Biophysical Properties and Ionic Signature of Neuronal Progenitors of the Postnatal Subventricular Zone In Situ

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Wang, D. D., D. D. Krueger, and A. Bordey. Biophysical properties and ionic signature of neuronal progenitors of the postnatal subventricular zone in situ. J Neurophysiol 90: 2291–2302, 2003. First published June 11, 2003; 10.1152/jn.01116.2002. Previous studies have reported the presence of neuronal progenitors in the subventricular zone (SVZ) and rostral migratory stream (RMS) of the postnatal mammalian brain. Although many studies have examined the survival and migration of progenitors after transplantation and the factors influencing their proliferation or differentiation, no information is available on the electrophysiological properties of these progenitors in a near-intact environment. Thus we performed whole cell and cell-attached patch-clamp recordings of progenitors in brain slices containing either the SVZ or the RMS from postnatal day 15 to day 25 mice. Both regions displayed strong immunoreactivity for nestin and neuron-specific class III β-tubulin, and recorded cells displayed a morphology typical of the neuronal progenitors known to migrate throughout the SVZ and RMS to the olfactory bulb. Recorded progenitors had depolarized zero-current resting potentials (mean more depolarized than −28 mV), very high input resistances (about 4 GΩ), and lacked action potentials. Using the reversal potential of K+ currents through a cell-attached patch a mean resting potential of −59 mV was estimated. Recorded progenitors displayed Ca2+-dependent K+ currents and TEA-sensitive-delayed rectifying K+ (K̂DR) currents, but lacked inward K+ currents and transient outward K+ currents. K̂DR currents displayed classical kinetics and were also sensitive to 4-aminopyridine and α-dendrotoxin, a blocker of Kv1 channels. Na+ currents were found in about 60% of the SVZ neuronal progenitors. No developmental changes were observed in the passive membrane properties and current profile of neuronal progenitors. Together these data suggest that SVZ neuronal progenitors display passive membrane properties and an ionic signature distinct from that of cultured SVZ neuronal progenitors and mature neurons.

INTRODUCTION

The postnatal subventricular zone (SVZ) is the largest germinatal center in the postnatal mammalian brain, composed of several distinct cell types including stem cells and progenitors committed to a neuronal or glial lineage (Doetsch et al. 1997, 1999; Garcia-Verdugo et al. 1998; Levison and Goldman 1997; Luskin 1998). Of particular interest in this region are the neuronal progenitors that migrate on a rostro-caudal axis to the olfactory bulb by the rostral migratory stream (RMS) (Doetsch et al. 1997; Luskin 1993, 1998). Understanding the biology of the SVZ neuronal progenitors might provide important insights into the development of the CNS and the factors regulating cell proliferation, migration, and differentiation, which constitute the process of neurogenesis. In addition these cells hold promise for future therapeutic intervention in neurodegenerative diseases using transplants and/or enhancement of endogenous neurogenesis. The different steps of neurogenesis can be influenced by a large variety of stimuli (Cameron et al. 1998; Gage et al. 1998; Heller et al. 1996; Morrison 2001; Nakashima and Taga 2002). Although many laboratories have started to reveal the extracellular factors regulating neurogenesis, intrinsic cellular properties such as ion channels, which are known to functionally regulate the different steps of neurogenesis in several cell types, have received very little attention.

K+ channels in particular are known to be involved in the regulation of two critical steps of neurogenesis: cell proliferation and migration (Dubois and Rouzaire-Dubois 1993; Puro et al. 1989; Schlüchter et al. 1996; Schwab 2001; Schwab et al. 1999). Moreover K+ channel properties are often influenced by factors known to regulate the distinct steps of neurogenesis, such as cytokines and growth factors (Bowlby et al. 1997; Gamper et al. 2002; Perillan et al. 2002; Timpe and Fantl 1994). Thus a detailed characterization of these channels in neuronal progenitors promises to contribute to an understanding of the mechanisms of action of the various factors stimulating neurogenesis in the SVZ and RMS. Moreover this knowledge could provide additional approaches for selectively regulating cell proliferation and/or migration in these regions. A recent report characterized the K+ channels in neuronal progenitors in primary cultures isolated from the SVZ (Stewart et al. 1999). However, cultured cells may take on a more differentiated phenotype and thus become unrepresentative of cells in their native environment. Therefore it is critical to study the intrinsic properties of progenitors in their near-intact physiological environment.

We thus conducted a comprehensive biophysical study of neuronal progenitors located in the SVZ and RMS. Neuronal progenitors exhibited high-input resistances and depolarized zero-current resting potentials similar to those of cultured SVZ neuronal progenitors (Stewart et al. 1999). However, using the single K+ channel reversal potential the resting potential was estimated at −59 mV. SVZ progenitors were found to express Ca2+-dependent K+ channels and delayed rectifying K+ (K̂DR) channels but lack transient A-type K+ channels that were reported in cultured SVZ progenitors (Stewart et al. 1999).

