEIMER
Department of Neurobiology, University of Alabama at Birmingham, Birmingham, Alabama 35294

Bordey, Angélique and Harald Sontheimer. Postnatal development of ionic currents in rat hippocampal astrocytes in situ. J. Neurophysiol. 78: 461–477, 1997. Developmental changes in ion channel expression and cell morphology were studied in glial cells with the use of whole cell patch-clamp recordings in rat postnatal day (P)5–P50 hippocampal slices. Recordings were obtained from 234 cells, presumed to be glia, in stratum radiatum and stratum lacunosum-molecular of the CA1 region. Of 66 recorded cells filled with Lucifer yellow, 48 stained positive for glial fibrillary acidic protein, which identified them as astrocytes. All glial cells studied were of a stellate morphology, and developmental changes primarily comprised an increase in the length and number of cell processes associated with an overall increase in cell size and membrane capacitance. Two distinct outward potassium currents could be identified: a transient 4-aminopyridine-sensitive current (Iₖ) and a persistent outward current sensitive to tetraethylammonium (Iₒ₆). Iₖ activated at −40 mV, and steady-state activation and inactivation midpoints were −16 and −74 mV, respectively. Decay time constants ranged from 7 ms at −30 mV to 19 ms at +80 mV. Iₒ₆ activated at −30 mV. A third K⁺ current sensitive to cesium activated with hyperpolarizing command voltages and showed strong inward rectification. Transient, voltage-activated sodium currents (Iₛ₆) were tetrodotoxin sensitive (100 nM) and activated at about −40 mV, peaked at about −10 mV, and reversed at +63 mV. Iₒ₆ was half-inactivated at −49 mV and half-activated at −19 mV. During the first 2 wk of postnatal development, the percentage of cells showing inwardly rectifying K⁺ current (Iₖ), Iₛ₆, and Iₒ₆ increased significantly from 40% (at P5) to 90% (at P20–P50). By contrast, almost all cells independent of age expressed Iₖ. Specific conductances for Iₖ (gₖ) and Iₛ₆ increased significantly between P5 and P20, concomitant with a decrease in input resistance. By contrast, specific conductance of the outwardly rectifying K⁺ current (gₒ₆) decreased threefold between P5 and P20. Specific Na⁺ conductance was always <1/4 of the total potassium conductance. These results indicate that CA1 hippocampal astrocytes are characterized by expression of voltage-activated Na⁺ channels and three types of K⁺ channels showing changes in their relative expression during early postnatal development: 1) the number of cells expressing Iₛ₆, Iₒ₆, and Iₒ₆ increases significantly and 2) their specific conductance changes such that gₒ₆, predominant at P5–P20, is gradually replaced by gₖ, the predominant conductance in adult astrocytes. Adult morphological and electrophysiological phenotypes are established at about P20. These data suggest that previous studies in which cultured or acutely isolated cells from immature or embryonic rats were used were not adequately reflecting the properties of hippocampal astrocytes in situ.

In introduction

Mammalian brain development is not complete at birth. Numerous structural and functional changes and/or refinements occur in early postnatal life. These include, for example, changes in the subunit composition of Na⁺, K⁺, and Ca²⁺ channels (Beckh et al. 1989; Drew et al. 1992), γ-aminobutyric acid-A and N-methyl-D-aspartate receptors (Farrant et al. 1994; Laurie et al. 1992), protein kinase C isoforms (Jiang et al. 1994), the relative expression of Na⁺/K⁺-ATPase (Fukuda and Prince 1992), and the extent of electrical coupling (Pei et al. 1993). Postnatal development has been particularly well investigated in hippocampus, a structure with well-defined synaptic connections. Hippocampal pyramidal cells develop much of their dendritic arbor postnatally (Lauder and McCarthy 1986). Electrophysiological studies of hippocampal neurons have demonstrated significant developmental changes in ionic current expression during this period (Spigelman et al. 1992; Spitzer 1991), specifically the late onset of expression of slowly activating, outwardly rectifying currents and a gradual decline in transient K⁺ currents.

Voltage-activated currents are not restricted to neurons, but are also present in glial cells in vitro (Barres et al. 1990b; Nowak et al. 1987; Ritchie 1992; Sontheimer 1994) and in situ (Sontheimer and Waxman 1993; Steinhauser et al. 1992, 1994). Like neurons, glial cells undergo significant changes during postnatal development. These include morphological changes (Butt and Ransom 1993), myelination of axons (Bunge and Waxman 1985), increased gap-junctional coupling (Binning and Muller 1992), changes in cytokine and growth factor receptor expression (Merrill and Janakait 1995; Mizuno et al. 1994; Otero and Merrill 1994; Scherer and Schnitzer 1994; Schwartz and Nishiyama 1994), and significant changes in antigenic phenotypes (Duchala et al. 1995; Herman et al. 1993; Sancho-Tello et al. 1995; Schachner 1982). These changes may be associated with age-dependent changes in the expression and/or activity of voltage-activated ion channels in glial cells. Indeed, developmental changes in glial current expression have been observed to some extend in cultured or acutely isolated cells (Barres et al. 1990b; Sontheimer et al. 1989, 1991b, 1992b), and one recent study suggests developmental changes in sodium and potassium channels in cells presumed to be glial cells in mouse hippocampal slice (Kress et al. 1995). None of the aforementioned studies has investigated hippocampal postnatal development in situ spanning from early postnatal life to adulthood.

To fill this void, we conducted a comprehensive study of glial cell development in which we identify a significant percentage of cells as glial fibrillary acidic protein (GFAP)-positive astrocytes and show age-dependent changes in their ion channel complements. Unlike previous studies, ours included animals from postnatal day (P)5 to P50. All studied glial cells were located in the CA1 region of rat hippocampal...
slices and recorded with the whole cell patch-clamp method. Our results indicate that glial cell express 4-aminopyridine (4-AP)-, tetraethylammonium (TEA)-, and cesium (Cs⁺)-sensitive potassium currents and tetrodotoxin (TTX)-sensitive sodium currents at all stages of postnatal development. However, the relative contribution of each channel to whole cell currents and the relative channel complement change markedly with development. Adult physiological and morphological phenotypes are established by about P20. Some of these findings are in sharp contrast to previous studies of acutely isolated or cultured astrocytes.

