Kv3-like potassium channels are required for sustained high frequency firing in basal ganglia output neurons

Running title: Potassium channels in basal ganglia output neurons

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Abstract

The GABA projection neurons in the substantial nigra pars reticulata (SNr) are key output neurons of the basal ganglia motor control circuit. These neurons fire sustained high frequency, short duration spikes that provide a tonic inhibition to their targets and are critical to movement control. We hypothesized that a robust voltage-activated $K^+$ conductance that activates quickly and resists inactivation is essential to the remarkable fast-spiking capability in these neurons. Semi-quantitative RT-PCR (qRT-PCR) analysis on laser capture-microdissected nigral neurons indicated that mRNAs for Kv3.1 and Kv3.4, two key subunits for forming high activation threshold, fast-activating, slow-inactivating, 1 mM tetraethylammonium (TEA)-sensitive, fast delayed rectifier ($I_{DR$-fast}$) type Kv channels, are more abundant in fast-spiking SNr GABA neurons than in slow-spiking nigral dopamine neurons. Nucleated patch clamp recordings showed that SNr GABA neurons have a strong Kv3-like $I_{DR$-fast}$ current sensitive to 1 mM TEA that activates quickly at depolarized membrane potentials and is resistant to inactivation. $I_{DR$-fast}$ is smaller in nigral dopamine neurons. Pharmacological blockade of $I_{DR$-fast}$ by 1 mM TEA impaired the high frequency firing capability in SNr GABA neurons. Taken together, these results indicate that Kv3-like channels mediating fast-activating, inactivation-resistant $I_{DR$-fast}$ current are critical to the sustained high frequency firing in SNr GABA projection neurons and hence, movement control.
**Introduction**

The substantia nigra (SN) is a key component of the basal ganglia motor control circuitry. The major cell type in the SN pars compacta (SNc, **Fig. 1A**) is the dopamine (DA) neuron (Nelson et al. 1996; Haber et al. 2000; Parent et al. 2000). The main neuron type in the SN pars reticulata (SNr, **Fig. 1A**) is the γ-amino butyric acid (GABA) projection neuron (SNr GABA neurons hereafter) (Bolam et al. 2000; Gonzalez-Hernandez and Rodriguez, 2000; Deniau et al. 2007). The SNr also contains scattered DA neurons. SNr GABA neurons and nigral DA neurons have strikingly different neurophysiological properties. Nigral DA neurons fire low frequency (1-5 Hz), long duration spikes (around 2.5 ms under in vitro conditions) (Hyland et al. 2002; Zhou et al. 2006). In contrast, the SNr GABA neurons fire sustained high frequency (10-70 Hz depending on recording condition and animal species), short duration spikes (around 1 ms) (Hikosaka & Wurtz 1983; Schultz, 1986; Atherton et al. 2005; Zhou et al. 2006). The sustained high frequency firing in these GABA output neurons provides a tonic inhibition to their targets and is critical to movement control (Hikosaka et al. 2000). The mechanisms underlying this remarkable fast firing capability in SNr GABA neurons are not entirely understood.

Voltage-activated K⁺ (Kv) channels are important for controlling spike frequency and duration (Bean 2007). Four Kv channel families (Kv1, Kv2, Kv3 and Kv4) are commonly expressed in mammalian brain (Pongs 1992; Rudy et al. 1999; Birnbaum et al. 2004; Chang et al. 2007; Constantinople et al. 2009). Each of these Kv channel families has multiple subtypes with each channel formed by 4 identical or similar subunits, leading to diverse biophysical, pharmacological, and functional properties (Gutman et al. 2005). Evidence suggests that due to their fast activation and slow inactivation kinetics, Kv3 channels, Kv3.1-containing channels in particular, are essential to high frequency neuronal firing (Gan and Kaczmarek 1998; Rudy and McBain 2001; Baranauskas et al. 2003; Lien and Jonas 2003). In contrast, Kv4 channels often form I₄ type channels that activate at subthreshold
membrane potentials and thus reduce firing frequency (Tkatch et al. 2000; Liss et al. 2001).

Tissue level studies have detected Kv3.1, Kv3.2, Kv3.3 and Kv3.4 mRNAs and also Kv3.1, Kv3.2, and Kv3.3 proteins in the SNr region, whereas these Kv channel signals were very low in SNc region (Perney et al. 1992; Weiser et al. 1994, 1995; Ozaita et al. 2002; Chang et al. 2007). Kv4.3 mRNA is prominently expressed in the SNc region (Tsaur et al. 1997; Serodio et al. 1998). Since the main cell type in SNr and SNc are the GABA neurons and DA neurons, respectively, the Kv3 mRNAs are likely in SNr GABA neurons, and Kv4.3 mRNA is likely in SNc DA neurons. Indeed, in SNc DA neurons, Kv4.3 channel-mediated I_A type current has been implicated in regulating SNc DA neuron firing (Liss et al. 2001; see Koyama and Appel 2006 and Khaliq and Bean 2008 on I_A in ventral tegmental area DA neurons). But the precise functional roles of Kv channels in the fast-spiking SNr GABA neurons were not clear. We hypothesize that a fast-activating and slow-inactivating Kv current mediated by Kv3.1-containing channels enables these basal ganglia output neurons to fire sustained high frequency spikes.

Methods

Preparation of midbrain slices

Wild type, 16-21 days old male and female Sprague-Dawley rats were used. All procedures were carried out following NIH guidelines and were approved by the Institutional Animal Care Committee of The University of Tennessee Health Science Center. The procedures to prepare coronal midbrain slices containing the substantia nigra have been described in detail (Atherton et al. 2005; Zhou et al. 2006, 2008, 2009). Briefly, rats were deeply anesthetized with urethane. After transcardiac perfusion with an oxygenated ice-cold high sucrose cutting solution (see below), their brains were quickly dissected out and immersed in the ice-cold
oxygenated cutting solution for 2 min. Coronal midbrain slices (300 μm thickness) containing the midrostral part of substantia nigra were prepared in the cutting solution using a Vibratome 1000 Plus (Vibratome Company, St Louis, USA) or Leica Zero Z VT1200S vibratome (Leica Microsystems, Wetzlar, Germany). Slices were transferred to a holding chamber containing the normal extracellular solution (see below) at 30 °C for 45 min and then kept at room temperature (25 °C).

**Nucleated patch clamp recording**

Recordings were made at 30 °C under visual guidance of a video microscope (Olympus BX51WI and Zeiss Axiocam MRm digital camera) equipped with Nomarski optics and a 60X water immersion lens. Patch pipettes with resistances of 1-3 MΩ were pulled from borosilicate glass capillary tubing (KG-33, 1.1 mm i.d., 1.65 mm o.d., King Precision Glass, Claremont, CA) using a PC-10 puller (Narishige, Tokyo, Japan). A Multiclamp 700B amplifier, pClamp 9.2 software and Digidata 1322A interface (Molecular Devices, Sunnyvale, CA) were used to acquire and analyze data. After electrophysiologically fingerprinting SNr GABA and nigral DA neurons with conventional whole cell patch clamp (Fig. 1), gentle negative pressure was applied and the patch pipette was withdrawn slowly to isolate nucleated membrane patches (Fig. 4A-C) (Martina et al. 1998). As can be seen in the sequential images in Fig. 4A-C, the formation of spherical nucleated membrane patches free of axonal and dendritic processes can be clearly documented by including fluorescent lucifer yellow or Alexa 594 in the pipette solution. The key advantage of nucleated patch clamp is the substantially reduced spatial and voltage clamp problems.

Nucleated membrane patches were commonly held at –90 or –100 mV under voltage clamp mode. Stimulation voltage waveforms were generated by pClamp 9.2 software. Signals were digitized at 20 KHz and filtered at 10 kHz using the built-in 4-pole low-pass Bessel filter in the patch clamp amplifier. Pipette capacitance was nulled after forming giga-ohm seal onto the cell and before rupturing the membrane into whole cell mode. Series resistance and cell
membrane capacitive transients were compensated with the auto compensation functions of the Multiclamp 700B. The capacitance readings were taken as the estimated capacitance of the nucleated membrane patch to calculate current and conductance density. A P4 procedure was used to subtract ohmic leak current online. Ca\(^{2+}\) was replaced by Mg\(^{2+}\) to eliminate Ca\(^{2+}\) currents and Ca\(^{2+}\)-activated potassium currents. Voltage-activated sodium currents were blocked by 1 \(\mu\)M tetrodotoxin (TTX). GABA\(_A\) receptors and ionotropic glutamate receptors were also routinely blocked.

### Composition of solutions

The high sucrose cutting solution contained (in mM): 220 sucrose, 2.5 KCl, 1.25 Na\(_2\)HPO\(_4\), 25 NaHCO\(_3\), 0.5 CaCl\(_2\), 7 MgCl\(_2\), 20 D-glucose. The normal extracellular solution contained (in mM): 125 NaCl, 2.5 KCl, 25 NaHCO\(_3\), 1.25 Na\(_2\)HPO\(_4\), 2.5 CaCl\(_2\), 1.3 MgCl\(_2\), and 10 D-glucose with pH maintained at 7.4 by continuously bubbling with 95% O\(_2\) and 5% CO\(_2\). When recording Kv currents, 2.5 mM CaCl\(_2\) was replaced by 2.5 mM MgCl\(_2\). When 20 mM TEA-Cl was used, NaCl was reduced by 20 mM to keep osmolarity constant. Normal intracellular solution contained (in mM): 135 KCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 4 Na\(_2\)-phosphocreatine. pH was adjusted to 7.25 with KOH and osmolarity was 280-290.