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METHODS

Preparation of acute slices

Slices containing the SVZ or RMS were acutely prepared as previously described for hippocampal and cortical slices (Bordey and Sontheimer 1997; Bordey et al. 2001). Briefly, 15- to 25-day-old CD-1 mice were anesthetized using pentobarbital (50 mg/kg) and decapitated. The brain was quickly removed and chilled (0–4°C) in 95% O₂–5% CO₂ saturated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 125; KCl 140; CaCl₂ 1.0; MgCl₂ 2.0; ethylene glycol-bis-(amino-ethyl) ether)–N,N,N’,N’-tetraacetic acid (EGTA) 10; Hepes 10; pH adjusted to 7.2 with Tris-base. When noted in the text, EGTA and Ca²⁺ were removed from the intracellular solution. The osmolarities of the intracellular and extracellular solutions were 295–300 and 305–310 mOsm, respectively. The osmolarity of all solutions was measured with a vapor pressure osmometer 5500 (Wescor) and was adjusted by addition of n-mannitol. For morphological identification, 0.1% Lucifer yellow (LY, dilithium salt) was added to the pipette solution. When a Ca²⁺-free extracellular solution was used, Ca²⁺ was replaced with Mg²⁺ and 1 mM of the Ca²⁺ chelator EGTA was added. In addition, the control solution contained only 1 mM Ca²⁺ instead of 2 mM to avoid any complication attributed to surface capacitances of thin-walled borosilicate glass (OD, 1.55 mm; ID, 1.2 mm; WPI, TW150F-40) on a PP-83 puller (Narishige, Japan). Pipettes had resistances of 6–8 MΩ when filled with the following solutions (in mM): KCl 140; NaCl 1.0; MgCl₂ 2.0; ethylene glycol-bis-(amino-ethyl) ether)–N,N,N’,N’-tetraacetic acid (EGTA) 10; Hepes 10; pH adjusted to 7.2 with Tris-base. When noted in the text, EGTA and Ca²⁺ were removed from the intracellular solution. The osmolarities of the intracellular and extracellular solutions were 295–300 and 305–310 mOsm, respectively. The osmolarity of all solutions was measured with a vapor pressure osmometer 5500 (Wescor) and was adjusted by addition of n-mannitol. For morphological identification, 0.1% Lucifer yellow (LY, dilithium salt) was added to the pipette solution. When a Ca²⁺-free extracellular solution was used, Ca²⁺ was replaced with Mg²⁺ and 1 mM of the Ca²⁺ chelator EGTA was added. In addition, the control solution contained only 1 mM Ca²⁺ instead of 2 mM to avoid any complication attributed to surface capacitance charge effect. Whole cell and cell-attached recordings were performed using an Axopatch-200B amplifier (Axon Instruments). Recordings were obtained ≤7 h after brain removal. Current signals were low-pass filtered at 2–5 kHz and digitized on-line at 5–20 kHz using a Digidata 1320 digitizing board (Axon Instruments) interfaced with an IBM-compatible computer system. Data acquisition, storage, and analysis were performed using PClamp version 8.0.2 (Axon Instruments). Whole cell parameters (capacitance Cₚ and series resistance) were determined by compensating the transients of a small (5 mV) 10-ms hyperpolarizing voltage step. Voltage-clamp data in this study were limited to cells with an input resistance Rᵢ > 200 MΩ (determined at –80 mV). The capacitance reading of the amplifier was used as the value for the whole cell capacitance. These values and the zero-current resting membrane potential Vᵢ were determined in the first 3 min of whole cell recording. Capacitive and leak conductances were not subtracted unless otherwise stated. Peak currents were determined using Clampfit (Axon Instruments), and statistical values were evaluated with a statistical graphing and curve-fitting program (Origin, MicroCal). Statistical comparison of means was performed with the Student’s t-test or ANOVA test (Statview, SAS Institute). Data are represented as means ± SD in the text and as means ± SE on the graphs. Ion channel blockers were diluted in ACSF and applied by a rapid bath application system. Blockers were diluted in ACSF in which Hepes replaced NaHCO₃ and the pH was adjusted to 7.4 by NaOH or Tris base. No correction of junction potentials was performed.

Whole cell recordings and drug application

Whole cell patch-clamp recordings were obtained as previously described (Edwards and Konnerth 1992). Patch pipettes were pulled from thin-walled borosilicate glass (OD, 1.55 mm; ID, 1.2 mm; WPI, TW150F-40) on a PP-83 puller (Narishige, Japan). Pipettes had resistances of 6–8 MΩ when filled with the following solutions (in mM): KCl 140; CaCl₂ 1.0; MgCl₂ 2.0; ethylene glycol-bis-(amino-ethyl) ether)–N,N,N’,N’-tetraacetic acid (EGTA) 10; Hepes 10; pH adjusted to 7.2 with Tris-base. When noted in the text, EGTA and Ca²⁺ were removed from the intracellular solution. The osmolarities of the intracellular and extracellular solutions were 295–300 and 305–310 mOsm, respectively. The osmolarity of all solutions was measured with a vapor pressure osmometer 5500 (Wescor) and was adjusted by addition of n-mannitol. For morphological identification, 0.1% Lucifer yellow (LY, dilithium salt) was added to the pipette solution. When a Ca²⁺-free extracellular solution was used, Ca²⁺ was replaced with Mg²⁺ and 1 mM of the Ca²⁺ chelator EGTA was added. In addition, the control solution contained only 1 mM Ca²⁺ instead of 2 mM to avoid any complication attributed to surface capacitance charge effect. Whole cell and cell-attached recordings were performed using an Axopatch-200B amplifier (Axon Instruments). Recordings were obtained ≤7 h after brain removal. Current signals were low-pass filtered at 2–5 kHz and digitized on-line at 5–20 kHz using a Digidata 1320 digitizing board (Axon Instruments) interfaced with an IBM-compatible computer system. Data acquisition, storage, and analysis were performed using PClamp version 8.0.2 (Axon Instruments). Whole cell parameters (capacitance Cₚ and series resistance) were determined by compensating the transients of a small (5 mV) 10-ms hyperpolarizing voltage step. Voltage-clamp data in this study were limited to cells with an input resistance Rᵢ > 200 MΩ (determined at –80 mV). The capacitance reading of the amplifier was used as the value for the whole cell capacitance. These values and the zero-current resting membrane potential Vᵢ were determined in the first 3 min of whole cell recording. Capacitive and leak conductances were not subtracted unless otherwise stated. Peak currents were determined using Clampfit (Axon Instruments), and statistical values were evaluated with a statistical graphing and curve-fitting program (Origin, MicroCal). Statistical comparison of means was performed with the Student’s t-test or ANOVA test (Statview, SAS Institute). Data are represented as means ± SD in the text and as means ± SE on the graphs. Ion channel blockers were diluted in ACSF and applied by a rapid bath application system. Blockers were diluted in ACSF in which Hepes replaced NaHCO₃ and the pH was adjusted to 7.4 by NaOH or Tris base. No correction of junction potentials was performed.