**METHODS**

**Slice preparation**

Methods used for preparation of thin hippocampal slices used in this study were essentially as previously described (Sontheimer and Waxman 1993). Briefly, 5- to 50-day-old Sprague-Dawley rats were anesthetized with the use of pentobarbital sodium salt (50 mg/kg) and decapitated. The brain was quickly removed and placed in ice-cold (4°C) calcium-free artificial cerebrospinal fluid (ACSF-Ca free) containing (in mM) 116 NaCl, 4.5 KCl, 0.8 MgCl₂, 26.2 NaHCO₃, 11.1 glucose, and 5 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), continuously oxygenated with 95% O₂-5% CO₂. The brain was hemisected, and a block of tissue containing the hippocampus was glued (cyanoacrylate glue) to the stage of a Vibratome. Transverse hippocampal slices (150 μm thick) were cut in cold oxygenated ACSF-Ca free and transferred to a beaker filled with ACSF-Ca free at room temperature. After a recovery period of ≥1 h in ACSF-Ca free, slices were placed in a flow-through chamber continuously perfused with oxygenated ACSF (see composition below) containing 1.8 mM CaCl₂ at room temperature. The chamber was mounted on the stage of an upright microscope (Nikon Optiphot2) equipped with a ×40 (working distance 2 mm) water immersion objective and Nomarski optics.

**Whole cell recordings and data analysis**

Whole cell patch-clamp recordings were obtained as previously described for hippocampal slices (Edwards et al. 1989). Patch pipettes were pulled from thin-walled borosilicate glass (1.55 mm OD, 1.2 mm ID; WPI, TW150F-40) on a PP-83 puller (Narishige, Tokyo, Japan). Pipettes had resistances of 4–7 MΩ when filled with the following solution composition, in mM: 145 KCl, 0.2 CaCl₂, 1.0 MgCl₂, 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, and 10 HEPES (sodium salt), pH adjusted to 7.2 with tris (hydroxymethyl) aminomethane. To label cells for later morphological and antigenic identification, 0.1% Lucifer yellow (LY, dilithium salt) was added to the pipette solution. Voltage-clamp recordings were performed with the use of an Axopatch-200A amplifier (Axon Instruments). Current signals were low-pass filtered at 5 kHz and digitized on-line at 25–100 kHz with the use of a Digidata 1200 digitizing board (Axon Instruments) interfaced with an IBM-compatible computer system. Data acquisition, storage, and analysis were done with the use of pClamp version 6 (Axon Instruments). For all measurements, capacitance compensation and series resistance compensation (40–80%) were used to minimize voltage errors. Settings were determined by compensating the transients of a small (5-mV) 10-ms hyperpolarizing voltage step; the capacitance reading of the amplifier was used as value for the whole cell capacitance. After compensation, series resistances ranged from 7 to 12 MΩ. Alternatively, input membrane resistance (Rm) and membrane time constants were determined from the average response to 50 hyperpolarizing (10-mV) current pulses (20 ms). The resulting membrane current charging curves were fitted (fitting procedures described below) either to a single or double exponential.

Unless indicated otherwise, capacitive and leak conductances were substituted on-line by a modified P5 protocol. Peak currents were determined with the use of Clampfit (Axon Instruments), and statistical values (means ± SE, with n being the number of cells tested) were evaluated with a statistical graphing and curve-fitting program (Origin, MicroCal). Time constant for capacitive charging curves and the time constant of activation and inactivation of the whole cell currents were fitted to either a monoexponential or biexponential function of the form

\[
y(t) = yo + a[\exp\left(\frac{-t}{\tau}\right)] + b[\exp\left(\frac{-t}{\tau}\right)]
\]

**Steady-state parameters for activation and inactivation**

To establish steady-state activation or inactivation curves, the peak current, I, was measured at each potential and the corresponding conductance, G, was calculated with the use of the following equation

\[
G = I/(V - V_r)
\]

where V is the membrane command potential and V_r is the equilibrium (Nernst) potential for the ion under consideration (for potassium, −86 mV; for sodium, +68 mV). The measured peak amplitudes and the calculated peak conductance were then normalized with respect to the maximum values and plotted as a function of the membrane potential during the test pulse. The resulting inactivation and activation curves were fitted to the Boltzmann equation

\[
\frac{G}{G_{\text{max}}} = \frac{1}{1 + \exp\left(\frac{V_r - V - V_s}{k}\right)}
\]

where \( G_{\text{max}} \) is the maximum ionic conductance at peak current, \( V_r \) is the voltage at which \( G \) is half of \( G_{\text{max}} \), and \( k \) is the slope factor, determines the voltage-dependent relation of \( G \).

**Hodgkin and Huxley parameters**

Time constants for current activation and inactivation were obtained by fitting data to the empirically derived Hodgkin-Huxley function (Hodgkin et al. 1952) of type m̃ñ. The transient outward currents were fit by the following equation

\[
I(t) = I_o + I_a\left[1 - \exp\left(-\frac{t - t_0}{\tau_{a}}\right)\right] \cdot \exp\left(-\frac{t - t_0}{\tau_{r}}\right)
\]

where \( I_a \) and \( I_o \) are amplitude factors, \( t_0 \) is a time offset factor, \( \tau_{a} \) and \( \tau_{r} \) are the time constants for activation and inactivation, respectively, and \( p \) is the power factor for the activation (a) term. To reconstruct the current, \( p \) and \( \tau_{r} \) were varied to give the best fit to the experimental data. \( \tau_{r} \) was obtained from the experimental data by fitting the decay phase with a single-exponential function. The experimental data were best described by using \( p = 4 \).