### Electrophysiology data analysis

With 135 mM K\(^+\) in the pipette internal solution, 2.5 mM K\(^+\) in extracellular solution and recording temperature at 30 °C, the reversal potential was estimated to be about –100 mV, according to the Nernst equation. Conductance (G) was calculated using Ohm’s law and, when necessary, normalized to the maximum conductance (G\(_{\text{max}}\)). To construct steady-state inactivation curves, currents were normalized to the maximum currents and plotted against the conditioning potentials. Both activation and inactivation curves were fitted with the Boltzmann
equation in the form $f = 1/[1 + \exp[±(V-V_{1/2})/K]]$, where $V$ is the membrane potential, $V_{1/2}$ is the midpoint potential, and $K$ is the slope factor.

The liquid junction potential between the normal extracellular solution, 0 mM Ca$^{2+}$, 1 mM TEA-Cl, and 20 mM TEA-Cl-containing extracellular solutions and the 135 mM KCl-based intracellular solution was calculated to be 3.7 mV, 3.8 mV, 3.6 mV, and 4.2 mV, respectively. These relatively small liquid junction potentials were not corrected in the data presented below.

Data are reported as mean ± SEM. Two-sample independent t tests were used to make comparisons with $p$ value < 0.05 being considered statistically significant.

**Single cell reverse transcription PCR (scRT-PCR) of recorded neurons**

scRT-PCR procedures generally followed the well established methods (Surmeier et al. 1996; Liss et al. 2001; Zhou et al. 2008, 2009). Patch pipettes were autoclaved to eliminate RNase. Intracellular solution containing (in mM) 135 KCl, 0.5 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, and 4 Na$_2$-phosphocreatine was prepared using DNase-RNase free water. After electrophysiologically fingerprinting SNr GABA and nigral DA neurons, gentle suction was applied to aspirate the cytoplasm without disrupting the seal. The aspirated cell content was expelled into a 0.2 ml PCR tube and treated with DNase I (5 min at 25 °C) to remove genomic DNA. cDNA was synthesized using SuperScript III reverse transcriptase-based Cells-Direct cDNA Synthesis kit (Invitrogen). The synthesized cDNA was amplified using a hot-start Platinum PCR SuperMix (Invitrogen). RT-minus controls, in which the reverse transcriptase was omitted while all other reaction components were exactly the same, were performed to verify complete removal of genomic DNA. Negative controls were performed by lowering patch pipettes into the tissue and taking them out again, without seal formation and suction, to exclude nonspecific harvesting of surrounding tissue components.

Two-stage PCR amplification was used as described previously (Zhou et al. 2008, 2009). Briefly, 5 μl of the 30 μl cDNA sample was amplified for 45 cycles in
the presence of the specific primer pair for the first stage (see Supplemental Table S1 for primer pair sequences). The thermal cycling protocol was 2 min at 94 °C for the initial denaturation, than 45 cycles of 15 s at 94 °C to denature, 30 s at 48 °C to anneal, and 50 s at 72 °C to extend, followed by a 10 min final extension.

In the second stage PCR, the product of 1 μl from the first stage PCR amplification was used as template and the same primer pair that used in the first stage was used, and 40 cycles were run.

We first detected GAD1 (glutamate decarboxylase 1) and TH (tyrosine hydroxylase) mRNAs to identify GABA and DA neurons and to confirm the success of cytoplasm aspiration and cDNA synthesis. Then we used 5 μl of the remaining cDNA from the original 30-μl cDNAs to detect the target genes. All the products from the second stage amplification were separated by 1.5% agarose gel electrophoresis, visualized by ethidium bromide under UV light or Gelgreen also under UV light and photographed. The positive bands were then cut out and extracted using a Qiagen extraction kit. The extraction products were sequenced at the Molecular Resource Center of The University of Tennessee Health Science Center in Memphis, Tennessee and positively identified with the target gene sequences.

Primers were designed according to the sequences in GenBank and done using the web-based Primer3 software (http://fokker.wi.mit.edu/primer3/input.htm) (MIT, Cambridge, MA). Whenever possible, intron-spanning primer pairs were used that help detect genomic DNA contamination. Primer pair sequences were listed in Supplemental Table S1. In cases where negative scRT-PCR results were obtained, the effectiveness of the primer pairs was verified by whole brain mRNAs that yielded positive products (Supplemental Fig. S1). All primers were synthesized by Integrated DNA Technologies (Coralville, Iowa, USA).

qRT-PCR assay on laser capture microdissected-nigral neurons

Rats were deeply anesthetized with urethane (1.5g/Kg). Brains were rapidly removed and frozen with Freeze Spray. Coronal cryostat midbrain sections (10
μm) were collected and stored at –80 ºC. Tyrosine hydroxylase (TH) and parvalbumin (PV) were used as markers for DA and GABA neurons, respectively (Rajakumar et al. 1994; Gonzalez-Hernandez and Rodriguez 2000). To quickly immunostain DA neurons or GABA neurons, slide-mounted cryostat sections of unfixed rat midbrain sections were removed from –80 ºC storage and allowed to thaw before fixation in ice-cold 100% methanol for 3 min. The slide-mounted sections were briefly dipped in cold 0.02 M PBS and incubated for 3 min with rabbit anti-TH polyclonal antibody (Novus Biologicals, Littleton, CO) or mouse anti-PV monoclonal antibody (Sigma, St. Louis, MO), both diluted at 1:25 in PBS. This was followed by four brief rinses in cold PBS. The sections were then incubated for 3 min in red fluorescent secondary donkey anti-rabbit antibody (Alexa 568) or separately in green fluorescent secondary donkey anti-mouse antibody (Alexa 488) (InVitrogen), both diluted at 1:50 in PBS. The slide-mounted sections were washed four times in cold PBS and dehydrated (1 min each in 75%, 95% and 100% EtOH), followed by 5 min in xylene 2 times. After air-drying for 10 min, brain sections were visualized on an Arcturus PixCell fluorescent laser capture microdissection (LCM) system. TH-positive or PV-positive cells were picked by LCM and collected on the Arcturus Capsures sample caps (Molecular Devices). Approximately 300-400 TH-positive or PV-positive neurons from six coronal nigral sections were collected and pooled into a single cap. Six caps were used to collect TH-positive or PV-positive neurons for each animal. RNA was extracted and purified using the Arcturus PicoPure RNA isolation kit (Molecular Devices). RNA samples were further treated with DNase to remove any potential genomic DNA contamination and then used as template to generate cDNA using SuperScript III CellsDirect cDNA Synthesis kit (InVitrogen). Levels of mRNA for Kv1.1-1.6, Kv2.1-2.2, Kv3.1-3.4, Kv4.1-4.3 and β-actin (internal control) were analyzed by using a Roche LightCycler 480 (LC 480) quantitative real-time PCR system and the Universal ProbeLibrary probes and primers (Roche Applied Science, Indianapolis, Indiana). The sequences for these primers are listed in Supplemental Table S2. Using β-actin mRNA as internal control, Kv mRNA quantification was performed employing the
comparative crossing point (Cp) method in the form of $2^{-\Delta Cp}$ (Luu-The et al. 2005). The Cp values of the real-time fluorescence intensity curve were calculated using the second derivative method. The calculation was performed by the built-in software on LC 480. For Kv mRNA levels in DA neurons, $\Delta Cp,_{\text{Kv,DA neuron}} = Cp,_{\text{Kv,DA neuron}} - Cp,_{\beta\text{-actin,DA neuron}}$. For Kv mRNA levels in GABA neurons, $\Delta Cp,_{\text{Kv,GABA neuron}} = Cp,_{\text{Kv,GABA neuron}} - Cp,_{\beta\text{-actin,GABA neuron}}$. Finally, $2^{-\Delta Cp}$ values for Kv mRNAs were normalized to those in SNr GABA neurons.