Using the reversal potential of K⁺ currents through cell-attached patches to monitor the cell resting potential

The method to measure Vᵢ from cell-attached K⁺ currents is identical to that described by Verheugen et al. (1995, 1999). For these recordings, 150 mM K⁺ was used in the pipette solution. With a 150 mM K⁺ pipette solution, which is close to the estimated intracellular [K⁺] in other cell types (150 mM; Hille 1992), the equilibrium potential for K⁺ (E_K) across the patch is approximately 0 mV, and K⁺ currents will reverse when the pipette potential (V_pip) cancels Vᵢ out. Therefore the cell-holding potential (−V_pip) at which the K⁺ current reverses direction gives a direct quantitative measure for Vᵢ (at K⁺ channel reversal, V_pip = −V_R = E_K about 0 mV). Depolarizing voltage ramps (Fig. 3) were applied to activate voltage-gated K⁺ channels. For analysis of currents evoked by ramp stimulation, a correction was made for a leak component by linear fit and extrapolation of the closed level.

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 using the following equation: G = I/(V − Vᵢ), where V is the membrane command potential and Vᵢ is the equilibrium (Nernst) potential for the ion under consideration (for potassium, V_K = –101 mV). The measured peak amplitudes and the calculated peak conductances were then normalized with respect to the maximum values and plotted as a function of the membrane potential during the test pulse. The resulting activation and inactivation curves were fitted to the Boltzmann equation: G/G_{max} = 1/(1 + exp[(V_{1/2} - V)/k]), where G_{max} is the maximum ionic conductance at peak current V_{1/2} is the voltage where G is one half of G_{max}, and k is the slope factor that determines the voltage-dependent relation of Gᵢ.

Cell identification

Images of cells visually chosen for recordings were archived using LG3 frame grabber (Scion Corporation, Frederick, MD) for later (off-line) comparison to LY fills. After recordings, slices were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight. Slices were washed three times in 2× Tris-buffered saline (TBS) over 1 h, permeabilized for 15 min with 1.25% Triton X-100 and 10% bovine serum albumin (BSA, Sigma) in 2× TBS, and then blocked for 1 h in blocking solution containing 0.2% Triton and 10% BSA in TBS. Slices were subsequently incubated with primary antibodies specific for nestin (Rat-401, supernatant, Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa) or neuron-specific class III β-tubulin (TuJ1, monoclonal anti-β-tubulin Cyt3 conjugate; Sigma, St. Louis, MO). For TuJ1 immunostaining, slices were incubated with Cy3-conjugated antibody against TuJ1 (1:100) for 2 h at room temperature in blocking solution. For nestin immunostaining, slices were incubated overnight at 4°C with primary antibody in blocking solution. Slices were subsequently washed three times with TBS for 5 min and incubated with secondary antibody (goat anti-mouse IgG) conjugated to Texas red (dilution 1:200; Vector) for 2 h at room temperature. For both stainings, slices were then washed in TBS and mounted on glass coverslips with fluorescent mounting medium ( Vectashield; Vector) and were finally viewed on an epifluorescence microscope (Olympus microscope BX51) using standard procedures. Images were captured with a DVC color camera and printed on an Epson color printer.

Chemicals were purchased from Sigma except the recombinant peptide toxins, agatoxin-2 and α-dendrotoxin, which were purchased...
from Alomone Labs (Jerusalem, Israel). Toxins were stored lyophilized at −20°C and made up in extracellular solution containing 0.1% BSA.

RESULTS

Whole cell recordings were obtained from 186 visually identified progenitors located either in the early (posterior) RMS or in the SVZ (Fig. 1).

The location and morphology of recorded cells identify them as neuronal progenitors

LOCATION. Coronal slices containing either the RMS or the SVZ were located between sections 9 and 16 (both approximately) based on the brain map described by Swanson (2002), as shown in a sagittal section of the brain with the section number on a rostro-caudal axis (Fig. 1A, left panel). Images of slices shown in Fig. 1 were taken before or during the recordings. At section 11 (approximately) on the brain map, the RMS took on an elongated shape, distinguishable from the surrounding tissue by its darker appearance, as indicated by the location of the two patch pipettes in Fig. 1B. In more rostral slices, the RMS had a round appearance (data not shown). In section >12, the lateral ventricle could be observed together with the SVZ [Fig. 1C, section 15 (approximately)]. At this level, the SVZ is located along the lateral wall of the ventricle and striatum and extends between the striatum and the corpus callosum (see diagram in Fig. 1A, right panel).

CELLS RECORDED IN THE SVZ AND RMS DISPLAY SIMILAR MORPHOLOGIES, TYPICAL OF IMMATURE CELLS. Cells in the RMS (Fig. 1D) and the SVZ (Fig. 1E) were densely packed, with a morphological appearance distinct from that of mature cells. They were characterized by a small round cell body with a main diameter of 6–8 μm, as well as occasional short processes that could be observed particularly when the patch pipette was penetrating inside the tissue. In the RMS they formed a channel of cells that often appeared to protrude from

![Diagram of brain sections](http://jn.physiology.org/)

FIG. 1. Location and morphology of SVZ cells. A: schematic view of a sagittal section of the adult mouse brain. The lateral ventricle is located under the corpus callosum (CC), drawn in black. The rostral migratory stream (RMS) is highlighted in gray. Most slices used for recording were chosen between sections 9 and 16 on rostro-caudal axis. B and C: 4× magnification photographs of a coronal section of the RMS (B) and the subventricular zone (SVZ, C) observed under infrared DIC optics. B: in section 11 (approximately), the RMS, located around two patch pipettes, is elongated and can be distinguished from the surrounding tissue by its darker appearance. C: in section 15 (approximately), the SVZ lines the lateral ventricle and extends between the CC and striatum. D and E: photographs of cells in the RMS (D) and the SVZ (E) taken at 60× magnification during recording.
the surrounding mature tissue (Fig. 1D). In contrast, the SVZ consisted of a 30- to 50-μm-wide layer of cells, separated from the ventricle by ependymal cells displaying characteristic cilia. This layer of cells narrowed in more caudal sections. To permit further visualization of the cell morphology, cells were routinely filled with Lucifer yellow (LY) during the recording, and a photograph of each recorded cell was taken and stored. Most of the recorded cells in the SVZ and RMS had a similar morphology characterized by a round to fusiform cell body with one or occasionally two processes (Fig. 4A, top panel). These recorded cells closely resemble neuronal progenitors described in vivo (Doetsch et al. 1999) and in vitro (Stewart et al. 1999; Wichterle et al. 1997). A few recorded cells had a larger cell body with one long process and were found only in the SVZ; other recorded cells were found both in the SVZ or RMS but had a more elaborated morphology with 2 or 3 long processes. Data from these morphologically distinct cells were not included in the present study.