**Cell identification**

Images of cells visually chosen for recordings were archived with the use of a charge-coupled device camera (Water Instruments) in combination with a video printer (Sony) for later (offline) comparison with LY fills. After recordings, slices were transferred to a fixation medium containing 4% paraformaldehyde in phosphate-buffered saline (PBS) and several slices were collected over 3- to 4-day periods. Slices were washed three times in PBS for 1 h and were incubated for 10 min with 1% Triton X-100 (Kodak), 1% normal goat serum (Vector) in PBS. Slices were then incubated for 24 h at 4°C with the primary polyclonal antibody to GFAP (IncStar; rabbit anti-mouse GFAP, dilution 1:100) in PBS in the presence of 1% normal goat serum and 0.2% Triton.
Slices were washed three times with PBS for 5 min and incubated with the secondary antibody (goat-anti-rabbit immunoglobulin G), conjugated to Rhodamine (dilution 1:100, Atlantic Antibodies) for 2 h at room temperature. Slices were mounted in Gel Mounting Medium (Fisher) on glass coverslips and were viewed on an epifluorescence microscope with the use of standard procedures. LY labeling and GFAP staining were visualized on a Leica microscope at ×20–40–100 magnification. Images were captured with an Optronics DEI-750 integrating camera and printed on a Kodak color printer. No bleedthrough was observed between the Rhodamine and fluorescein isothiocyanate fluorescence channels in control experiments. Some cells were imaged by the use of a Molecular Dynamics confocal microscope system. High-magnification (×100) serial optical sections were taken at consecutive focal levels of 1–2 μm. Addition of data from all section series enabled the three-dimensional morphology of the cell to be represented in a two-dimensional image.

Chemicals were purchased from Sigma, unless otherwise noted.

RESULTS

Whole cell recordings were obtained from 234 visually identified cells located in the stratum radiatum (sr) and stratum lacunosum-moleculare (slm) of the CA1 region of rat hippocampus. The approximate location for each cell was documented through a low-power fluorescence image after recordings. The location of each of the 234 cells is shown superimposed on a representative hippocampal slice in Fig. 1. Voltage-clamp data in this study were limited to cells that had an input resistance (determined at −80 mV) >50 MΩ (see METHODS). Cells had a mean resting membrane potential (Vr) of −59.4 ± 1.2 (SE) mV (ranging from −36 to −89 mV, n = 234), an Rm of 221.9 ± 187.1 MΩ (ranging from 50 to 1,880 MΩ, n = 229) and whole cell membrane capacitance (Cm) of 27.5 ± 1.2 pF (ranging from 7 to 100 pF, n = 234). The time constants for charging the capacitive transients resulting from either a mono- or a double-exponential fit were 0.513 ± 0.045 ms (n = 62) or 0.109 ± 0.005 ms and 0.726 ± 0.037 ms (n = 173), respectively. These values were determined in the first 3 min of whole cell recording.

Cell morphology and identification

Cells were routinely filled with LY during recordings. Of 234 cells included in this study, 66 cells were recovered for immunohistochemical identification after intracellular injection of LY. In 14 cells the cell body adhered so strongly to the pipette that it was removed by pulling the pipette out of the slice and only filled processes remained. Of the 66 cells, 48 stained positive for GFAP, an astrocyte-specific cytoskeletal protein (Eng 1985). Figure 1B illustrates representative examples of LY-filled cells that were GFAP positive at 21 days (Fig. 1Ba), at 31 days (Fig. 1Bb), and at 33 days (Fig. 1Bc). In very young rats (P5), cells (n = 5) had round cell bodies ~10–11 μm diam. These cells were characterized by short, unbranched processes that were confined to an area <90 μm diam. In older animals, cell bodies were either round, oval, or elongated and typically had long, branched processes. Between the ages of P11–P19, 8 of 13 cells were GFAP positive. Figure 2A shows representative examples of these two types of cells in the same slice. Of these, 10 cells had round or oval cell bodies with diameters of ~7.5–9 μm (Fig. 2A, left). Processes extended into all three planes, covering an area ~120 μm diam and giving cells a stellate appearance. Three of the cells were fusiform (~6 × 11 μm2 in size) and bipolar with two main processes of ~70 μm that remained in the same plane (Fig. 2A, right). Later in development (P21–P29), 24 of 30 recorded cells could be identified as GFAP positive. They maintained similar cell body shapes and sizes, but had much longer (up to 200 μm) and more complex cell processes. Again, processes typically extended in all three planes, defining a stellate morphology. Representative examples of two GFAP-positive cells are shown in Fig. 2B. In slices from animals >30 days old, 16 of 19 cells were GFAP positive. Cell body size was ~8.5 μm for round cells and 5.5–7 and 8.5–11 μm for fusiform cells. All of the cells had stellate appearances with processes extending in all axes and into an area 150 μm diam (Fig. 2C).

Biophysical and pharmacological properties of whole cell currents

Whole cell currents were activated in voltage-clamped cells by applying hyper- and depolarizing voltage steps. Currents were identified on the basis of a combination of kinetic and pharmacological properties. To limit inaccuracies in the kinetic analysis due to voltage-clamp errors, we only included recordings in which access resistance was <10 ΜΩ and input resistance was >50 MΩ. None of the recorded cells considered to be glial displayed any spontaneous action potentials or could be induced to fire action potentials after current injection. They also did not show any spontaneous or 4-AP-induced synaptic currents that are characteristic for sr and slm CA1 hippocampal neurons (Williams et al. 1994). Outward potassium currents. To activate outward currents, cells were held at −80 mV and depolarizing voltage steps were applied from a holding potential of ~70 mV (Fig. 3, A and D) or after a 200-ms conditioning prepulse to −110 nV (Fig. 3, B and E). In recordings without a prepulse, 185 of 234 cells displayed outward currents that showed delayed activation and slow inactivation (Fig. 3A). Both the initial amplitude and the current remaining at the end of a 30-ms pulse were much larger if activated from a prepulse potential to −110 mV (Fig. 3B). Bath application of 10 mM TEA selectively reduced the sustained component of the current (Fig. 3, D vs. A) without significantly affecting the transient component (Fig. 3, E vs. B). The selective block of TEA is more readily visible after a point-by-point subtraction of the sustained currents from the total currents, which results in the isolation of the transient currents (Fig. 3, F vs. C).

