Biophysical properties and ionic signature of neuronal progenitors of the postnatal subventricular zone in situ

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ABSTRACT

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 15-25 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 (~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 delayed rectifying K⁺ (KDR) currents, but lacked inward K⁺ currents and transient outward K⁺ currents. KDR currents displayed classical kinetics, and were also sensitive to 4-aminopyridine and α-dendrotoxin, a blocker of Kv1 channels. Na⁺ currents were found in ~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 germinal center in the postnatal mammalian brain, composed of several distinct cell types including stem cells and progenitors committed to a neuronal or glial lineage (Levison and Goldman 1997; Doetsch et al. 1997, 1999; Garcia-Verdugo et al. 1998; Luskin 1998). Of particular interest in this region are the neuronal progenitors that migrate on a rostro-caudal axis to the olfactory bulb via the rostral migratory stream (RMS, Luskin 1993, 1998; Doetsch et al. 1997). Understanding the biology of the SVZ neuronal progenitors might provide important insights into the development of the central nervous system 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 (Heller et al. 1996; Gage et al. 1998; Cameron et al. 1998; 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 (Puro et al. 1989; Dubois and Rouzaire-Dubois 1993; Schlichter et al. 1996; Schwab et al. 1999; Schwab 2001). In addition, K+ channel properties are often influenced by factors known to regulate the distinct steps of neurogenesis, such as cytokines and growth factors. 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 Ca$^{2+}$-dependent $K^+$ channels and delayed rectifying $K^+$ ($K_{DR}$) channels but lack transient A-type $K^+$ channels that were reported in cultured SVZ progenitors (Stewart et al. 1999).
MATERIAL AND 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 2.5; CaCl₂ 2; MgCl₂ 2; NaHCO₃ 25; glucose 10. The tissue of interest was glued to the stage of a Vibratome and 250 μm thick slices were cut in cold oxygenated ACSF. After a recovery period of 1 hour in ACSF, slices were placed in a flow-through chamber, held in position by a nylon mesh glued to a U-shaped platinum wire and continuously superfused with oxygenated ACSF at room temperature. The chamber was mounted on the stage of an upright microscope (Olympus BX50WI) equipped with a 60x water immersion objective and infra-red optics.

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 (o.d., 1.55mm; i.d., 1.2mm; 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 (-aminoethyl 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 mOsm and 305-310 mOsm, respectively. The osmolality of all solutions was measured with a vapor pressure osmometer 5500 (Wescor) and was adjusted by addition of D-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 complication due to surface charge effect. Whole-cell and cell-attached recordings were performed using an Axopatch-200B amplifier (Axon Instruments). Recordings were obtained up to 7 hours 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 $C_M$ 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 $R_M$ greater than 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 $V_R$ 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 t-test or ANOVA test (Statview, SAS Institute Inc.). Data are represented as mean ± SD in the text and as mean ± SEM 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 NaHCO$_3$ 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 $V_R$ 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 (155 mM, Hille 1992), the equilibrium potential for K$^+$ ($E_K$) across the patch is ~0 mV, and K$^+$ currents will reverse when the pipette potential ($V_{pip}$) cancels $V_R$ out. Therefore, the cell holding potential ($-V_{pip}$) at
which the K⁺ current reverses direction gives a direct quantitative measure for Vm (at K⁺ channel reversal, Vpatch= Vₚ -Vpip= Eₖ≈-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, Vₖ=-101 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 activation and inactivation curves were fitted to the Boltzmann equation: Gᵢ/Gᵢ(max)= 1/(1+exp[(V 1/2-V)k]), where Gᵢ(max) is the maximum ionic conductance at peak current;V 1/2 is the voltage where Gᵢ is one half of Gᵢ(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, Maryland) 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 2X Tris-buffered saline (TBS) over 1 hour, permeabilized for 15 min with 1.25% Triton X-100 and 10% bovine serum albumin (BSA, Sigma) in 2X TBS, and then blocked for 1 hour 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 Cy3 conjugate, Sigma). For TuJ1 immunostaining, slices were
incubated with Cy3-conjugated antibody against TuJ1 (1:100) for 2h 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-rabbit IgG) conjugated to Texas red (dilution 1:200, Vector) for 2 hours 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 (Saint Louis, MO) except the recombinant peptide toxins, agitoxin-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 based on the brain map described by Swanson (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 prior to or during the recordings. At section ~11 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). 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 the surrounding mature tissue (Fig 1D). In contrast, the SVZ consisted of a 30-50 μm-wide layer of cells, separated from the ventricle by ependymal cells displaying characteristic cilia. This layer of cells narrowed in more caudal sections. In order 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 (Wichterle et al. 1997; Stewart et al. 1999). A few recorded cells had a larger cell body with one long process and were only found 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 which 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, section ~12-13). At high magnification, nestin staining in the dorsolateral SVZ had a honeycomb appearance, probably due to 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 theses regions as neuronal progenitors, as previously reported (Doetsch et al. 1997; Brock et al. 1998; Luskin 1998). As expected, the corpus callosum, the striatum and the olfactory limb of the anterior commissure stain 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 (Luskin 1993, 1998; Lois et al. 1996; Gritti et al. 2002). 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 input resistances (mean of $\sim 4000 \, \text{M}\Omega$) and depolarized zero-current $V_R$ (Table 1). The mean zero-current $V_R$ values were more depolarized than $-28 \, \text{mV}$. Both SVZ and RMS progenitors also displayed similar and small $C_M$ (mean $<8 \, \text{pF}$, Table 1), indicating their small cell size.