Results

Electrophysiological identification of nigral neuron types

The SNc and SNr were identified reliably based on their anatomical location and characteristic cell distribution (Fig. 1A, B). The differences in electrophysiological properties between SNr GABA neurons, SNr DA neurons, and SNc DA neurons are well established (Tepper et al. 1995; Richards et al. 1997; Atherton et al. 2005; Blythe et al. 2007; Lee and Tepper 2007; Yung et al. 1991; Zhou et al. 2006, 2008). We were able to reliably identify SNr GABA and DA neurons according to their electrophysiological characteristics with conventional whole cell patch clamp (Fig. 1C-F). Putative SNr GABA neurons exhibited spontaneous spiking at $8.4 \pm 0.3$ Hz (n = 59) with an action potential base duration of $1.15 \pm 0.05$ ms and weak or no “sag” in response to hyperpolarizing current injections (Fig. 1D). In contrast, the presumed SNr DA neurons spiked spontaneously at $1.4 \pm 0.1$ Hz (n = 18) with an action potential base duration of $2.85 \pm 0.14$ ms and exhibited a pronounced sag in response to hyperpolarizing current injection (Fig. 1E). There was no overlap in the firing frequency and spike width between the two neuron types, as reported previously (Zhou et al. 2006). Additionally, we also recorded DA neurons in SNc. These SNc DA neurons spiked spontaneously at $1.5 \pm 0.1$ Hz (n = 23) with action potential base duration of $2.84 \pm 0.13$ ms and exhibited a pronounced sag in response to hyperpolarizing current injection. These action potential parameters and also the Kv current parameters...
that will be described below were not different between SNc and SNr DA neurons. Thus, data from SNr and SNC DA neurons were pooled and these DA neurons will be collectively referred to as nigral DA neurons.

Our electrophysiological identification of SNr GABA neurons and nigral DA neurons was further confirmed by scRT-PCR detection of GAD1 but not TH in electrophysiologically identified SNr GABA neurons and TH but not GAD1 mRNA in electrophysiologically identified nigral DA neurons. Therefore, our electrophysiological identification of SNr GABA neurons and nigral DA neurons was reliable.

Clearly, SNr GABA neurons and nigral DA neurons differ substantially in their action potential firing patterns and action potential waveforms, and we can identify these two neuron types reliably. So now we can study Kv channels that may underlie these differences in these two types of neighboring neurons.

Kv channel gene expression in identified nigral GABA and DA neurons

We first performed scRT-PCR on electrophysiologically identified SNr GABA neurons and nigral DA neurons. As shown in Figure 2, our qualitative scRT-PCR data indicated that Kv3.1 and Kv3.4 mRNAs were more frequently detected in SNr GABA neurons than in nigral DA neurons, whereas Kv4.2 and Kv4.3 mRNAs were more frequently detected in nigral DA neurons than in SNr GABA neurons. The detection of other Kv channel mRNAs was similar in SNr GABA and nigral DA neurons. These results suggested that the expression level of a few key Kv channel mRNAs is different in these two neuron types. Thus, quantitative comparison of Kv channel mRNA levels between SNr GABA neurons and nigral DA neurons were required. However, the quantity of mRNA obtained in a single neuron was not sufficient for qRT-PCR.

To overcome this difficulty, we collected and pooled SNr GABA neurons and nigral DA neurons, separately, by using laser capture microdissection (LCM) and performed qRT-PCR analysis on the common neuronal Kv (Kv1-Kv4) channel mRNAs isolated from these neurons. SNr GABA neurons and nigral DA neurons
were identified by parvalbumin (PV) and tyrosine hydroxylase (TH) fluorescent immunoreactivity, respectively (Gonzalez-Hernandez and Rodriguez 2000; Zhou et al. 2009). As expected, in our tissue sections, we did not see any over-lapping immunofluorescence in these two cell types, indicating the reliability of our immunofluorescence-based cell identification method (Zhou et al. 2009). Under these conditions, we found that the levels of Kv3.1 mRNA and Kv3.4 mRNA in SNr GABA neurons were 2.8 and 2.7 folds of those in nigral DA neurons, respectively (Fig. 3). In contrast, the levels of Kv4.2 and Kv4.3 mRNAs in nigral DA neurons were 2.7 and 3.3 folds of those in SNr GABA neurons, respectively (Fig. 3). No significant difference was detected in the level of mRNA for Kv1.1, Kv1.2, Kv1.3, Kv1.4, Kv1.6, Kv2.1, and Kv2.2 between the two cell types. Also, detection of mRNA for Kv1.5 and Kv4.1 occurred at CP values higher than 35, indicating low abundance and thus potentially unreliable quantification. The effectiveness of the primers for these mRNAs was positively verified using whole brain total RNA. Consequently, these two low abundance Kv channel mRNAs were excluded from analysis.

These results indicate a quantitatively differential co-expression of a few Kv channel mRNAs in the fast-spiking SNr GABA neurons and slow-spiking nigral DA neurons, potentially leading to quantitatively different expression of a few key types of Kv channels with diverse functional properties. Based on these qRT-PCR data, we predicted that SNr GABA neurons may express prominent Kv3.1- and Kv3.4-containing channels that mediate a fast delayed rectifier type Kv current ($I_{DR-fast}$) that activates rapidly at depolarized membrane potentials and inactivates slowly. These properties of the $I_{DR-fast}$ current may be crucial to the sustained high frequency spiking in SNr GABA neurons. We also predicted that nigral DA neurons may express prominent Kv4-containing channels that mediate an $I_A$ type Kv current that activates at subthreshold membrane potentials and inactivates quickly, contributing to the slow spiking in nigral DA neurons. In the following sections, we will present our data to support these hypotheses.
Composite somatic Kv currents differ between SNr GABA neurons and DA neurons

Our first experiment was to investigate the total depolarization-activated Kv current that is likely composed of multiple component Kv currents. Characterization of this composite total Kv current will give us an overall understanding of repolarizing forces in these fast-spiking SNr GABA neurons and slow-spiking nigral DA neurons. Characterization of individual component Kv currents will be presented in the later parts of this paper.

We first electrophysiologically identified SNr GABA neurons, SNr DA neurons, and SNc DA neurons as described earlier (Fig. 1D-F). Then we isolated nucleated membrane patches from these neurons (Fig. 4). These relatively small, spherical membrane patches free of neuronal processes provide adequate space clamp condition that is critical to studying Kv currents (Martina et al. 1998). Tetrodotoxin (TTX, 1 μM) was used to block voltage-gated Na current and 2.5 mM Ca\(^{2+}\) was replaced by 2.5 mM Mg\(^{2+}\) to remove the interference of voltage-activated Ca\(^{2+}\) current and Ca\(^{2+}\)-activated K currents. Under these conditions, we first compared the density of the composite peak Kv current at +40 mV where the activation curve had reached its peak and all available Kv channels should be open. When evoked from a holding potential of –100 mV (Fig. 5A1-A3), the peak composite current density was 772.8 ± 91.8 pA/pF or 5520 ± 656 pS/pF for SNr GABA neurons (n = 21) and 716.5 ± 110.9 pA/pF or 5118 ± 792 pS/pF for nigral DA neurons (n = 16) (Table 1). The density of the peak composite current or conductance was not statistically different between the two cell types (p > 0.05). (However, when evoked from a more physiologically relevant membrane potential, e.g. –50 mV, the peak current density was substantially higher in SNr GABA neurons than in nigral DA neurons. See next section.)

Second, we compared the voltage-dependence of the composite peak Kv current activation in SNr GABA neurons and nigral DA neurons, evoked from a holding potential of –100 mV. As illustrated in Fig. 5A2,3, the peak Kv currents in the two neuron types differed in their voltage dependence of activation: it was
apparently activated at more negative membrane potentials in nigral DA neurons than in SNr GABA neurons. These differences were more precisely described by the midpoint potentials ($V_{1/2}$) and slope factors (K) of activation curves obtained through Boltzmann function fitting. Specifically, SNr GABA neurons showed an activation curve with a more positive $V_{1/2}$ and steeper slope or voltage dependence than that of nigral DA neurons. The midpoint voltage $V_{1/2}$ was $-11.6 \pm 1.0$ mV ($n = 18$) for SNr GABA neurons and $-20.2 \pm 2.2$ (n = 12) for DA neurons ($p < 0.01$); the slope factor K was $12.4 \pm 0.6$ for SNr GABA neurons and $18.3 \pm 1.8$ for nigral DA neurons ($p < 0.01$) (Fig. 5A4). These results are consistent with our qRT-PCR profiling data showing that SNr GABA neurons express more Kv3.1 and Kv3.4 mRNAs that are known to form heteromeric $I_{DR}$ fast Kv channels that activate at relatively high membrane potentials (Rudy and McBain 2001), whereas nigral DA neurons express more Kv4.2 and Kv4.3 mRNAs that may form $I_{A}$ type Kv channels activating at more hyperpolarized membrane potentials. Since these Boltzmann equation fittings were obtained from the composite peak Kv currents, the parameters were approximations, although they indicated the overall differences in the total peak Kv current profiles in the two cell types.