Overall, application of TEA reduced the sustained current by 46% (n = 13, Table 1), increased Rm from 451 to 598 MΩ (n = 6), and led to a positive shift in Vr, from −59 to −53 mV (n = 12). Point-by-point subtraction of the sustained current in the presence (Fig. 3D) and absence (Fig. 3A) of TEA isolated the TEA-sensitive current component (Fig. 4A). This current activated slowly and showed kinetics reminiscent of delayed rectifying potassium currents (I钾) in other cells. The current-voltage (I-V) relationship of the TEA-sensitive current suggested an activation threshold close to −30 mV (Fig. 4B).

Transient “A-type” outward currents. To study the phar-
FIG. 1.  A: low-power magnification photograph of hippocampal slice. Location of recorded cells is superimposed on representative hippocampal slice. B, left: recorded cells filled with Lucifer yellow (LY). B, right: anti-glial fibrillary acidic protein (GFAP) antibody labeling of recorded cells. Bc and Bd were obtained by averaging 3 photos taken at different planes, which led to a bigger cell body for the LY-filled picture.
macology and kinetics of the transient current without contamination by the sustained current, we subtracted recordings with and without prepulse as described above (Fig. 3C). Bath application of 2 mM 4AP and Cs$^+$ (to inhibit inwardly rectifier potassium currents, see below) on average led to a 51% reduction ($n = 16$, Table 1) of transient currents (Fig. 5A). The $I$-$V$ curve of the 4-AP-sensitive current (Fig. 5C) was more negative than the corresponding $I$-$V$ curve for $I_d$ (Fig. 4C), with an activation threshold near $-40$ mV. Kinetic properties of transient 4-AP-sensitive current ($I_a$) were studied in more detail in some cells in the presence of extracellular Cs$^+$ (1 mM) and TEA (10 mM) to reduce contamination by $I_d$. Each steady-state activation curve of $I_a$ (METHODS) was fit to the Boltzmann equation, and averages of the fitted values gave $V_{1/2} = \pm 16.3 \pm 5.3$ mV and $k = 16.4 \pm 2.9$ mV$^{-1}$ ($n = 9$; Fig. 6B, ○). The steady-state inactivation properties of $I_a$ were determined by measuring peak current amplitudes at $+10$ mV after prepulse potentials ranging from $-110$ to $-20$ mV (Fig. 6A). Fit of each inactivation curve of $I_a$ (Fig. 6B, ○) to the Boltzmann equation yielded averaged values of $-74.0 \pm 1.3$ mV for $V_{1/2}$ and $9.4 \pm 0.7$ mV$^{-1}$ for $k$ ($n = 11$). The time course of $I_a$ activation and inactivation was determined at each holding potential by fitting currents to an m$h$ function (see METHODS; Fig. 7) for six cells. Activation time constants were between $0.86$ and $1.98$ ms at $-30$ mV and between $0.50$ and $1.21$ ms at $+80$ mV. Inactivation time constants were between $7.6$ and $14.6$ ms for a depolarizing pulse to $-30$ mV and between $14.6$ and $19.5$ ms for a depolarizing pulse to $+80$ mV. Time constants for activation and inactivation differed in their dependence on the membrane potential: the inactivation time constant increased whereas the activation time constants decreased with more positive potentials (Fig. 7B). To examine recovery from inactivation, membrane potential was repeatedly stepped from $-110$ to $+10$ mV while increasing the time that the cell was held at $-110$ mV between repetitions (Fig. 7C). The resulting peak currents were normalized to the largest current amplitude observed and plotted as a func-

Fig. 2. Developmental morphology of CA1 astroglial cells. A: at postnatal day (P) 11, 2 types of GFAP-positive cells have been filled with LY during recordings. Cell on left has oval cell body and processes extend in 3 directions. Cell on right has a more fusiform cell body with 2 main processes that remain in same plane. B: at P25 and P22, long processes extended into 3 planes, defining a stellate morphology. C: at P30 and P40, filled cells have a stellate morphology with shorter processes than at P25. Image at P30 is a confocal image reconstructed from 11 section sections. Scale bar: 50 μm.
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FIG. 3. Transient and sustained currents. (A) Voltage command pulses from 70 to 80 mV from holding potential of 0 mV evoked sustained outward current. (B): conditioning prepulse to −110 mV reveals transient outward current in addition to sustained component of currents. (C): point-by-point subtraction of sustained currents (A) and transient currents (B) results in isolation of transient currents. (D): application of tetraethylammonium (TEA, 10 mM) blocks sustained currents displayed in A. (E): TEA does not significantly affect currents activated by depolarizing steps following conditioning prepulse to −110 mV. (F): point-by-point subtraction of sustained currents (D) and transient currents (F), showing that isolation of transient currents recorded in presence of TEA is not affected by TEA. Bottom: protocols for A-D and B-E.