Because developmental changes in the architecture of the SVZ occur during the third postnatal week in rats (Peretto et al. 1999), we questioned whether the passive membrane properties of the neuronal progenitors may also show developmental changes between postnatal day 15 to 25. To address this question, we plotted $C_M$, zero-current $V_R$ and $R_M$ as a function of the animal age (data not shown). There was no correlation between these parameters and the age of the animals as measured by plotting these respective parameters as a function of the postnatal day (linear coefficient of correlation $r=-0.06$ and 0.02 for $C_M$, -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 following a $-100 \, \text{pA}$-current injection at $-70 \, \text{mV}$. The membrane time constant of neuronal progenitors was $46.1 \pm 8.6 \, \text{ms}$ ($n=7$), which is between that of dentate granule neurons and CA3 pyramidal neurons ($40$ and $66 \, \text{ms}$ 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 ($\sim 4 \, \text{G}\Omega$) and the seal resistances were between 3 and 10 $\text{G}\Omega$. 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 artifactual 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 (-$V_{pip}$, Fig. 3C-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 due to 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, non-leak subtracted traces were similar and characterized by voltage-dependent outward currents, resulting in a strictly outwardly rectifying current-voltage (I-V) curve (Fig. 4A and B for a SVZ progenitor). The conductance density (conductance divided by $C_M$) of outward currents was similar in SVZ and RMS progenitors (mean of 270 and 340 pS/pF, respectively, Table 1). In addition, the density of outward current amplitude did not change with the age of the animal (r=-0.07 and -0.01 for RMS and SVZ cells, respectively). A tail current analysis of the outward currents gave a reversal potential of about -80 mV, close to $E_K$ in our recording conditions (Fig. 4C and D), and the sensitivity of these outward currents to TEA identified them as voltage-dependent K$^+$ currents (see next section and Fig. 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 following a pre-pulse to -50 mV (Fig. 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
(Fig. 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 Materials
and Methods), 100 µM TEA, and 100 nM charybdotoxin (ChTx) on the outward $K^+$ currents. 100 µM
TEA 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 µM TEA reversibly blocked outward $K^+$ currents by 11.6 ± 2.7 %
(n=3, Fig. 5) and 14.7 ± 2.3 % (n=3, data not shown), respectively. ChTx irreversibly 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\(_{\text{Ca}}\) 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\(_{\text{Ca}}\) channel activation, bath application of 40 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 IC\(_{50}\) of 3.2 mM (Fig. 6E and F). This IC\(_{50}\) is close to that reported for K\(_{\text{DR}}\) 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, \(\alpha\)-dendrotoxin.**