Third, we compared the rise and decay of the composite peak Kv currents in SNr GABA neurons and nigral DA neurons. As shown in Fig. 5A2, A3, the activation profiles were different in the two cell types with DA neurons having a prominent transient peak reminiscent of $I_{A}$ type current. In contrast, the Kv current in GABA neurons had a blunted peak, resembling the waveform of the heteromeric $Kv3.1$ and $Kv3.4$ channel-mediated current (Rudy and McBain 2001; Baranauskas et al. 2003). The composite peak Kv current activates more rapidly with increasingly high membrane potentials. At $+40$ mV where all available Kv channel should be open, the 20-80% rise time ($T_{20-80}$) was $0.44 \pm 0.04$ ms for SNr GABA neurons ($n = 18$) and $0.34 \pm 0.03$ ms for nigral DA neurons ($n = 12$, $p < 0.05$). (The faster rise of the composite peak Kv current in DA neurons was due to the fast $I_{A}$ current that predominates the peak Kv current evoked from very negative holding potentials in DA neurons, but this $I_{A}$ is mostly inactivated at physiologically relevant
membrane potentials. See next section.) The decay of the composite Kv current was clearly different in these two cell types. DA neurons had a prominent fast component. Consequently, the ratio of the Kv current at the end of 100 ms, +40 mV command pulse over the peak Kv current, as a measure of the decay, was 0.69 ± 0.06 in SNr GABA neurons (n = 18) and 0.31 ± 0.03 in nigral DA neurons (n = 12). These results clearly indicate that, compared with nigral DA neurons, the peak composite Kv current in SNr GABA neurons decayed more slowly and was more resistant to inactivation. These electrophysiological results are also consistent with our qRT-PCR Kv mRNA profiling data showing that SNr GABA neurons express more Kv3.1 and Kv3.4 mRNAs that are known to form Kv channels mediating a slow-inactivating I_{DR-fast} Kv current, whereas nigral DA neurons express more Kv4.2 and Kv4.3 mRNAs that may form Kv channels mediating a transient, fast-inactivating I_A current.

Finally, the steady-state inactivation of the composite peak Kv current was studied by using a 10 s conditioning prepulse at membrane potentials ranging from −110 mV to 0 mV and then to a testing potential at +50 mV for 100 ms (Fig. 5B1). As shown in Fig. 5B2-4, the peak composite Kv current was inactivated differently in SNr GABA neurons and nigral DA neurons. The steady-state inactivation curves and Boltzmann function fitting clearly demonstrated that compared with nigral DA neurons, the peak composite current in SNr GABA neurons started to inactivate at more positive potentials and had a less steep slope or voltage dependence (Fig. 5B4). The midpoint potential V_{1/2} was −52.3 ± 2.5 mV (n = 10) for SNr GABA neurons and −72.1 ± 2.7 mV (n = 8) for nigral DA neurons (p < 0.01). The slope factor K was 12.9 ± 0.9 for SNr GABA neurons and 8.2 ± 0.8 for nigral DA neurons (p < 0.01). These data indicate that the peak composite Kv current in SNr GABA neurons was more resistant to voltage-dependent inactivation than the peak composite Kv current in nigral DA neurons. This is consistent with our idea that the major component of the peak Kv current in SNr GABA neurons was probably an inactivation-resistant, Kv3.1-containing channel-mediated I_{DR-fast}, whereas the
major component of the peak Kv current in nigral DA neurons was a transient I_A-type current probably mediated by Kv4-containing channels.

Different subthreshold inactivation of the composite peak Kv current in SNr GABA and nigral DA neurons

Data presented above describe the composite peak Kv current evoked from very negative, non-physiological membrane potentials (–90 to –110 mV). However, SNr GABA neurons and nigral DA neurons usually stay above –60 mV and may only briefly dip below that membrane potential level during strong inhibitory synaptic inputs and afterhyperpolarization. Furthermore, before reaching action potential threshold, the membrane potential of SNr GABA neurons and especially nigral DA neurons is often around –50 mV (Fig. 6A,B). A sojourn at such a membrane potential may inactivate Kv channels and thus leaves only fractions of Kv channel populations available for regulating the initiation and repolarization of action potentials. Such subthreshold inactivation may affect different Kv channels differently in SNr GABA neurons and nigral DA neurons.

To explore these possibilities, we did the following experiment. As illustrated in Fig. 6C, we held the nucleated membrane patch at –90 mV, then stepped to a prepulse at –50 mV of increasing duration to mimic the subthreshold membrane potential, then stepped to a testing potential of +20 mV for 150 ms, and finally returned to –90 mV. As shown in Fig. 6D-F, large subthreshold inactivation of the peak Kv current occurred in nigral DA neurons but not in SNr GABA neurons. For example, after 50 ms at –50 mV that occurs frequently in both SNr GABA and nigral DA neurons, the composite peak Kv current was reduced to 82.2 ± 7.8 % (current density 597 ± 58 pA/pF) in SNr GABA neurons (n = 6) and to 40.4 ± 6.9 % (275 ± 48 pA/pF) in nigral DA neurons (n = 6, p < 0.01). After 200 ms at –50 mV that occurs commonly in nigral DA neurons but rarely in SNr GABA neurons, the composite peak Kv current was reduced to 75.1 ± 6.6 % (545 ± 49 pA/pF) in SNr GABA neurons (n = 6) and to 31.3 ± 5.7 % (212 ± 38 pA/pF) in nigral DA neurons (n = 6, p < 0.01). These data clearly indicate that the peak Kv
current had minimal subthreshold inactivation in SNr GABA neurons, whereas the peak Kv current in nigral DA neurons was inactivated substantially before the membrane reaches action potential threshold. As we will address later, it is likely the Kv4-containing channel-mediated $I_A$ type current that undergoes prominent subthreshold inactivation. Since nigral DA neurons often experience membrane potentials between $-50$ mV to $-45$ mV for 50 ms or longer before they spike, the subthreshold inactivation of these Kv4-containing channels likely contributes to the relatively slow repolarization in nigral DA neurons. These findings also indicate that under physiological conditions with resting potentials fluctuating around $-50$ mV, more Kv channels are available to repolarize action potentials in SNr GABA neurons than in nigral DA neurons. These differential Kv channel properties are ideally suited for the fast- and slow-spiking behaviors in these two neuron types.

**Pharmacological dissection reveals a strong Kv3-like $I_{DR-fast}$ current in fast-spiking SNr GABA neurons**

Experiments described so far examined the properties of the composite peak Kv current in SNr GABA and nigral DA neurons. Characterization of the composite peak Kv current is essential to understanding the vastly different firing behaviors in these two neuron types. In the following sections, we will investigate the individual component Kv currents that comprise the composite peak Kv current.

It is well established that Kv3.1-containing channel-mediated $I_{DR-fast}$ is highly sensitive to external TEA and blocked by 1 mM external TEA, whereas the potentially Kv1-containing channel-mediated slow delayed rectifier ($I_{DR-slow}$) is inhibited by 20 mM TEA (Rettig et al. 1992; Martina et al. 1998; Coetzee et al. 1999; Rudy et al. 1999; Hernández-Pineda et al. 1999; Gutman et al. 2005). In contrast, the Kv4-containing channel-mediated transient $I_A$ type current is resistant to high concentrations (e.g., 20 mM) of TEA (Pak et al. 1991; Coetzee et al. 1999; Rudy et al. 1999; Gutman et al. 2005). Thus, after replacing extracellular Ca$^{2+}$ with Mg$^{2+}$ to eliminate the interference from Ca$^{2+}$ currents and Ca$^{2+}$-activated K
channels, we used 1 mM and 20 mM TEA as the pharmacological tools to dissect different components in the composite Kv current. We reasoned that if Kv3.1-containing channels mediate the $I_{\text{DR-fast}}$ current in SNr GABA neurons and nigral DA neurons, then 1 mM TEA will likely block this $I_{\text{DR-fast}}$ current. Also, since SNr GABA neurons express more Kv3.1 mRNA and hence more Kv3.1-containing $I_{\text{DR-fast}}$ channels than nigral DA neurons, 1 mM TEA may inhibit a larger portion of the composite peak Kv current in SNr GABA neurons than in nigral DA neurons.

Indeed, as illustrated in Fig. 7, we detected 1 mM TEA-sensitive $I_{\text{DR-fast}}$, 20 mM TEA-resistant $I_A$, and 20 mM TEA-sensitive $I_{\text{DR-slow}}$ in both SNr GABA neurons and nigral DA neurons. Furthermore, we found that there were major differences in the amplitudes of $I_{\text{DR-fast}}$ and $I_A$ currents in the two cell types (Fig. 7A1-A4, B1-B4, Table 1). Specifically, in SNr GABA neurons ($n = 10$), the 1 mM TEA-sensitive $I_{\text{DR-fast}}$ was strong, contributing $58.8 \pm 5.3\%$ to the composite peak Kv current, evoked from a holding potential of $-100$ mV to a testing potential of $+20$ mV that is commonly experienced by SNr GABA neurons and nigral DA neurons near or at their spike peak (Fig. 7A1-A4). In nigral DA neurons ($n = 9$), this 1 mM TEA-sensitive $I_{\text{DR-fast}}$ was weak, contributing only $11.1 \pm 3.5\%$ to the composite peak Kv current evoked by the same voltage protocol used in SNr GABA neurons ($p < 0.01$, Fig. 7A2, B2, Table 1). The situation with the fast-inactivating, 20 mM TEA-resistant $I_A$ type Kv current evoked at a testing potential of $+20$ mV from a holding potential of $-100$ mV was the opposite: $I_A$ current contributed only $14.7 \pm 2.9\%$ to the composite peak Kv current in SNr GABA neurons ($n = 10$) but $64.5 \pm 5.8\%$ to the composite peak Kv current in nigral DA neurons ($n = 9$, $p < 0.01$, Fig. 7A4, B4, Table 1). The contribution of the $I_{\text{DR-slow}}$ Kv current sensitive to 20 mM TEA to the composite $K^+$ current was similar between the two cell types ($26.5 \pm 3.4\%$ for 10 SNr GABA neurons, $24.4 \pm 5.1\%$ for 9 DA neurons, $p > 0.05$, Fig. 7A3, B3, Table 1). Taken together with our qRT-PCR results (Fig. 3), these data clearly indicate that the fast-spiking SNr GABA neurons express more functional Kv3.1-containing channel-mediated $I_{\text{DR-fast}}$ current, whereas the slow-spiking nigral DA neurons express more Kv4-containing channel-mediated $I_A$ current.
We also tested the effect of 100 nM α-dendrotoxin (α-DTX), known to inhibit Kv1 channels (Gutman et al. 2005), on the composite Kv currents evoked in somatic nucleated membrane patches. The composite Kv current in SNr GABA neurons and nigral DA neurons was not or only minimally reduced by 100 nM α-DTX (data not shown), indicating that α-DTX-sensitive Kv1 channels probably only make minimal contribution to the composite somatic Kv currents in these two types of neurons, similar to the situation in hippocampal fast-spiking GABA interneurons (Lien et al. 2002).