IMMUNOSTAININGS FOR NESTIN AND TUJ1. To verify that the described regions contained immature cells, immunostaining was performed for nestin, a class VI intermediate filament protein that distinguishes progenitors from more differentiated cells (Hockfield and McKay 1985; Lendahl et al. 1990). In our slices, nestin staining was selectively observed in the SVZ and RMS (Fig. 2A, left panel, sections 12–13 (approximately)). At high magnification, nestin staining in the dorsolateral SVZ had a honeycomb appearance, probably as a result of the round cell somata and the lack of staining in the nucleus (Fig. 2A, right panel). Virtually every cell in the SVZ was nestin-positive, identifying them as progenitors. In addition, to verify that the described regions contained neuronal progenitors, immunostaining was performed for TuJ1, a marker of neuronal progenitors. Slices containing the RMS and the rostral (or anterior) SVZ displayed strong immunostaining for TuJ1 (Fig. 2B), identifying a large proportion of the cells in these regions as neuronal progenitors, as previously reported (Brock et al. 1998; Doetsch et al. 1997; Luskin 1998). As expected, the corpus callosum, the striatum, and the olfactory limb of the anterior commissure stained positive for TuJ1.

Thus the location, morphology, and immunostaining data strongly suggest that the cells recorded in the SVZ and RMS correspond to the neuronal progenitors known to migrate to the olfactory bulb in postnatal animals (Gritti et al. 2002; Lois et al. 1996; Luskin 1993, 1998). In addition, because glial progenitors have not been reported in the RMS (Doetsch et al. 1997), recorded cells are unlikely to be glial progenitors. In the following sections, we set out to determine and compare the electrophysiological properties of RMS and SVZ neuronal progenitors.

Neuronal progenitors in the SVZ and RMS display similar passive membrane properties and current profile

PASSIVE MEMBRANE PROPERTIES. The zero-current $V_R$, $C_M$, and $R_M$ were determined for 49 and 112 neuronal progenitors recorded in the RMS and the SVZ, respectively (Table 1). Both SVZ and RMS progenitors exhibited similar and very high

FIG. 2. Nestin and TuJ1 stainings in the SVZ. A: nestin and TuJ1 immunostainings of cells in mouse corticostriatal slices containing the SVZ (sections 11–12 (approximately)). Scheme representing location of immunostaining is shown in the inset between both panels. B: higher magnification of nestin- and TuJ1-positive cells in the SVZ.
FIG. 3. Estimation of the cell resting potential using the reversal potential of K⁺ channels in patches. A: plot of the input resistances as function of zero-current Vᵦ (n = 161). Linear fit of the data gave a coefficient of correlation of 0.3. Open circles: zero-current Vᵦ of cells in the SVZ and RMS, respectively. B: schematic representation of cell-attached recording conditions. C: a cell-attached record in response to depolarizing ramp command (shown above the trace) from -140 to +200 mV. Holding potential (-Vₚᵦ) was -100 mV with respect to the cell membrane potential. Voltage-gated K⁺ currents activated by the depolarizing voltage ramp were initially inward, and then reversed to become outward. Eₚ was determined from the intersection of the fit (straight line) to the linear leak and the K⁺ current. Cell resting membrane potential was measured from the reversal of cell-attached K⁺ currents. D: a cell-attached record in response to the same ramp command as in C. Inward and outward presumably K⁺ channels were activated and the reversal potential of channels gave an estimate of the cell resting potential.

TABLE 1. Electrophysiological properties of progenitors in the SVZ and RMS

<table>
<thead>
<tr>
<th>Cell Type Parameters</th>
<th>SVZ Progenitors</th>
<th>RMS Progenitors</th>
<th>P values (SVZ-RMS)</th>
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</thead>
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<tr>
<td>Zero-current Vᵦ (mV)</td>
<td>-23.2 ± 9.0 (n = 112)</td>
<td>-28.1 ± 12.9 (n = 49)</td>
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<td>Rₘ (MΩ)</td>
<td>4308 ± 1841 (n = 112)</td>
<td>4022 ± 2072 (n = 49)</td>
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<td>Cᵦ (pF)</td>
<td>7.3 ± 2.1 (n = 49)</td>
<td>7.8 ± 1.8 (n = 49)</td>
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<tr>
<td>Gₑ/Cᵦ (pS/pF)</td>
<td>267.3 ± 146.9 (n = 111)</td>
<td>337.8 ± 239.7 (n = 48)</td>
<td>0.019</td>
</tr>
<tr>
<td>Ratio (peak/steady-state current amplitude)</td>
<td>1.24 ± 0.30 (n = 111)</td>
<td>1.3 ± 0.4 (n = 48)</td>
<td>0.27</td>
</tr>
<tr>
<td>Gₑ/Cₑ/Cᵦ (pS/pF)</td>
<td>99.2 ± 53.8 (n = 41)</td>
<td>106.2 ± 83.3 (n = 31)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of cells. SVZ, subventricular zone; RMS, rostral migratory stream.
these respective parameters as a function of the postnatal day (linear coefficient of correlation $r = -0.06$ and 0.02 for $C_{A1}$, $-0.07$ and 0.07 for zero-current $V_R$, and 0.07 and 0.03 for $R_M$ in the RMS and SVZ, respectively). In a few cells, we estimated the membrane time constant from a monoexponential fit to the voltage response after a $-100$-pA current injection at $-70$ mV. The membrane time constant of neuronal progenitors was $46.1 \pm 8.6$ ms ($n = 7$), which is between that of dentate granule neurons and CA3 pyramidal neurons [40 and 66 ms at their resting potentials (Spruston and Johnston 1992)].