2 mM 4-AP completely blocked K\(_{\text{DR}}\) currents in neuronal progenitors (Fig. 7A). 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 four major subfamilies, designed as Kv1, Kv2, Kv3, and Kv4 (Pongs 1992;Coetzee et al. 1999). Due to the similarity between the properties (kinetics, and TEA and 4-AP sensitivity) of K\(_{\text{DR}}\) currents found in neuronal progenitors and those of Kv1 currents (Coetzee et al. 1999), we tested two selective blockers of Kv1 channels. \(\alpha\)-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. 80 nM α-dendrotoxin reduced outward currents by 62 ± 21 % (data not shown). 10 nM Agitoxin-2, which is an extremely potent Kv1.3 blocker and less potent Kv.1.1 blocker (Garcia et al. 1994, 1997), reduced outward K⁺ currents in only 2/8 cells tested (70 ± 4 %, n=2, data not shown).

**60% of SVZ and 80% of RMS neuronal progenitors express Na⁺ channels**

Transient inward currents were activated with voltage steps more positive than -40 mV, peaked within less than 3 ms and inactivated rapidly (Fig. 8A). Current amplitudes were voltage-dependent, peaked between -10 mV 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⁻¹ (Fig. 8D, filled circles, Table 2). The steady-state inactivation properties of Na⁺ currents were determined by measuring peak current amplitudes at -10 mV after pre-pulse potentials ranging from -104 mV to 0 mV (Fig. 8C). Fit of each inactivation curve of Na⁺ currents (Fig. 8D, open circles) to a Boltzmann equation yielded average values of -61 mV for V₁/₂ 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, Chi Square and Fisher Exact test). The mean density of Na⁺ conductance was ~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). Fig. 8E displays a plot of $G_K/C_M$ as a function of $G_{NA}/C_M$ that shows the range of both $K^+$ and $Na^+$ conductances. In addition, this plot illustrates that $G_K > G_{NA}$. As for the passive membrane 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 day 15-19 (61% SVZ cells, $n=41/67$; 82% RMS cells, $n=14/17$) and postnatal day 21-25 (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 that: 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 $Ca^{2+}$- 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, and 3) ~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 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; Garcia-Verdugo et al. 1998; Luskin 1998). The presumed SVZ stem cells have a fairly complex astrocyte-like morphology, while 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 ~80% neuronal but not glial progenitors and ~20% astrocyte-like stem cells (Luskin 1993; Doetsch et al. 1997), 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 via the RMS (Luskin 1993, 1998; Doetsch et al. 1997).

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_R 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 $[\text{K}^+]$ of 155 mM a difference of 15 mM would result in an error of $\text{RT/F*ln(155/140)} < 3 \text{ mV}$. In addition, some neuronal progenitors had a relatively hyperpolarized resting potential ($<-50 \text{ mV}$, Fig. 3A). Postnatal neuronal progenitors have thus 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 due 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. $\text{K}_\text{Ca}$ and $\text{K}_\text{DR}$ currents activated at about $-20 \text{ mV}$ and $-40 \text{ mV}$, respectively. $\text{K}_\text{DR}$ currents were totally blocked by 40 mM TEA and were significantly reduced by 2 mM 4-AP. The IC$_{50}$ for TEA blockade of $\text{K}_\text{DR}$ 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$^+$ channels are assembled from distinct subunits of four major subfamilies (Kv1, Kv2, Kv3, and Kv4), which can form heterotetramers, and each different subunit composition can confer unique kinetic and pharmacological properties upon the resulting channel (Coetzee et al. 1999). A reduction of $\text{K}_\text{DR}$ currents by $\alpha$-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 hours 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 (~80%) than that in the SVZ (~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 (GK/GNa>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 (Stewart et al. 1999; Liu et al. 1999). While 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 KDR 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 due 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 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, due to 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:* While 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 (Puro et al. 1989; Gallo et al. 1996; Attali et al. 1997; Pappas and Ritchie 1998; Kotecha and Schlichter 1999). It is thought that blockade of K$^+$ channels influences intracellular Ca$^{2+}$ homeostasis, which in turn regulates cell proliferation (Platoshyn et al. 2000; Wohlrab et al. 2002). Thus, the potential regulation of K$_{DR}$ currents via growth factors or cytokines might influence the proliferation of neuronal progenitors.
ACKNOWLEDGMENTS

This work was supported by R21NS044161-01 and the ALS association.
**Table 1:** Electrophysiological properties of progenitors in the SVZ and RMS (Mean ± SD, number of cells).