**Similar kinetics of pharmacologically isolated component Kv currents in SNr GABA neurons and nigral DA neurons**

Since SNr GABA neurons and nigral DA neurons both expressed 1 mM TEA-sensitive $I_{DR-fast}$ Kv current, 20 mM TEA-sensitive $I_{DR-slow}$, and 20 mM TEA-resistant $I_A$ Kv current, we asked this question: besides the differences in expression levels, are there any differences in the kinetics of these component Kv currents between the two neuron types that contribute to their different firing behaviors?

First, we compared the kinetics of the 1 mM TEA-sensitive $I_{DR-fast}$ current in SNr GABA neurons and nigral DA neurons. 1 mM TEA-sensitive $I_{DR-fast}$ Kv currents at different membrane potentials were extracted by subtraction (Fig. 8A1-4, Fig. 8B1-4). To ensure the reliability of the subtraction procedure, we required that the currents recovered upon washing out 1 mM TEA such that the subtraction result was not experimental artifacts (Fig. 8A3). Under these rigorous conditions, we saw a large $I_{DR-fast}$ Kv current in SNr GABA neurons whereas this current was much smaller in nigral DA neurons (Fig. 8A1-4, Fig. 8B1-4, Table 1). As shown in Fig. 8C1, the activation midpoint of $I_{DR-fast}$ in these two neuron types was similar: $-8.5 \pm 1.6$ mV for SNr GABA neurons ($n = 12$) and $-6.2 \pm 1.2$ mV for nigral DA neurons ($n = 7$) ($p > 0.05$). The slope factor $K$ of voltage-dependent activation was $8.9 \pm 0.6$ in SNr GABA neuron ($n = 12$) and $10.8 \pm 0.7$ in nigral DA neuron ($n = 7$, $p > 0.05$). The steady-state inactivation of the 1 mM TEA-sensitive $I_{DR-fast}$ was also...
similar in these two cell types (Fig. 8C2): the \( V_{1/2} \) was \(-49.2 \pm 1.6 \) mV for SNr GABA neurons (\( n = 5 \)) and \(-53.2 \pm 1.4 \) mV for nigral DA neurons (\( n = 5 \)), respectively (\( p > 0.05 \)); the slope factor \( K \) was \( 8.7 \pm 0.6 \) for SNr GABA neurons and \( 11.2 \pm 1.1 \) for nigral DA neurons, respectively (\( p > 0.05 \)). These results indicate that the voltage-dependent activation and inactivation kinetics and perhaps the molecular composition of the 1 mM TEA-sensitive \( I_{DR\text{-fast}} \) current are similar in SNr GABA neurons and nigral DA neurons, although the amplitude of \( I_{DR\text{-fast}} \) and hence the expression level of the underlying \( K_v \) channels are different in these two cell types. These results also show that the robust \( I_{DR\text{-fast}} \) current activates at membrane potentials more positive than the spike threshold (\(-40 \) mV) in GABA neurons. Consequently, unlike \( I_A \), \( I_{DR\text{-fast}} \) does not impede the generation of spikes but can repolarize the membrane once a spike is fired.

We also investigated the deactivation kinetics of \( I_{DR\text{-fast}} \) in SNr GABA neurons by examining the decay of the \( I_{DR\text{-fast}} \) tail current (Fig. 9). Due to the small amplitude of the \( I_{DR\text{-fast}} \) tail current in nigral DA neurons, we were unable to reliably estimate its deactivation kinetics. So we focused on the deactivation kinetics of \( I_{DR\text{-fast}} \) in SNr GABA neurons. As illustrated in Fig. 9A, the nucleated membrane patch from SNr GABA neurons was held at \(-90 \) mV, then stepped to 20 mV for 100 ms to activated \( I_{DR\text{-fast}} \), then stepped to \(-30 \) mV, \(-40 \) mV, \(-50 \) mV, \(-60 \) mV, and \(-70 \) mV to deactivate \( I_{DR\text{-fast}} \). The 1 mM TEA-sensitive \( I_{DR\text{-fast}} \) was then obtained by subtraction (Fig. 9A). The tail currents were expanded and displayed in the middle of Fig. 9. The decay of the tail currents at these membrane potentials was fitted to single exponential functions and the time constants were used as a measure of the deactivation kinetics of the 1 mM TEA-sensitive \( I_{DR\text{-fast}} \). The decay time constants of the tail currents or the deactivation time constants of \( I_{DR\text{-fast}} \) were fast (Fig. 9A, B). For example, the tail current decay time constant at \(-60 \), \(-50 \) mV, and \(-40 \) mV, three physiologically relevant membrane potentials, was \( 0.82 \pm 0.06 \) ms, \( 1.35 \pm 0.12 \), and \( 1.87 \pm 0.16 \) ms, respectively. The deactivation became faster upon at more hyperpolarized membrane potentials (Fig. 9B). These deactivation characteristics of \( I_{DR\text{-fast}} \) observed in SNr GABA neurons are similar to those
reported for Kv3.1-containing channels (Grissmer et al. 1994; Hernández-Pineda et al. 1999; Baranauskas et al. 2003). Functionally, the fast deactivation of $I_{DR-fast}$ allows the SNr GABA neurons to depolarize again without much delay, contributing to the fast-spiking behavior in these basal ganglia output neurons.

Next, we examined the kinetics of the $I_{DR-slow}$ current sensitive to 20 mM TEA. We used two methods to obtain $I_{DR-slow}$. First, since $I_{DR-slow}$ is sensitive to 20 mM TEA, we used subtraction to extract the 20 mM TEA-sensitive $I_{DR-slow}$. Second, we used 5 mM 4-AP to block $I_A$ and 1 mM TEA to block $I_{DR-fast}$, the remaining current was $I_{DR-slow}$ that was also blocked by 20 mM TEA. The $I_{DR-slow}$ obtained with these two methods was similar and thus the data were pooled. We found that there was no detectable difference in the current density and activation and inactivation kinetics of $I_{DR-slow}$ in SNr GABA neurons and nigral DA neurons (Fig. 10, Table 1). These results indicate that this $I_{DR-slow}$ Kv current is probably conducted by similar or identical Kv1 and/or Kv2-containing channels in these two neuron types.

Third, we examined the kinetics of the 20 mM TEA resistant, $I_A$ type Kv current. In both SNr GABA neurons and nigral DA neurons, from a holding potential of −100 mV, $I_A$ was activated significantly at −60 mV, about 20 mV below the action potential threshold (Fig. 11A). The activation $V_{1/2}$ and slope factor K were similar among the two cell types: −33.7 ± 1.3 mV and 15.7 ± 1.1 in SNr GABA neurons (n = 6) and −39.2 ± 1.5 mV and 16.4 ± 1.2 for nigral DA neurons (Fig. 11A4, n = 7, Table 1, p>0.05). The steady-state inactivation $V_{1/2}$ and slope factor K were also similar among the two cell types: −78.5 ± 1.5 mV and 8.9 ± 0.8 in SNr GABA neurons (n = 6) and −83.2 ± 1.7 mV and 10.3 ± 1.2 for nigral DA neurons (Fig. 11B, n = 6, p>0.05). However, when evoked at +40 mV from a holding potential of −100 mV, the peak $I_A$ current or conductance density in SNr GABA neurons was only about ¼ of that in nigral DA neurons: 110.4 ± 18.9 pA/pF or 778 ± 135 pS/pF (estimated driving force was 140 mV) for SNr GABA neurons (n = 6) and 453.2 ± 57.3 pA/pF or 3237 ± 409 pS/pF for nigral DA neurons (n = 7) (p < 0.01). These data indicate that while SNr GABA neurons and nigral DA
neurons both express $I_A$ type Kv4-containing channels, the level of expression is much higher in DA neurons than in SNr GABA neurons, consistent with our qRT-PCR data.