Table 1. TEA, Cs⁺, and 4-AP effects on Vᵣ, Rₑ, Iᵣ, and Iₑ amplitudes

<table>
<thead>
<tr>
<th></th>
<th>TEA, 10 mM (13)</th>
<th>Cs⁺, 1 mM (7)</th>
<th>Cs⁺ + 4-AP, 2 mM (16)</th>
</tr>
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<tbody>
<tr>
<td>Vᵣ</td>
<td>91.2 ± 13.5 (12)</td>
<td>86.5 ± 8.7 (6)*</td>
<td>91.8 ± 7.0 (16)</td>
</tr>
<tr>
<td>Rₑ</td>
<td>128.7 ± 12.6 (6)</td>
<td>224.5 ± 102.2 (4)*</td>
<td>180.2 ± 170.0 (12)</td>
</tr>
<tr>
<td>Iᵣ</td>
<td>38.6 ± 21.3 (7)*</td>
<td>29.0 ± 21.2 (16)†</td>
<td>159.4 ± 216.8 (11)</td>
</tr>
<tr>
<td>Iₑ</td>
<td>54.3 ± 34.1 (13)*</td>
<td>84.0 ± 21.4 (5)</td>
<td>49.2 ± 35.1 (16)†</td>
</tr>
<tr>
<td>Iₑ</td>
<td>56.8 ± 43 (6)</td>
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Values given are means ± SD, with number of cells in parentheses and represent the relative changes as compared to control values. TEA, tetraethylammonium; 4-AP, 4-aminopyridine; Vᵣ, resting membrane potential; Rₑ, input membrane resistance; Iᵣ, inward-rectifying K⁺ current; Iₑ, persistent outward current sensitive to TEA; Iₑ, transient 4-AP–sensitive current. Significant changes at *P < 0.01 and †P < 0.001.
were completely abolished with 100 nM TTX (Fig. 10C) of the inactivation curves gave an exponential decay time constant of 5 ± 7 ms, and inactivated rapidly (Fig. 9B). Current amplitudes measured at 0 mV on average decreased by 58 ± 9.8% (n = 10) during the first 4–5 min of recording (data not shown).

SODIUM CURRENT. Transient, fast inward currents were observed in 206 of 234 recorded cells. Currents were activated with voltage steps more positive than −40 mV, peaked within <3 ms, and inactivated rapidly (Fig. 9A). Current amplitudes were voltage dependent, peaked between −10 and 0 mV, and decreased with more depolarized voltage steps. I-V curves (Fig. 9B) were generated from seven cells recorded in the presence of Cs⁺ (1 mM) and 4-AP (2 mM) to minimize contributions from K⁺ currents. We extrapolated current reversal potential for seven cells and obtained a value of +63.3 ± 3.7 mV (n = 7), which is in good agreement with the predicted Nernst potential for Na⁺ ions (+68 mV), suggesting that these fast, transient inward currents were Na⁺ currents. Steady-state activation and inactivation curves were constructed as described above for Iᵩ and are shown in Fig. 9D. Boltzmann fits of the activation curves gave values of 37 ± 1 mV for V½ and 5.2 ± 0.4 mV⁻¹ for k (n = 7). Sodium currents demonstrated typical steady-state inactivation behavior (Fig. 9, C and D). The Boltzmann fits of the inactivation curves gave an e-fold reduction per 8.1 ± 0.6 mV (n = 9) and a mean V½ of −49.2 ± 1.1 mV (n = 9). Inactivation kinetics of Na⁺ currents were well fit by a single exponential (see METHODS) with time constants ranging from 4.2 to 0.9 ms over the voltage range of −30 to +30 mV (Fig. 10B). Figure 10A shows two representative current traces elicited by a voltage step from a holding potential of −110 to −30 mV (top trace) and to +20 mV (bottom trace). The monoexponential decay time constant was determined and plotted versus the command potential (Fig. 10B). The decay time constant was voltage dependent and decreased with membrane depolarization from a maximum to a minimum value of 4.8 ms at −30 mV and 0.5 ms at +30 mV, respectively. In each cell tested, the inward currents were completely abolished with 100 nM TTX (Fig. 10C). TTX block was partially reversible and did not significantly alter the resting potential of the cell. In light of two previous studies (Sontheimer et al. 1991b, 1992b) showing a switch in Na⁺ current expressed in hippocampal astrocytes about P7, we examined TTX inhibition in six cells at P23–P24 and three cells at P5–P6. Currents were always totally eliminated by 100 nM TTX, suggesting that currents were TTX sensitive irrespective of postnatal age.

Developmental changes in expression of ionic currents

To determine whether there are developmental changes in channel expression, we calculated specific conductances for each of the currents by dividing ionic conductances for each cell by the Cᵦ. This eliminates cell-to-cell variabilities and allows a more accurate comparison. We also determined the percentage of cells expressing currents for each current and each age group. The resulting data were graphed for each of the currents separately in Fig. 11 as a function of animal age to illustrate developmental changes in both the relative percentage of cells (#) displaying the particular current and the specific conductances (●) for each current component. Data were pooled in age groups of 5–7, 11–13, 14–15, 16–19, 20–21, 22, 23–24, 25–27, 30–32, 33–37, and 40–50 days and means ± SE were plotted for each age group.

POTASSIUM CURRENTS. During the first 2 wk of postnatal development, the percentage of cells displaying Iᵩ (Fig. 11A) and Iᵩ (Fig. 11C) increased markedly from 50% and 40% at P5 to almost 100% at P14. For individual cells, the specific conductance for Iᵩ (gᵩ) did not change significantly and values were close to 400 pS/pF. The specific conductance for Iᵩ increased slightly but not significantly up to P19, but dropped to significantly lower values (P < 0.01) of ~200 pS/pF at P20–P21. For the specific conductance of the outwardly rectifying K⁺ current (gᵦ), there was a progressive and significant (P < 0.001) decrease from 600 pS/pF at P5 to a plateau of 200 pS/pF at older than P20 (Fig. 11B). The percentage of cells expressing delayed rectifier channels was relatively invariable and >90% of cells exhibited Iᵩ at all times studied.