<table>
<thead>
<tr>
<th>Cell types Parameters</th>
<th>SVZ progenitors (112)</th>
<th>RMS Progenitors (49)</th>
<th>p values (SVZ – RMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero-current $V_R$ (mV)</td>
<td>-23.2 ± 9.0 (112)</td>
<td>-28.1 ± 12.9 (49)</td>
<td>0.007</td>
</tr>
<tr>
<td>$R_M$ (MΩ)</td>
<td>4308 ± 1841 (112)</td>
<td>4022 ± 2072 (49)</td>
<td>0.39</td>
</tr>
<tr>
<td>$C_M$ (pF)</td>
<td>7.3 ± 2.1 (112)</td>
<td>7.8 ± 1.8 (49)</td>
<td>0.12</td>
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<tr>
<td>$G_{K/M}$, pS/pF</td>
<td>267.3 ± 146.9 (111)</td>
<td>337.8 ± 239.7 (48)</td>
<td>0.019</td>
</tr>
<tr>
<td>Ratio (peak/steady-state current amplitude)</td>
<td>1.24 ± 0.30 (111)</td>
<td>1.3 ± 0.4 (48)</td>
<td>0.27</td>
</tr>
<tr>
<td>$G_{Na/M}$, pS/pF</td>
<td>99.2 ± 53.8 (41)</td>
<td>106.2 ± 83.3 (31)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Properties of delayed rectifying K⁺ and Na⁺ currents in neuronal progenitors.

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


**Figure 1. Location and morphology of SVZ cells.** A. Schematic view of a sagittal section of an adult mouse brain. The lateral ventricle is located under the corpus callosum (CC), which is drawn in black. The rostral migratory stream (RMS) is highlighted in gray. Most of the slices used for recording were chosen between sections 9-16 on the rostro-caudal axis. **B** and **C**. 4x magnification photographs of a coronal section of the RMS (B) and the subventricular zone (SVZ, C) observed under infra-red DIC optics. (B) In section ~11, the RMS, which is located around the two patch pipettes is elongated and can be distinguished from the surrounding tissue by its darker appearance. (C) In section ~15, the SVZ lines the lateral ventricle and extends between the CC and the striatum. **D** and **E**. Photographs of cells in the RMS (D) and the SVZ (E) taken at 60x magnification during the recording.

**Figure 2. Nestin and TuJ1 stainings in the SVZ.** **A.** Nestin and TuJ1 immunostainings of cells in mouse corticostriatal slices containing the SVZ (section ~11-12). A scheme representing the location of the immunostaining is shown in the inset between both panels. **B.** Higher magnification of nestin- and TuJ1-positive cells in the SVZ.

**Figure 3. Estimation of the cell resting potential using the reversal potential of K⁺ channels in patches.** **A.** Plot of the input resistance as a function of the zero-current $V_R$ (n=161). A linear fit of the data gave a coefficient of correlation of 0.3. The open and filled circles refer to the zero-current $V_R$ of cells in the SVZ and RMS, respectively. **B.** Schematic representation of the cell-attached recording conditions. **C.** A cell-attached record in response to a depolarizing ramp command (shown above the trace) from -140 to +200 mV (-Vpip). The holding potential (-Vpip) 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. $E_K$ was determined from the intersection of the fit (straight line) to the linear leak and the K⁺ current. The 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 have been activated and the reversal potential of the channels gave an estimate of the cell resting potential.