It is important to point out that the zero-current $V_R$ of neuronal progenitors likely does not represent the true resting potential of these cells because neuronal progenitors have a very high input resistance (about 4 GΩ) and the seal resistances were between 3 and 10 GΩ. In these circumstances, the seal conductance value is close to that of the membrane conductance (estimated as the input conductance minus the seal conductance), which results in an artificial depolarization of recorded cells (Pongracz et al. 1991). In fact, there was a weak correlation of the input resistance plotted as a function of the zero-current $V_R$ ($r = 0.4$, $n = 121$, Fig. 3). To estimate the $V_R$ of neuronal progenitors with another method more appropriate in this circumstance, we performed cell-attached recordings and used the reversal potential of $K^+$ channels as a monitor of $V_R$, as previously described for lymphocytes (Verheugen et al. 1995) and hippocampal interneurons (Verheugen et al. 1999) (Fig. 3B, schematic of the recording conditions). To activate $K^+$ channels, voltage ramps from $-140$ to $+200$ mV ($V_{ \text{pip}}$, Fig. 3, C and D) were repeated at $0.5$ Hz without any significant inactivation of $K^+$ channels. Sufficiently large outward currents were activated in about $20\%$ of the patches. Only patches displaying inward $K^+$ currents in addition to outward $K^+$ currents were analyzed (Fig. 3C). In addition, in 2 patches single-channel currents likely the result of $Ca^{2+}$-dependent $K^+$ channel activity were observed (Fig. 3D). At the $K^+$ channel reversal, the cell membrane potential, which represents the cell resting potential at rest was $-59.1 \pm 2.4$ mV ($n = 7$).

**CURRENT PROFILE.** Whole cell currents were activated in voltage-clamped progenitors by applying hyperpolarizing and depolarizing voltage steps from $-100$ mV to $+100$ mV from a holding potential of $-80$ mV. In both RMS and SVZ cells, nonleak subtracted traces were similar and characterized by holding potential of $80$ mV, close to $E_k$ in our recording conditions (Fig. 4, C and D), and the sensitivity of these outward currents to TEA identified them as voltage-dependent $K^+$ currents (see next section and Figs. 5 and 6). The $K^+$ currents recorded in the RMS and SVZ were similar in their extent of voltage-dependent inactivation. To quantify this observation, the ratio of the peak current amplitude to the current at the end of a $300$-ms pulse to $+20$ mV was calculated. This ratio was similar in both groups of neuronal progenitors (ratio of $1.3$ in RMS cells and $1.2$ in SVZ cells, Table 1). A substantial cumulative inactivation of the $K^+$ currents for voltage steps applied every $4$ s was also observed in both RMS and SVZ neuronal progenitors (data not shown).

In summary, neuronal progenitors in the SVZ and RMS display similar morphology, passive membrane properties, and current profile, confirming that they belong to the same progenitor type (i.e., neuronal progenitors).

**Biophysical and pharmacological properties of $K^+$ currents in neuronal progenitors.**

To activate outward currents, cells were held at $-80$ mV and depolarizing voltage steps were applied after a prepulse to $-50$ mV (Figs. 5 and 6) or to $-110$ mV (Fig. 7) to allow the activation of transient A-type $K^+$ currents in addition to delayed-rectifying $K^+$ ($K_{DR}$) currents. In our recordings, neuronal progenitors displayed outward currents that showed delayed activation and slow inactivation (Figs. 5 and 6). Virtually no transient A-type $K^+$ currents were observed in SVZ and RMS neuronal progenitors.

**PRESENCE OF TEA AND CHARYBDOTOXIN-SENSITIVE $K^+$ CURRENTS.** Stewart et al. (1999) showed that a step to $+0$ mV resulted in opening of $Ca^{2+}$-dependent $K^+$ channels ($K_{Ca}$) as a result of $Ca^{2+}$ entry through voltage-dependent $Ca^{2+}$ channels. Thus we tested the effects of a $Ca^{2+}$-free extracellular solution (see METHODS), $100 \mu M$ TEA, and $100 \mu M$ charybdotoxin (ChTx) on the outward $K^+$ currents. TEA ($100 \mu M$ has been shown to block $K_{Ca}$ channels without affecting $K_{DR}$ channels (Iwatsuki and Petersen 1985). ChTx is known to block $K_{Ca}$ channels. Using a $Ca^{2+}$-free extracellular solution or bath applying TEA or ChTx did not significantly reduce the outward current amplitude measured at $+20$ mV, suggesting that in our recording conditions $K_{Ca}$ currents did not significantly contribute to the current amplitude measured at $+20$ mV. However, when cells were recorded with an intracellular solution containing no EGTA/$Ca^{2+}$, a $Ca^{2+}$-free extracellular solution and $100 \mu M$ TEA reversibly blocked outward $K^+$ currents by $11.6 \pm 2.7\%$ ($n = 3$, Fig. 5) and $14.7 \pm 2.3\%$ ($n = 3$, data not shown), respectively. ChTx reversibly reduced outward currents when cells were recorded without internal EGTA. The $Ca^{2+}$-sensitive $K^+$ currents were voltage dependent and activated at about $-10$ mV (Fig. 5A). These data suggest the presence of $Ca^{2+}$ and voltage-dependent $K^+$ channels in neuronal progenitors, as previously shown by Stewart et al. (1999). All the following experiments were performed with an intracellular solution containing EGTA to limit $K_{Ca}$ channel activation.