Taken together, these data on pharmacologically isolated component Kv currents suggest that SNr GABA neurons express predominantly Kv3-containing channel-mediated $I_{DR}$-fast with lower levels of $I_{DR}$-slow and $I_A$, whereas DA neurons express predominantly Kv4-containing channel-mediated $I_A$ with lower levels of $I_{DR}$-fast and $I_{DR}$-slow.

**Inhibition of Kv3-like $I_{DR}$-fast current impairs sustainable high frequency spiking in substantia nigra reticulata GABA projection neurons**

In other neuronal systems that are capable of high frequency firing, pharmacological (by 1 mM TEA) or genetic inactivation of members of Kv3 channel family has been shown to impair the ability for neurons to fire high frequency spikes (Wang et al. 1998; Erisir et al. 1999; Lau et al. 2000; Rudy and McBain 2001; Lien and Jonas 2003; Macica et al. 2003). Based on our qRT-PCR and voltage clamp data presented in above sections, we hypothesized that blocking the Kv3-like $I_{DR}$-fast will impair the ability for SNr GABA neurons to fire sustained high frequency spikes. To test this idea, we performed whole cell current clamp recording. According to literature evidence and our voltage clamp data, we used 1 mM TEA to block $I_{DR}$-fast. Indeed, as illustrated in Fig. 12A1-A8, upon intracellular injection of depolarizing currents, SNr GABA neurons were able to fire sustained high frequency fast spikes. The maximum rise rate (Fig. 12A7) and the peak amplitude (Fig. 12A1) of the spikes were also largely sustained, indicating that the Kv currents were able to quickly repolarize the cell and thus recover voltage-dependent Na channels from inactivation. Bath application of 1 mM TEA modestly increased the firing frequency at low intensity current injection (Fig. 12A8). The most prominent effect of 1 mM TEA was that, as the current injection intensity increased and the initial firing frequency increased, the action potential amplitude decreased progressively, eventually leading to a complete cessation of firing (Fig.
12A2, A6, A7). In the presence of 1 mM TEA, the mean firing frequency over the
10 s period was also drastically decreased when 300 and 400 pA currents were
injected (Fig. 12A8). These results indicate that SNr GABA neurons were unable
to fire sustained high frequency spikes in the presence of 1 mM TEA that blocked
I_{DR-fast} leading to broadened spikes and reduced membrane hyperpolarization (Fig.
12A5). As indicated by the rapid decrease in the maximum spike rise rate, the
delayed and shallow repolarization after blocking I_{DR-fast} likely caused cumulative
inactivation of voltage-dependent Na channels, leading to the eventual loss of
spiking capability (Fig. 12A8). Similar results were obtained when the cell was
hyperpolarized to around −75 mV by injecting negative currents (Supplemental
Fig. S2). At that membrane potential, significant amounts of I_A were available that
were not inhibited by 1 mM TEA. Thus, these results indicate that it is probably the
Kv3-containing I_{DR-fast}, not I_A, that is most critical for maintaining the high frequency
firing in SNr GABA neurons.

To remove or minimize the potential confounding inhibitory effect of 1 mM
TEA on Ca^{2+}-activated K channels (Wei et al. 2005), we further examined the
effects of 1 mM TEA on the spike firing in SNr GABA neurons in 0 mM Ca^{2+}
extracellular bathing solution (Fig. 12B1-B8). After replacing extracellular Ca^{2+}
with Mg^{2+}, SNr GABA neurons were able to fire sustained fast spikes at even
higher frequencies than under normal 2.5 mM Ca^{2+} (Fig. 12B1, B2, B6, B8), likely
due to the removal of inhibitory influences of Ca^{2+}-activated K channels. When 1
mM TEA was applied to the 0 mM Ca^{2+}-containing bathing solution, SNr GABA
neurons were able to spike only within the first few seconds upon strong
depolarizing current injection (Fig. 12B3, B6, B8). Furthermore, the spikes within
the few seconds became progressively smaller in amplitude and maximum rise
rate, leading to an eventual cessation of the spikes (Fig. 12B3, B6, B7). In the
presence of 1 mM TEA, the mean firing frequency over the 10 s period was also
dramatically decreased when 300 and 400 pA currents were injected (Fig. 12B7).
Additionally, as illustrated in Fig. 12B5, B8, removing Ca^{2+} from the bathing
solution only modestly broadened the spike duration, reduced the fast
afterhyperpolarization (fAHP), and decreased maximum spike rise rate. In contrast, addition of 1 mM TEA completely blocked the fAHP and drastically broadened the spike duration, indicating it’s probably the $I_{\text{DR-fast}}$, not Ca$^{2+}$-dependent K channels, that are primarily responsible for spike repolarization in SNr GABA neurons. The rapid decrease in the maximum spike rise rate indicate that the delayed and shallow repolarization caused a progressive inactivation of voltage-dependent Na channels and eventual loss of spiking capability (Fig. 12B8). These results provide compelling evidence that the Kv3-containing $I_{\text{DR-fast}}$, not Ca$^{2+}$-activated K channels, is critical for SNr GABA neurons to rapidly repolarize and fire sustained high frequency spikes.
Discussion

The main finding of this study is that, in comparison with nigral DA neurons, SNr GABA neurons express more high-threshold, fast-activating, inactivation-resistant, and fast-deactivating delayed rectifier ($I_{\text{DR-fast}}$) type Kv3-like current. Pharmacological blockade of this Kv3-like $I_{\text{DR-fast}}$ impairs SNr GABA neurons’ capability to fire sustained high frequency spikes.

Fast-spiking SNr GABA projection neurons express more Kv3.1 and Kv3.4 mRNAs and stronger Kv3-like $I_{\text{DR-fast}}$ than slow-spiking nigral DA neurons

In this study, we performed qualitative scRT-PCR in patch clamped cells and also semi-quantitative qRT-PCR analysis on the expression of 15 common neuronal Kv channel subunits including Kv1.1-6, Kv2.1-2, Kv3.1-4, and Kv4.1-3 in immunohistochemically identified, laser capture-microdissected SNr GABA neurons and nigral DA neurons. We found that in comparison with slow-spiking nigral DA neurons, fast-spiking SNr GABA neurons express more Kv3.1 and Kv3.4 mRNAs that can form the fast delayed rectifier type K channels and conduct $I_{\text{DR-fast}}$. In contrast, nigral DA neurons express more Kv4.2 and Kv4.3 mRNAs that may form $I_A$ type channels and conduct $I_A$. These molecular findings were supported by our electrophysiological data. Using the nucleated patch clamp technique that provides outstanding voltage clamp conditions, we found that SNr GABA neurons express a prominent $I_{\text{DR-fast}}$ Kv current that starts to activate near $–30$ mV, that is above the action potential threshold; the activation was fast but the decay or inactivation was slow. These properties of this $I_{\text{DR-fast}}$ Kv current are reminiscent of the currents mediated by cloned heteromeric Kv3.1 and Kv3.4 channels (Weiser et al. 1994; Rudy and McBain 2001; Baranauskas et al. 2003). The kinetic and pharmacological properties of the Kv3-like $I_{\text{DR-fast}}$ current in fast-spiking SNr GABA neurons are also similar to the Kv3-like $I_{\text{DR-fast}}$ currents in fast-spiking GABA neurons in other brain areas (Martina et al. 1998; Rudy and McBain 2001; Baranauskas et al. 2003; Lien and Jonas 2003). The deactivation of $I_{\text{DR-fast}}$ in SNr GABA neurons was very fast with a time constant of about 1 ms at $–60$ mV.
This is similar to the deactivation kinetics of cloned homomeric Kv3.1 channels, heteromeric Kv3.1 and Kv3.4 channels (Grissmer 1994; Hernández-Pineda et al. 1999; Baranauskas et al. 2003) and native Kv3.1-containing $I_{\text{DR-fast}}$ in fast-spiking neurons (Du et al. 1996; Martina and Jonas 1998; Hernández-Pineda et al. 1999; Baranauskas et al. 2003). In addition to the prominent $I_{\text{DR-fast}}$, SNr GABA neurons also express small amounts of the $I_{\text{DR-slow}}$ and classic $I_A$ type current. In contrast, nigral DA neurons express a robust subthreshold-activating, subthreshold-inactivating, and fast-inactivating, classic $I_A$ current likely mediated by Kv4.2 and/or Kv4.3-containing channels. This $I_A$ current is ideally suited for regulating the low frequency firing of long duration spikes in nigral DA neurons (Liss et al. 2001).