**Figure 4. SVZ neuronal progenitor morphology and current profile.** A. Photographs of a typical LY-filled progenitor recorded in the SVZ. B. Representative traces of the recorded cell shown in (A) obtained by depolarizing the cell from -100 mV to +60 mV from a holding potential of -80 mV. The traces display an outwardly rectifying current profile. C. Plots of the density of peak (filled circles) and steady-state (open circles) current (current/capacitance) as a function of the voltage for traces shown in (B). D. Tail current analysis of outward K$^+$ currents. The cell membrane was stepped from −120 to +60 mV following a pre-pulse to 0 mV. Only traces from -120 to +20 mV spaced by 20 mV are displayed. E. I-V curve of the current amplitude measured at the time point indicated by the arrow in (D).

**Figure 5. Neuronal progenitors express 100 μM TEA- and charybdotoxin-sensitive outward K$^+$ currents.** A. Records of outward K$^+$ currents activated by depolarizing the cells from -70 to +80 mV following a pre-pulse to -50 mV before (a), after bath application of 100 μM TEA (b), after wash out of TEA (c), and after bath application of 100 nM charybdotoxin (d). These recordings were obtained with an intracellular solution containing no EGTA and no added Ca$^{2+}$. Both 100 μM TEA and charybdotoxin reduced the outward K$^+$ currents in neuronal progenitors, suggesting the presence of large conductance Ca$^{2+}$-dependent K$^+$ currents. B. 100 μM TEA-sensitive currents obtained from point-by-point subtraction of traces in (b) from traces in (a). C. I-V curves of the 100 μM TEA-sensitive currents.

**Figure 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 following a pre-pulse 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 as delayed rectifying K⁺ currents (K_{DR}). B. **Top panel:** 40 mM TEA-sensitive currents obtained from point-by-point subtraction of traces in (b) from traces in (a). **Left panel:** I-V curves of the steady-state outward currents under control conditions (filled symbols) and in the presence of TEA (open symbols), and of the TEA-sensitive currents (open stars). C. Mean I-V curves of the steady-state outward currents in 5 neuronal progenitors. D. Mean steady-state activation curves of TEA-sensitive outward currents from 5 neuronal progenitors. This curve was fitted with a Boltzmann function shown in dashed line. The midpoint potentials was -4.7 mV and the slope value was 9.9 mV⁻¹. E. Currents obtained by applying 10 s-interval voltage ramps from -100 to +100 mV from a holding potential of -70 mV (ramp). Notice the successive increase in the blockage by increasing the concentration of TEA. F. Concentration-percent inhibition curve for TEA. Data were fitted with a sigmoidal curve and gave an IC₅₀ of 3.2 mM and a slope factor of 0.4. The number above each data point indicates the number of cells tested for each TEA concentration.

**Figure 7.** Delayed rectifying K⁺ currents are completely blocked by 4-aminopyridine (4-AP) in neuronal progenitors. A. K_{DR} 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.

**Figure 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 following a 200 ms pre-pulse to -110 mV. Capacitive and leak conductances were subtracted on-line by a modified P/5 protocol for this protocol and that in (C). B. Mean I-V curves of the peak currents in 7 neuronal progenitors. The 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 pre-pulse potential. The pre-pulse potentials started at -104 mV and were
incremented in 8-mV steps while 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 mV 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. The Na$^+$ currents in the cell displaying the slow action potentials are shown in the inset.
A. Nestin

250 µm

20 µm

B. TuJ1

Corpus callosum

Striatum

200 µm

20 µm

Anterior commissure, olfactory limb
Input resistance, $\Omega$:

Zero-current resting potential, mV:

$V_{pip}$

$V_{patch} = V_m - V_{pip} = E_K \approx 0 \text{ mV}$

At $K^+$ channel reversal potential:

$V_{pip}$

$155 \text{ mM } K^+$

$150 \text{ mM } K^+$

$r = 0.4, \ n = 161$

Input resistance, $M$

$\sim 155 \text{ mM } K^+$

$\sim 155 \text{ mM } K^+$

$V_{pip}$

$V_{patch} = V_m - V_{pip} = E_K \approx 0 \text{ mV}$
A  Intracellular solution: 0 EGTA/0 Ca^{2+}

B  Intracellular solution: 10 mM EGTA/1 mM Ca^{2+}
A  a: Control  b: 4-AP 2mM

200 pA
40 ms

B  a-b: 4-AP-sensitive

200 pA
40 ms

C  I, pA

n=7

V, mV