To evaluate the relative contribution of outwardly rectifying versus inwardly rectifying K⁺ currents, we divided gᵩ by gᵩ (Fig. 11D). This ratio (gᵩ/gᵩ) indicates that the relative contribution of Iᵩ is initially significantly higher (at P5–P15) than that of Iᵩ, and subsequently decreases to its adult...
FIG. 5. 4-aminopyridine (4-AP) sensitivity and I-V curve of transient current. A: whole cell currents induced by a step depolarization starting at −70 mV, incremented by 10 mV from holding potential of −110 mV. Currents recorded in control (left), in presence of 4-AP, and after 20-min washout of 4-AP. 4-AP was bath applied at 2 mM for 10 min. Holding potential: −80 mV. B: illustrated currents represent 4-AP-sensitive component of outward current obtained by subtracting control currents from current in presence of 4-AP. C: I-V curve of control current displayed in A and 4-AP-sensitive current displayed in B. Current amplitude was measured at peak of current, as indicated by arrow.

FIG. 6. Steady-state activation and inactivation of transient outward current. A: whole cell transient outward currents under influence of 200-ms inactivating prepulse potential. Prepotentials started at −110 mV and were incremented in 10-mV steps while command potential was kept at +10 mV (30 ms). B: activation (○) and inactivation (●) curves. Data obtained from subtracted values as described in Fig. 3C are plotted as an activation curve for peak current according to Eq. 2. Dotted line: best fit of mean curve according to Eq. 2 (see METHODS) with \( V_{1/2} = -16.7 \text{ mV}, k = 19.0 \text{ mV}^{-1} \). Fraction of peak current measured from 7 cells is plotted vs. inactivation voltage. Dotted curve: best fit to a Boltzmann distribution with values of −74.4 mV for \( V_{1/2} \) and 9.8 mV \(^{-1} \) for \( k \). \( I_{\text{max}} \), peak current amplitude measured at −10 mV divided by the maximum peak current amplitude measured at +10 mV after a prepulse to −110 mV; \( g/g_{\text{max}} \), conductance of the peak current amplitude divided by the conductance of the maximum peak current amplitude obtained at +80 mV.
**FIG. 7.** Time course of transient outward current. A: transient currents obtained as explained in Fig. 3C are well described by a Hodgkin-Huxley-type function. Current recordings at +70 and −40 mV. Superimposed dotted curves are calculated with the use of the m⁴h model. B: activation (○) and inactivation (●) time constants estimated with the use of the m⁴h model are plotted against membrane potential (n = 6). Time constants differ in their dependency on membrane potential: inactivation time constant increases while activation time constant decreases. C: to determine recovery of inactivation for transient 4-AP-sensitive current (Ia), membrane potential was repeatedly stepped from −110 to +10 mV while the time the cell was held at −110 mV was varied between repetitions. Recorded currents are at bottom; protocol is displayed at top. D: resulting peak currents (C), normalized to largest current amplitude observed, were averaged for 6 cells and plotted as a function of conditioning pulse duration. Resulting data were fitted with a double exponential (see text), giving recovery time constants of 6.8 ms (a = 0.84) and 11.9 ms (b = 0.15).

value of 0.3 at older than P30 (Fig. 11D). Thus there appears to be a quantitative switch in relative potassium current contribution from predominantly I_d at birth to predominantly I_r in astrocytes from rats above P20.

**SODIUM CURRENT.** Similar developmental changes characterize the transient, voltage-activated sodium current (I_na). During the first 2 wk, the percentage of cells expressing sodium channels increased continuously from 60% at P5 to ~90% at P14 (Fig. 11E, ○). Between 80 and 90% of cells continued to display I_na in the adult, albeit on a cell-by-cell basis; the relative Na⁺ channel expression must have been reduced because values for specific Na⁺ conductance (g_na) dropped from 300 pS/pF at P5 to ~240 pS/pF at older than P40. At every stage of the postnatal development, the specific total potassium conductance (g_K), calculated by the addition of all potassium conductances and then divided by C_m, was at least fourfold higher than g_na (Fig. 11F), with mean values ranging from 4 to 8 without significant variations during development. In the adult, g_na is <1/4 of g_K. This explains why these cells were incapable of producing regenerative responses.

**Developmental changes of passive membrane properties**

We also studied changes in passive membrane properties, including V_r, C_m, and R_m (determined at −80 mV), as a function of development. Resting potential values were spread between −58 and −66 mV and did not vary much with age (Fig. 12A). Consistent with an increase in overall cell size, capacitance values (Fig. 12B) increased from a minimum of 14 pF at P13 to a maximum of 46 pF at P32 with adult values ~32 pF. Consistent with an increase in the overall contribution of I_r to whole cell currents, R_m decreased significantly from ~500 MΩ at P5 to ~120 MΩ at older than P20.

**DISCUSSION**

Our results suggest that CA1 astrocytes in the rat hippocampus undergo considerable changes in cell morphology,
ion channel complement, and passive membrane properties during the first month of postnatal development. Several of
the observed changes are in contrast to those seen previously in culture studies, stressing the importance of studying develop-
mental changes in cells within their native environment. We divided development into 11 age groups and included
representative samples of ≈13 cells at each of the time points for study, spanning early postnatal (P5) to adult (P30–P50)
life, and evaluated data from a total of 234 cells. To eliminate regional differences, we restricted this analysis to the CA1
region of hippocampus.