**TEA-SENSITIVE DELAYED RECTIFYING $K^+$ CURRENTS CONTRIBUTE TO OUTWARD $K^+$ CURRENTS.** In the presence of EGTA in the intracellular solution to limit $K_{Ca}$ channel activation, a bath application of $40 \text{mM}$ TEA completely blocked voltage-dependent outward currents in all neuronal progenitors tested ($n = 7$, Fig. 6A). TEA-sensitive currents and their respective $I-V$ curves are displayed in Fig. 6B. TEA-sensitive currents activate at $-40$ mV and display voltage-dependent inactivation as observed on their mean $I-V$ curves (Fig. 6C). The mean steady-state activation curves (see METHODS) of TEA-sensitive currents gave a mean half activation voltage $V_{1/2}$ of $-4.5$ mV and a mean slope $k$ of $8.6$ mV$^{-1}$ (Table 2, Fig. 6D). In neuronal
progenitors, the mean TEA-sensitivity curve could be fitted with a sigmoidal curve that gave an IC50 value of 3.2 mM (Fig. 6, E and F). This IC50 is close to that reported for KD currents in cultured neuronal progenitors (4.1 mM) (Stewart et al. 1999).

OUTWARD K+ CURRENTS ARE SENSITIVE TO 4-AMINOPYRIDINE (4-AP) AND A KV1 CHANNEL BLOCKER, -DENDROTOXIN. 4-AP (2 mM) completely blocked KD currents in neuronal progenitors (Fig. 7A), and 4-AP–sensitive currents (Fig. 7B) displayed kinetic characteristics similar to those of TEA-sensitive currents. They activated at −40 mM, displayed voltage-dependent inactivation (Fig. 7C), and their steady-state activation curve parameters were similar to those obtained for TEA-sensitive currents (Table 2). Voltage-gated K+ channels are assembled from subunits of 4 major subfamilies, designated as Kv1, Kv2, Kv3, and Kv4 (Coetzee et al. 1999; Pongs 1992). Because of the similarity between the properties (kinetics, and TEA and 4-AP sensitivity) of KD currents found in neuronal progenitors and those of Kv1 currents (Coetzee et al. 1999), we tested two selective blockers of Kv1 channels, α-Dendrotoxin (80 and 200 nM), which potently blocks Kv1.1, Kv1.2, and Kv1.6 (Coetzee et al. 1999), reduced outward K+ currents in 6/8 neuronal progenitors tested. α-Dendrotoxin (80 mM) reduced outward currents by 62 ± 21% (data not shown). Agitoxin-2 (10 nM), an extremely potent Kv1.3 blocker and less potent Kv1.1 blocker (Garcia et al. 1994, 1997), reduced outward K+ currents in only 2/8 cells tested (70 ± 4%, n = 2, data not shown).

SVZ (60%) and RMS (80%) neuronal progenitors express Na+ channels

Transient inward currents were activated with voltage steps more positive than −40 mV, peaked within <3 ms, and inactivated rapidly (Fig. 8A). Current amplitudes were voltage-dependent, peaked between −10 and 0 mV, and decreased with more depolarized voltage steps (Fig. 8B). The mean I–V curve was generated from 3 cells in normal solution without TEA and from 4 cells in the presence of 40 mM TEA to minimize contributions from K+ currents. Data obtained with and without TEA were combined to obtain a mean I–V curve. Extrapolation of the linear fit of the mean I–V curve between −10 and +40 mV gave a current reversal potential of +60 mV (n = 7). The mean steady-state activation curve of Na+ currents gave a mean V1/2 of −20 mV and a mean slope of −6 mV/m (Fig. 8D, filled circles; Table 2). The steady-state inactivation properties of Na+ currents were determined by measuring peak current amplitudes at
after prepulse potentials ranging from \(-10^4\) to 0 mV (Fig. 8C). Fitting each inactivation curve of Na\(^+\) currents (Fig. 8D, open circles) to a Boltzmann equation yielded average values of \(-61\) mV for \(V_{1/2}\) and 7.4 mV\(^{-1}\) for the slope \((n = 4)\). In 6 cells in which it was tested, 100 nM TTX blocked the inward currents, confirming that these currents are Na\(^+\) currents \((n = 3\) cells in the SVZ and 3 cells in the RMS, data not shown). TTX block was partially reversible.

Na\(^+\) currents were observed in 59% of SVZ (54/92) and 79% of RMS (35/44) neuronal progenitors, which was significantly different \((P < 0.02, \text{chi-square and Fisher exact test})\). The mean density of Na\(^+\) conductance was about 100 pS/pF in both SVZ and RMS neuronal progenitors (Table 2). Although overall 65% (89/136) of both SVZ and RMS neuronal progenitors express Na\(^+\) channels, Na\(^+\) conductances were too low to allow these progenitors to generate a fast action potential from \(-70\) mV in response to current injections (Fig. 8F).

\[ G_{Na}(V) = \frac{G_{Na}^{max}}{1 + e^{(V - V_{1/2})/kT}} \]

**FIG. 6.** Neuronal progenitors possess delayed rectifying K\(^+\) channels. A: representative traces of outward K\(^+\) currents activated by depolarizing the cells from \(-70\) to \(+80\) mV after a prepulse to \(-50\) mV before (a) and after bath application of 40 mM TEA (b). TEA completely blocked the outward K\(^+\) currents in neuronal progenitors, identifying them as delayed rectifying K\(^+\) currents (K\(_{DR}\)).