The precise subunit composition of the Kv channel conducting the Kv3-like $I_{\text{DR-fast}}$ in SNr GABA neurons remains unknown. In heterologous expression systems, all homomeric Kv3 channels start to activate at membrane potentials above action potential threshold (Rudy and McBain 2001; Baranauskas et al. 2003). Homomeric Kv3.1 and Kv3.2 channels conduct fast-activating, slow- or non-inactivating type currents, whereas homomeric Kv3.3 and Kv3.4 channels mediate fast-inactivating $I_A$ type current that is different from the classic $I_A$ current in that it activates at more positive potentials (Rettig et al. 1992; Weiser et al. 1994; Rudy and McBain 2001). More important, heteromeric Kv channels formed by Kv3.1 and Kv3.4 subunits conduct Kv currents with kinetics very similar to or virtually identical to those of the Kv3-like $I_{\text{DR-fast}}$ in SNr GABA neurons (Fig. 8A4) (Weiser et al. 1994; Rudy and McBain 2001; Baranauskas et al. 2003). Thus, the prominent 1 mM TEA–sensitive Kv3-like $I_{\text{DR-fast}}$ we recorded in fast-spiking SNr GABA may be mediated by heteromeric Kv3.1- and Kv3.4-containing Kv channels. This interpretation is also consistent with our qRT-PCR data showing a positive association between the expression level of Kv3.1 and Kv3.4 mRNAs and the amplitude of $I_{\text{DR-fast}}$: SNr GABA neurons express more Kv3.1 and Kv3.4 mRNAs and also have a stronger $I_{\text{DR-fast}}$, whereas nigral DA neurons express less Kv3.1 and Kv3.4 mRNAs and also have a weaker $I_{\text{DR-fast}}$. Certainly, resemblance is not
proof of molecular identity, and there is also a possibility that the $I_{DR\text{-fast}}$ is a summation of homomeric Kv3.1 and Kv3.4 channels.

We also detected Kv3.2 and Kv3.3 mRNAs in SNr GABA neurons and nigral DA neurons with no difference between the two cell types. Published immunostaining and in situ hybridization studies have detected the mRNAs and/or proteins for Kv3.2 and Kv3.3 in the SNr area and/or SNr GABA neurons (Rudy et al. 1999; Chang et al. 2007). However, due to their similar mRNA expression levels in the two cell types, Kv3.2 and Kv3.3 may not be the main Kv3 channel subunits that mediate the $I_{DR\text{-fast}}$ that is much larger in SNr GABA neurons than in nigral DA neurons. In contrast, Kv3.1 and Kv3.4 mRNA expression levels in the two cell types match the Kv3-like $I_{DR\text{-fast}}$ amplitude, supporting the idea of Kv3.1 and Kv3.4 being the main subunits mediating the Kv3-like $I_{DR\text{-fast}}$. How Kv3.2 and Kv3.3 may contribute to the Kv3-like $I_{DR\text{-fast}}$ recorded in the somatic membrane is not known. One possibility is that the absolute expression level of Kv3.2 and Kv3.3 that we did not measure may be much lower than Kv3.1 and Kv3.4 such that the Kv3 current mediated by potential Kv3.2 and Kv3.3 homomers and/or heteromers is very small. This is because our qRT-PCR data were semi-quantitative and only indicated the relatively abundance of a particular mRNA in different cell types. These qRT-PCR experiments were not designed to determine if the expression level of one mRNA is higher or lower than another mRNA, because the amplification efficiency is often different for different primer pairs. Combined with our patch clamp data, we suggest that Kv3.2 and Kv3.3 are expressed at lower levels than Kv3.1 and Kv3.4 in SNr GABA neurons. Certainly, mRNA levels and protein levels are not always positively correlated. Posttranslational modification and targeting may also alter the cell surface expression of ion channels (Lai and Jan 2006). Thus, a possibility exists that Kv3.2 and Kv3.3 may make a larger contribution to the Kv3-like $I_{DR\text{-fast}}$ in the fast-spiking SNr GABA neurons than that we discussed above. However, regardless of subunit composition, the assembled Kv3-like channels must be more abundant and conduct a stronger $I_{DR\text{-fast}}$ in fast-spiking SNr GABA neurons than in slow-spiking nigral DA neurons as
demonstrated by our present study. Future studies are needed to determine the absolute expressions of Kv3 subunits in both SNr GABA neurons and DA neurons and the precise subunit composition of $I_{DR}$-fast in SNr GABA neurons.

Nigral DA neurons express more Kv4.2 and Kv4.3 mRNAs and stronger $I_A$ than SNr GABA projection neurons

In comparison with SNr GABA neurons, our qRT-PCR analysis indicated that nigral DA neurons express more Kv4.2 and Kv4.3 mRNAs (Fig. 3). Kv4.2 and Kv4.3 mRNAs can form Kv channels with classic $I_A$ current characteristics, that is, fast subthreshold activation and inactivation (Stuhmer et al. 1989; Pak et al. 1991; Schroter et al. 1991, Rettig et al. 1992; Weiser et al. 1994; Serodio et al. 1996; Timothy et al. 1991; Tsaur et al. 1997; Norris & Nerbonne 2010). Our nucleated patch clamp recording revealed that SNr GABA neurons had a very weak $I_A$; in contrast, nigral DA neurons had a very strong $I_A$ that activates and inactivates quickly at subthreshold membrane potentials, resembling the classic $I_A$ current. Our qRT-PCR data and electrophysiological data suggest that the 20 mM TEA resistant $I_A$ current is likely to be mediated by Kv4.2 and/or Kv4.3-containing channels. Its subthreshold activation can keep the firing of DA neurons at low rates. However, there is one difference between our results and those of Liss et al. (2001). In their pioneering study, Liss et al. (2001) detected only Kv4.3 mRNA but not Kv4.2 mRNA and concluded that Kv4.3 is the main subunit for $I_A$ in SNc DA neurons. In our hands, both Kv4.2 and Kv4.3 mRNAs are frequently detected by scRT-PCR in patch clamped single cells and by qRT-PCR in pooled LCM-captured cells (Figs. 2 and 3). We did proper control to prevent false positive detection. We also sequenced and positively identified the PCR products. Another scRT-PCR study (Tkatch et al. 2000) detected both Kv4.2 and Kv4.3 in basal ganglia and basal forebrain neurons, with Kv4.2 being the main subunit forming $I_A$.

So the difference between our results and Liss et al. (2001) can only be resolved by independent studies in the future.
Similar expression of other Kv members in SNr GABA neurons and nigral DA neurons

Our scRT-PCR and qRT-PCR analyses also detected mRNAs for Kv1.1-4, Kv1.6, and Kv2.1-2 at similar levels in SNr GABA neurons and nigral DA neurons. Members of Kv1 and Kv2 may form the slow delayed rectifier Kv channels (Coetzee et al. 1999; Gutman et al. 2005). Consistent with this idea, the $I_{\text{DR-slow}}$ was recorded at similar amplitude in SNr GABA neurons and nigral DA neurons. Due to a lack of pharmacological means to selectively block $I_{\text{DR-slow}}$, the functional roles of $I_{\text{DR-slow}}$ in SNr GABA neurons and nigral DA neurons were not examined. However, we speculate that $I_{\text{DR-slow}}$ may be only a minor repolarizing force in SNr GABA neurons under normal condition, because of its slow kinetics and small amplitude, whereas $I_{\text{DR-slow}}$ may be a relatively more important repolarizing force in nigral DA neurons because their slow action potential allows more $I_{\text{DR-slow}}$ activation.

Functional implications

The prominent expression of $I_{\text{DR-fast}}$ in SNr GABA neurons meets the physiological requirements of the fast, high-frequency spiking properties in these neurons. First, SNr GABA neurons fire spontaneous, sustained action potentials around 25 Hz in rodents and 65 Hz in primates. To sustain this high frequency firing, these neurons must repolarize quickly to remove Na channel inactivation. So the repolarizing Kv ($I_{\text{DR-fast}}$) channels must activate quickly to repolarize the membrane to its resting potential and further to the more negative afterhyperpolarization. Second, these repolarizing Kv ($I_{\text{DR-fast}}$) channels also should not activate before the neuron reaches action potential threshold. Instead, they should activate at relatively positive membrane potentials, for example, $-40$ or $-30$ mV when the generation of action potential has reached the point-of-no-return. Otherwise, a subthreshold activation of repolarizing Kv ($I_{\text{DR-fast}}$) channels will prevent or interfere with the generation of spikes. Third, the repolarizing Kv ($I_{\text{DR-fast}}$) channels should also be resistant to inactivation such that they are available at the...
physiological membrane potential around –50 mV. Our data show that in the fast-spiking SNr GABA neurons, the $I_{\text{DR-fast}}$ current, likely mediated by Kv3.1-containing channels, meets all of these functional requirements. Thus, the $I_{\text{DR-fast}}$ channels in SNr neurons can effectively keep the action potential brief without impeding the action potential initiation, enabling and sustaining fast-spike firing.