Identification of glial cells

Cells were routinely filled with LY for subsequent morphological and immunohistochemical identification. Of
234 cells filled with LY, we recovered 66 cells. All others escaped our subsequent immunohistochemistry because
they either did not retain LY, lost their cell body on withdrawal of the pipette, or were simply too weakly fluorescent to be found by microscopic inspection. The majority of the recovered cells were GFAP positive (48 of 66), which unequivocally identified them as astrocytes (Eng 1985). Other recorded cells that either lacked GFAP (18 cells) or were not recovered for immunohistochemistry displayed similar electrophysiological characteristics and were presumed to be astrocytes rather than local circuit neurons (interneurons). Several morphological and electrophysiological criteria are consistent with this assumption. 1) Glial cell bodies were mainly round, oval, or elongated with a relatively small somatic diameter (8–10 μm). Interneurons, by contrast, typically have fusiform or bipolar cell bodies (Buhl et al. 1994; Lang and Frotscher 1990; Schwartzkroin and Kunkel 1985) and are usually

![Figure 8](image-url)

**FIG. 8.** Cesium sensitivity and time-dependent inactivation of inwardly rectifying currents. **A:** currents recorded in control (left), in presence of CsCl, and after 20-min washout of CsCl. CsCl was bath applied at 1 mM for 3 min. Holding potential was −80 mV. This rectifying inward current was induced by depolarizing the cell to 0 mV for 100 ms before giving a hyperpolarizing voltage step from −180 mV to 0 mV for 40 ms. B: because of inactivation for potentials more negative than −130 mV, peak (filled symbols) and steady-state (open symbols) current amplitudes of trace represented in A are drawn separately. Squares and circles: control condition (A, left) and cesium block (A, middle) of current. At concentration of 1 mM, cesium block is independent of voltage.
much bigger than glial cells (somatic diameter of 20 µm) (Williams et al. 1994) (somatic diameter of 30 µm for CA1 “giant cell”) (Maccarelli and McBain 1996). 2) Additionally, interneurons have a much larger and complex dendritic tree than glial cells in our study. Processes of glial cells at all ages were restricted to an area of <200 µm in overall length, whereas most hippocampal interneurons display much longer dendrites (Lang and Frotscher 1990), even at P5. Morphological changes that were observed in glial cells during postnatal development are consistent with those demonstrated by Nixdorf-Bergweiler et al. (1994).

Considering electrophysiological criteria, for the distinction of glial cells from interneurons, several differences were apparent: 1) sr-slm CA1 interneurons display spikes after current injection, and many interneurons show continuous firing at rest (Buhl et al. 1994; Kawaguchi and Hama 1987; Maccarelli and McBain 1996), features never encountered in glial cells in our studies. 2) A common feature of neuronal development is an increase in sustained currents (Spigelman et al. 1992). By contrast, our recordings of glial cells suggest the opposite, namely, we observed a decrease in sustained currents and an increase in transient currents during postnatal development.

**K⁺ currents**

We observed expression of three types of potassium currents that can be classified on the basis of kinetics and pharmacological features as $I_d$, $I_a$, and $I_r$. A major emphasis of this study was to evaluate changes in channel expression during development, and pronounced changes in the relative expression of K⁺ channels were observed.

**DELAYED RECTIFYING ($I_d$) AND TRANSIENT ($I_a$) K⁺ CURRENTS.**

Outwardly rectifying, noninactivating currents ($I_d$) were similar to the delayed rectifier currents present in numerous excitable and nonexcitable cells (Rudy 1988) and...
typically activated around $-30 \text{ mV}$ and showed a steep voltage activation. They were sensitive to TEA but insensitive to 4-AP. By contrast, the kinetic features and 4-AP sensitivity of the fast transient current correspond well with those first described for $A$ currents ($I_a$) in snail neurons (Connor and Stevens 1971) and in cultured hippocampal neurons (Segal and Barker 1984) and many other cell types (Rudy 1988) including cultured and acutely dissociated hippocampal astrocytes (Barres et al. 1990a; Tse et al. 1992).

Astrocytes expressed $Cs^+$-sensitive inwardly rectifying potassium currents at all ages, although their relative expression and importance increased with age. The relative contribution of $I_r$ typically exceeded those of $I_d$ and $I_a$, and $I_r$ was the current type that contributed most to the establishment of the cell resting potential, as previously shown for cultured glial cells (Newman 1993; Ransom and Sontheimer 1995). Blockade of inwardly rectifying currents by $Cs^+$ ions led to a 10-mV depolarization.

**Developmental changes in $K^+$ channel expression**

Major changes in the relative expression of $K^+$ channels were observed. Most notably, we observed a marked decrease of $g_K$ and in the number of cells expressing $I_d$ concomitant with a significant increase of $I_r$ and $I_s$, and an increase in the number of cells expressing the corresponding channels. This suggests that although $I_d$ is the dominating conductance early in development, it is gradually replaced by $I_r$, by far the dominant current in adult astrocytes. It has been speculated that outwardly rectifying currents are important in growth control and cell proliferation in glial cells (Sontheimer 1994), whereas inwardly rectifying $K^+$ currents are more characteristic of differentiated cells (Roy and Sontheimer 1995; Sontheimer and Fernandez-Marques 1996). Consistent with the notion that $I_d$ may be important in astrocyte proliferation, our results show that at P5, a time at which a significant number of astrocytes may still be mitotically active (Korr 1986), $\sim50\%$ of cells express only $I_d$ or $I_s$, but lack $I_r$. $I_a$, by contrast, is being implicated in
FIG. 11. Developmental changes in expression of ionic currents. For each current and each age group, specific conductance [ionic conductance divided by membrane capacitance ($C_m$)] and percentage of cells expressing currents ($s$) are represented for (A) inwardly rectifying $K^+$ current ($I_{\text{ir}}$), (B) persistent outward current sensitive to TEA ($I_{\text{id}}$), (C) $I_{\text{a}}$, and (E) transient, voltage-activated sodium current ($I_{\text{Na}}$). Bars: means ± SE. Data were pooled by age group [5–7 days ($n = 18$), 11–13 days ($n = 24$), 14–15 days ($n = 13$), 16–19 days ($n = 19$), 20–22 days ($n = 20$), 23–24 days ($n = 27$), 25–27 days ($n = 29$), 30–32 days ($n = 28$), 33–37 days ($n = 22$), and 40–50 days ($n = 14$)] and plotted as a mean age per group. Statistical evaluation was performed by comparing with value at age 40–50 days and significant difference at $P < 0.01$ is shown by an asterisk or, for lower degrees of significance, $P$ value is given above graph point.