**FIG. 7.** Delayed rectifying K\(^+\) currents are completely blocked by 4-aminopyridine (4-AP) in neuronal progenitors. A: K\(_{360}\) currents in neuronal progenitors before (a) and after the application of 2 mM 4-AP (b). B: point-by-point subtractions of traces in b from traces in a to isolate 4-AP-sensitive K\(^+\) currents. C: mean I–V curve of 4-AP-sensitive steady-state currents in 7 neuronal progenitors.
TABLE 2. Properties of delayed rectifying K+ and Na+ currents in neuronal progenitors

<table>
<thead>
<tr>
<th>Cell Type Parameters</th>
<th>SVZ Neuronal Progenitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEA-sensitive K+ currents</td>
<td>-4.5 ± 6.8 (n = 5)</td>
</tr>
<tr>
<td>V_{1/2}, mV</td>
<td>8.6 ± 1.5</td>
</tr>
<tr>
<td>k, mV^{-1}</td>
<td>8.1 ± 4.2 (n = 6)</td>
</tr>
<tr>
<td>4-AP-sensitive K+ currents</td>
<td>7.6 ± 1.3</td>
</tr>
<tr>
<td>Activation Na+ currents</td>
<td>-20.1 ± 4.1 (n = 6)</td>
</tr>
<tr>
<td>V_{1/2}, mV</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>k, mV^{-1}</td>
<td>-60.6 ± 2.8 (n = 4)</td>
</tr>
<tr>
<td>Inactivation</td>
<td>7.4 ± 1.4</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = number of currents.

properties and the K+ current amplitude, there was no change in the amplitude of the Na+ currents over the age period studied (r = 0.05 for the density of Na+ current amplitude in cells from both regions plotted as a function of the postnatal age). Similarly, there was no significant difference between the numbers of cells expressing Na+ channels between postnatal days 15 and 19 (61% SVZ cells, n = 41/67; 82% RMS cells, n = 14/17) and postnatal days 21 and 25 (56% SVZ cells, n = 13/25; 78% RMS cells, n = 21/27).

DISCUSSION

Our study provides the first electrophysiological characterization of neuronal progenitors of the SVZ and RMS in their near-intact environment; more specifically, our data show the following: 1) Neuronal progenitors have high-input resistances and depolarized zero-current resting potentials (−25 mV). Importantly, using the reversal potential of K+ currents in cell-attached patches provided an alternative method to estimate the resting potential, which gave a resting potential of −59 mV. 2) Neuronal progenitors express Ca2+ and voltage-dependent K+ channels and delayed rectifying K+ channels, but they lack inward K+ currents and transient A-type outward K+ currents, which have been reported in cultured SVZ neuronal progenitors. 3) Approximately 60% of SVZ and 80% of RMS neuronal progenitors express Na+ channels at a lower density than K+ channels.

Identification of neuronal progenitors in the SVZ

Cells were recorded in both the anterior SVZ and the RMS. These two regions are essentially composed of cells positive

FIG. 8. Voltage dependency of activation and inactivation of inward sodium currents. A: transient inward currents are shown in response to 8-ms step depolarizations from −70 to +60 mV after 200-ms prepulse to −110 mV. Capacitive and leak conductances were subtracted on-line by a modified P/5 protocol for this protocol and that in C:K: mean I–V curves of the peak currents in 7 neuronal progenitors. Extrapolated reversal potential E_{REV} was obtained by fitting the curve between −10 and +60 mV with a linear equation, which gave a value of +60 mV. C: Na+ currents under the influence of 200-ms inactivating prepulse potential. Prepulse potentials started at −104 mV and were incremented in 8-mV steps, whereas the command potential was kept at −10 mV. D: steady-state activation (filled circles) and inactivation (open circles) curves for Na+ currents. Boltzmann fits (dotted line) of the inactivation and activation curves gave V_{1/2} of −60.5 and −20.4 mV, and slopes of −7.4 mV^{-1} (n = 4) and 6.4 mV^{-1} (n = 7), respectively. E: plot of the density of Na+ conductance (G_{Na}/C_m) as a function of the density of K+ conductance (G_{K}/C_m). A linear fit of the data gave a coefficient of correlation of 0.3. F: voltage traces in responses to current injection pulses in a SVZ neuronal progenitor. The amount of current injected is shown (in pA) on the right of the displayed current-clamp trace. Inset: Na+ currents in the cell displaying slow action potentials.
for nestin, a marker of progenitors cells (Hockfield and McKay 1985; Lendahl et al. 1990). Indeed LY-filled cells recorded in these regions displayed a morphology typical of immature cells and were thus considered to be progenitors. The SVZ is known to contain distinct progenitor types, including stem cells, bipotential progenitors, and committed progenitors, that can be distinguished morphologically (Doetsch et al. 1997, 1999; García-Verdugo et al. 1998; Luskin 1998). The presumed SVZ stem cells have a fairly complex astrocyte-like morphology, whereas bipotential progenitors have no processes and are relatively rare (Doetsch et al. 1997). Cells recorded in our study resembled neither of these two types, but were characterized by a relatively small soma (6–8 µm in diameter) and one or occasionally two processes, identifying them as committed progenitors, which could be either neuronal or glial progenitors. Staining for class III β-tubulin, a marker of neurons and neuronal progenitors, showed that a large proportion of the SVZ cells were neuronal progenitors. In addition, the RMS has been shown to be composed of about 80% neuronal but not glial progenitors and about 20% astrocyte-like stem cells (Doetsch et al. 1997; Luskin 1993), identifying the cells recorded in the RMS as neuronal progenitors. The similar morphology of the cells recorded in the RMS and SVZ suggest that the recorded SVZ cells are the neuronal progenitors known to originate and migrate through the SVZ and reach the olfactory bulb by the RMS (Doetsch et al. 1997; Luskin 1993, 1998).

RMS and SVZ neuronal progenitors display similar passive membrane properties and current profile

PASSIVE PROPERTIES. Neuronal progenitors have depolarized zero-current resting membrane potentials (mean > −28 mV), a very high input resistance (mean >4 GΩ) and a low mean cell capacitance consistent with their small size. The mean zero-current V_R is close to that reported in cultured SVZ neuronal progenitors (−32 mV; Stewart et al. 1999). However, because the V_R measured as the zero-current potential is affected by the seal conductance in high-resistance cells, we estimated the resting potential with another method. Using the reversal of K^+ channels in patches V_g was estimated at −59 mV. It is worth mentioning that this method gives us an estimate and not the true resting potential because we do not know the intracellular K^+ levels. However, as mentioned by Verheugen et al. (1999), with a intracellular [K^+] of 155 mM a difference of 15 mV would result in an error of RT/F × ln (155/140) < 3 mV. In addition, some neuronal progenitors had a relatively hyperpolarized resting potential (< −50 mV, Fig. 3A). Postnatal neuronal progenitors thus have a resting potential similar to that of embryonic neuronal progenitors (−65/−60 mV; Owens et al. 1996).