D: plotting ratio of specific conductance of outwardly rectifying $K^+$ current ($g_d$) divided by specific conductance for $I_{\text{ir}}$ reveals a quantitative switch from predominantly $I_{\text{id}}$ at birth to $I_{\text{ir}}$ for rats above P20. For each age group, number of cells having both current was: 10, 20, 8, 27, 21, 20, 25, 16, and 13.

F: specific total potassium conductance ($g_K$, calculated by addition of all potassium conductances and then divided by $C_m$) was $\approx 4$-fold higher than specific Na$^+$ conductance ($g_{\text{Na}}$) at every stage of postnatal development.

the glial buffering of extracellular $K^+$ concentration ($[K^+]_o$) (Barres et al. 1990a; Newman 1993; Orkand and Opava 1994; Philippi et al. 1996; Ransom and Sontheimer 1995). This function of glial cells also appears to develop over a 2-wk time period after birth (Jendelova and Sykova 1991; Sykova 1983). In the spinal cord, $[K^+]_o$ can fluctuate over a large range initially and becomes tightly controlled at older than P14 (Jendelova and Sykova 1991; Somjen 1979). Likewise, in optic nerve, $[K^+]_o$ changes are more significant at birth than in the adult (Jendelova and Sykova 1991). The developmental appearance of $I_{\text{ir}}$ in astrocytes at older than P14 is thus in perfect agreement with the observed changes in $[K^+]_o$ if one subscribes to the thesis that astrocytic $I_{\text{ir}}$ channels play a role in the process of $K^+$ buffering. During postnatal development, juvenile tissues have a greater ability to develop seizures than adult tissues. These seizures are accompanied by abnormally large elevations of $[K^+]_o$ (Hablitz and Heinemann 1987, 1994; Swann et al. 1986) that may result from changes in extracellular space or from a reduced ability of glial cells to buffer $[K^+]_o$. The latter could be explained by the relatively low levels of astrocytic $g_{\text{ir}}$ expressed at birth.

$\text{Na}^+$ currents were observed in 88% of glial cells in our study, a much larger percentage than previously reported for cultured or acutely isolated astrocytes (reviewed in Sontheimer 1994). Currents were TTX sensitive and consistently showed kinetic properties of so-called neuronal sodium currents (Barres et al. 1989), present in most neurons and in stellate, type 2 astrocytes in vitro. By contrast, protoplastic or type 1 astrocytes express a glial form of sodium channel (Barres et al. 1989; Gautron et al. 1992; Sontheimer and Waxman 1992; Sontheimer et al. 1991a,b, 1992a,b, 1996) characterized by much reduced TTX sensitivity (Barres et
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value of sodium channels in rat hippocampal astrocytes after 5 days in vitro (about −60 mV) (Sontheimer et al. 1991b) and identical to half-inactivation in most neurons (Barres et al. 1989). Also, we did not find any differences in TTX sensitivity between cells or as a function of development. Currents were always completely blocked by 100 nM TTX.

A recent study of glial cells in mouse hippocampal slices could not demonstrate Na$^+$ channels in cells from animals older than P20 and concluded that Na$^+$ channels were a selective feature of immature astrocytes (Kressin et al. 1995). In our hands, Na$^+$ channel expression was present in the majority of astrocytes in adult hippocampus, albeit at much lower levels than in neurons. The conductance ratio of K$^+$ to Na$^+$ conductance ($g_K/g_{Na}$) was always ≥4, insufficient to support the generation of action potential as previously described in cultured astrocytes (Sontheimer and Waxman 1992). Consequently it is clear that astrocytic Na$^+$ channels must serve different roles in the brain. Although no clear function has been demonstrated, several suggestions for the role of glial sodium channels have been put forward (Sontheimer et al. 1996). Glial cells may serve as a donor of Na$^+$ channels to be transferred to and inserted into axonal membranes (Shrager et al. 1985). Na$^+$ channels may be activated by adjacent neuronal activity through ephaptic coupling and allow glial cells to sense activity of adjacent neurons (Chao et al. 1994). Leakage of Na$^+$ ions through Na$^+$ channels at the resting potential may fuel the Na/K-ATPase (Sontheimer et al. 1994). Na$^+$ influx may play a role in pH regulation through the Na$^+$/H$^+$ cotransporter (Shrode and Putnam 1994) or Na$^+$/HCO$^-$ cotransport (Brookes and Turner 1994; Rose and Ransom 1996). Indeed, membrane depolarization through changes in [K$^+$]o, neuronal activity, or glutamate receptor activation may enhance Na$^+$ influx through voltage-dependent sodium channels and may translate into changes in transport activity of Na$^+$-dependent carriers.

Differences between in situ and primary culture: implications

During the time period over which we observed the most striking changes in glial physiology and morphology (e.g., P5–P20), CA1 neurons likewise exhibit significant changes with regards to cell morphology and arborization of processes (Lang and Frotscher 1990), electrophysiological differentiation (Spigelman et al. 1992), and synaptogenesis (Bayer 1980a,b). According to our observations, hippocampal astrocytes achieve their adult morphological and electrophysiological phenotype around P20. Coincident with this age, structural maturation of CA1-CA3 hippocampal neurons is completed (Lang and Frotscher 1990). Thus there appears to be parallel changes in these properties in CA1 neurons and glial cells. It has become evident that neuronal-glial interaction plays an important role in the development of glial cells and nerve cells (Barres and Raff 1993; Lauder and McCarthy 1986; Sontheimer 1995). Astrocytes are implicated in the guidance of neuronal migration (Gasser and Hatten 1990; Rakic 1990), influence neuronal growth and differentiation by the release of neurotrophic factors (Cendrelli et al. 1994; Müller et al. 1995; Rudge et al. 1994; Schwartz and Nishiyama 1994), and control the ionic neu-
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