When recorded cells have a high-input resistance, it is usually thought that they lack electrical coupling. However, postnatal SVZ cells have been shown to express connexin 43 (Miragall et al. 1997). In addition we occasionally observed spontaneous 20-mV– amplitude stepwise changes in the membrane potential similar to those reported in island of Calleja granule cells (Halliwell and Horne 1998). Halliwell and Horne suggested that these stepwise changes observed in granule cells that have a 3-GΩ input resistance are attributed to electrical coupling. Thus to conclude on the lack or presence of electrical coupling would require further investigation.

EXPRESSION OF Ca^2+-DEPENDENT K+ CHANNELS AND DELAYED RECTIFYING K+ CHANNELS. Voltage-dependent outward K^+ currents in neuronal progenitors displayed slow activation and inactivation. These outward K^+ currents were composed of charybdotoxin-sensitive Ca^2+-dependent K^+ currents and TEA-sensitive delayed rectifying K^+ currents. K_Ca and K_DR currents activated at about −20 and −40 mV, respectively. K_DR currents were totally blocked by 40 mM TEA and were significantly reduced by 2 mM 4-AP. The IC_{50} for TEA blockage of K_DR was 3.2 mV, which is similar to that reported for cultured SVZ neuronal progenitors (4.2 mM; Stewart et al. 1999). Voltage-gated outward K^+ currents are assembled from distinct subunits of 4 major subfamilies (Kv1, Kv2, Kv3, and Kv4), which can form heterotetramers, and each different subunit composition can confer unique kinetic and pharmacological properties on the resulting channel (Coetzee et al. 1999). A reduction of K_DR currents by β-dendrotoxin, a blocker of Kv1.1, Kv1.2, and Kv1.6 (Coetzee et al. 1999), suggests the presence of Kv1 subunits in these cells.

Recorded progenitors lacked fast activating and inactivating A-type outward K^+ currents and inward K^+ currents. The lack of A-type K^+ currents contrasts with a previous study reporting large transient A-type currents in cultured SVZ neuronal progenitors (Stewart et al. 1999). Potential explanations for this difference might involve a change in the subunit composition of K^+ channels or a different regulation of the K^+ channel properties in the two systems, culture and slice preparation. It is also possible that after 24 h in culture, SVZ neuronal progenitors acquire a more differentiated phenotype. Indeed, several studies reported the development of transient A-type outward K^+ currents during neuronal differentiation (Barish 1986; Wakazono et al. 1997).

Neuronal progenitors express Na^+ channels. Interestingly, the percentage of neuronal progenitors expressing Na^+ channels is significantly higher in the RMS (about 80%) than that in the SVZ (about 60%), which is consistent with the hypothesis that Na^+ channels are expressed as an early event of differentiation as cells begin to migrate in the RMS. Consistent with their immature phenotype and the smaller amplitude of Na^+ currents than that of K^+ currents (G_Na/G_K > 1), neuronal progenitors were not able to generate an action potential in response to current injections. This was also reported for cultured SVZ neuronal progenitors (Liu et al. 1999; Stewart et al. 1999). Although Na^+ channels contribute to the generation of action potentials in mature neurons, their function in neuronal progenitors is unclear. They have been shown to be important in fueling the Na^+/K^+ pump in astrocytes, which may be one of their functions in neuronal progenitors as well (Sontheimer et al. 1994).

It is interesting to point out that neuronal progenitors display differences in the pharmacological profiles of K_DR channels and in the amplitude of K^+ currents. In addition only a subset of cells expresses Na^+ channels. These differences may reflect the fact that recorded cells are at different stages of differentiation or proliferation. Differences in the pharmacological profile of K^+ currents could also be attributable to the presence of distinct neuronal progenitor populations. This is in fact expected because SVZ neuronal progenitors give rise to two...
types of olfactory bulb interneurons, granule cells and periglomerular cells (Luskin 1998). Our data do not allow us to distinguish between the distinct possibilities explaining differences in neuronal progenitor properties and further studies would be required to address this important issue.

Functional implications of the properties of neuronal progenitors

PASSIVE PROPERTIES. Changes in the cell resting potential have been implicated in the control of protein synthesis, proliferation/migration, and voltage-dependent channel and transport system activity. Regulating the resting membrane potential might provide a direct control of the behavior of the neuronal progenitors, which are known to migrate, proliferate, and progressively differentiate along the RMS (Baker et al. 2001; Coskun and Luskin 2002). Importantly, because of the very high input resistance of the neuronal progenitors, induction of a small current flow can induce a significant change in the cell membrane potentials and could thus strongly influence the behavior of the cells. Such a current flow might result from the opening and closing of K⁺ channels or the activation of GABA_A receptors, which are present in neuronal progenitors in vitro (Stewart et al. 2002). Thus the passive membrane properties of neuronal progenitors support the idea that progenitor behavior is likely to be tightly controlled by small local changes in extracellular signals, such as the neurotransmitter GABA.

ION CHANNEL EXPRESSION. Although ion channels are commonly involved in the generation of action potentials in mature neurons, their function in neuronal progenitors remains unclear. However, TEA-sensitive K⁺ channels are known to be functionally involved in the regulation of cell proliferation of immature cells and many cell types other than neurons (Attali et al. 1997; Gallo et al. 1996; Kotecha and Schlichter 1999; Pappas and Ritchie 1998; Puro et al. 1989). It is thought that blockade of K⁺ channels influences intracellular Ca²⁺ homeostasis, which in turn regulates cell proliferation (Platoshyn et al. 2000; Wohlrab et al. 2002). Thus the potential regulation of K_DG currents by growth factors or cytokines might influence the proliferation of neuronal progenitors.

DISCLOSURES

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REFERENCES