In summary, our data indicate that a strong fast-activating, slow-inactivating Kv3-like $I_{\text{DR-fast}}$ mediated probably by Kv3.1- and Kv3.4-containing channels is essential to the sustained high frequency firing in the GABA projection neurons in the substantia pars reticulata, a key basal ganglia output nucleus. Consequently, these Kv3-like channels are likely to be critical to the maintenance of sustained tonic inhibition of the targets of the basal ganglia output neurons and hence movement control.
Acknowledgements

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References


**Figure legends**

**Fig. 1.** Electrophysiological identification of SNr GABA neurons and nigral DA neurons. **A:** Photomicrograph (obtained with a 4X objective) of a Nissl-stained coronal midbrain section from a 20-day old rat. The anatomical location of SNc and SNr is evident. **B:** Photomicrograph (obtained with also a 4X objective) of a live coronal midbrain section from a 18-day old rat. The anatomical location of SNc and SNr is clearly identifiable. **C:** Photomicrograph (obtained with a 60X objective and DIC optics) of a commonly seen SNr GABA neuron being patch clamped. **D:** Electrophysiological properties of SNr GABA neurons. Current-clamp recordings show spontaneous high frequency activity and minimal $I_h$-mediated sag response to hyperpolarizing current injection. **E:** Electrophysiological properties of SNr DA neurons. Current-clamp recordings show spontaneous low frequency pacemaker activity and prominent $I_h$-mediated sag response (arrow) to hyperpolarizing current injection. **F:** Overlay of a GABA neuron spike and a DA neuron spike, showing the clear differences in the spike waveform between the two cell types. VTA, ventral tegmental area. 3n, third cranial nerve (oculomotor nerve).

**Fig. 2.** scRT-PCR detection of common neuronal Kv channel mRNAs in SNr GABA neurons and nigral DA neurons. **A:** Kv mRNAs detected in electrophysiologically identified SNr GABA neurons. No TH mRNA was detected in any of these neurons. For each Kv mRNA, 5-8 GAD1-mRNA positive SNr GABA neurons were tested. Numbers in the parentheses are the expected amplicon sizes in base pair. **B:** Kv mRNAs detected in electrophysiologically identified nigral DA neurons. For each Kv mRNA, 4-6 TH mRNA-positive DA neurons were tested. The expected amplicon sizes are the same as in **A.** In both cell types, Kv1.2, Kv1.4, Kv1.5, Kv1.6, and Kv4.1 were not detected, indicating low abundance. **C:** summary of scRT-PCR detection ratio of Kv channel mRNAs in SNr GABA neurons and nigral DA neurons.
**Fig. 3.** qRT-PCR analysis of common neuronal Kv channel mRNAs in laser microdissection-captured, immunofluorescence-identified SNr GABA neurons and nigral DA neurons. β-actin mRNA was used as internal control. mRNA semi-quantification was performed using the comparative $C_p$ method in the form of $2^{-[\Delta C_p]}$. $2^{-[\Delta C_p]}$ values for Kv mRNAs were normalized to those in SNr GABA neurons. Kv3.1 and Kv3.4 mRNAs were higher in SNr GABA neurons than in nigral DA neurons, whereas Kv4.2 and Kv4.3 mRNAs were lower in SNr GABA neurons than in nigral DA neurons. No difference was detected in other mRNA levels between the two cell types. 3-5 samples for each mRNA. * indicates p<0.01.

**Fig. 4.** Formation of nucleated somatic membrane patch clamp. **A, B, C** are sequential images demonstrating the formation of nucleated somatic membrane patch. **A:** Conventional whole cell patch clamp to electrophysiologically fingerprint SNr GABA and nigral DA neurons. **B:** Gentle negative pressure was applied and the patch pipette was withdrawn slowly to pull nucleated patches. Arrow indicates the remaining part of the neuron. Arrowhead indicates the forming nucleated membrane patch with a membrane string still connected to the parent neuron. **C:** Spherical nucleated patches free of axon and dendritic processes was formed as demonstrated by including fluorescent 20 µM Alexa 594 in the pipette solution.

**Fig. 5.** Voltage-dependent activation and steady-state inactivation of composite peak Kv currents in SNr GABA neurons and nigral DA neurons. **A1:** Activation protocol to evoke Kv currents in nucleated membrane patches. Holding potential was $-100$ mV, each step increment was 10 mV. **A2, A3:** Representative traces of Kv currents evoked by the activation protocol in nucleated membrane patches isolated from a GABA neuron (A2) and a DA neuron (A3). **A4:** Activation curves of peak Kv currents in SNr GABA neurons (filled circles) (n = 18) and nigral DA neurons (open circle) (n = 12). Data points were fitted with Boltzmann function.
**B1:** Steady-state inactivation protocol. Holding potential –90 mV, 10 s prepulse starting at –110 mV with 10 mV increment. The testing pulse was 100 ms at +50 mV. **B2, B3:** Representative traces of K currents evoked by steady-state inactivation protocol in nucleated membrane patches isolated from an SNr GABA neuron (B2) and an nigral DA neuron (B3). **B4:** Steady-state inactivation curve for GABA neurons (filled circles) (n = 10) and DA neurons (open circles) (n = 8). Data points were fitted with Boltzmann function.

**Fig. 6.** Different subthreshold inactivation of composite peak Kv currents in SNr GABA neurons and nigral DA neurons. **A, B:** membrane potential was typically around –50 mV for significant durations before reaching spike threshold in GABA neurons (A) and DA neurons (B). Note the longer time scale for the DA neuron in B. **A** and **B** were conventional whole cell recordings. **C:** voltage command protocol to test subthreshold inactivation. Holding potential was –90 mV. Inactivation duration at –50 mV was variable, increasing at 50 ms/step. Testing voltage was at 20 mV for 150 ms. **D, E:** Examples traces of Kv currents evoked in nucleated membrane patches isolated from a GABA neuron (D) and DA neuron (E).

**Fig. 7.** Pharmacological dissection of different Kv current components in SNr GABA neurons and nigral DA neurons. Holding potential was –100 mV. Testing potential was +20 mV that is commonly experienced by both SNr GABA neurons and nigral DA neurons at the peak of action potentials. **A, A1:** Representative traces of Kv currents of SNr GABA neurons under control condition (trace 1), in the presence of 1 mM TEA (trace 2), and 20 mM TEA (trace 3). **A2:** 1 mM TEA-sensitive currents in GABA neurons obtained by subtraction. **A3:** Kv currents sensitive to 20 mM TEA (intermediate sensitivity, indicating $I_{DR-slow}$) in GABA neurons obtained by subtraction. **A4:** 20 mM TEA resistant Kv currents (indicating $I_A$) in GABA neurons. **B, B1:** Representative traces of Kv currents of nigral DA
neurons under control condition (trace 1), in the presence of 1 mM TEA (trace 2), and 20 mM TEA (trace 3). **B2:** 1 mM TEA-sensitive currents in DA neurons obtained by subtraction. **B3:** Kv currents sensitive to 20 mM TEA (intermediate sensitivity, indicating $I_{DR-slow}$) in DA neurons obtained by subtraction. **B4:** 20 mM TEA resistant Kv currents (indicating $I_A$) in DA neurons.

**Fig. 8.** Voltage-dependent activation and steady-state inactivation of the 1 mM TEA-sensitive $I_{DR-fast}$ current in SNr GABA neurons and nigral DA neurons. **A, A1-A4:** Representative traces of Kv currents in a nucleated membrane patch isolated from a GABA neuron, evoked by the activation protocol described in **Fig. 5A1**, under control condition (**A1**), in the presence of 1 mM TEA (**A2**) and after washing out 1 mM TEA (**A3**). The 1 mM TEA-sensitive $I_{DR-fast}$ was obtained by subtraction and displayed in **A4. B, B1-B4:** Representative traces of Kv currents in a nucleated membrane patch isolated from a DA neuron, evoked by the activation protocol described in **Fig. 5A1**, under control condition (**B1**), in the presence of 1 mM TEA (**B2**) and after washing out 1 mM TEA (**B3**). The 1 mM TEA-sensitive $I_{DR-fast}$ was obtained by subtraction and displayed in **B4.** Note the 1 mM TEA-sensitive $I_{DR-fast}$ was much bigger in SNr GABA neurons (**A4**) than in nigral DA neurons (**B4**). **C, C1:** Activation curves of the 1 mM TEA-sensitive $I_{DR-fast}$ in SNr GABA neurons (filled circles, n = 12) and in nigral DA neurons (n = 7, open circles). The parameters (midpoint voltage $V_{1/2}$ and slope factor $K$) of the two Boltzmann curves were not statistically different. **C2:** Steady-state inactivation curves of the 1 mM TEA-sensitive $I_{DR-fast}$ in SNr GABA neurons (filled circles, n = 5) and in nigral DA neurons (n = 5, open circles). The parameters (midpoint voltage $V_{1/2}$ and slope factor $K$) of the two Boltzmann curves were not statistically different.

**Fig. 9.** Deactivation kinetics of the 1 mM TEA-sensitive $I_{DR-fast}$ current in SNr GABA neurons. **A:** example traces of 1 mM TEA-sensitive $I_{DR-fast}$ obtained by subtraction. The nucleated membrane patch was held at −90 mV, then stepped to...
20 mV for 100 ms to activated $I_{DR\text{-fast}}$, then stepped to $-30$ mV, $-40$ mV, $-50$ mV, $-60$ mV, and $-70$ mV to deactivate $I_{DR\text{-fast}}$. The tail currents were expanded and displayed in the middle. The decay of the tail current at these membrane potentials was fitted to single exponential functions and the time constants were used as a measure of the 1 mM TEA-sensitive $I_{DR\text{-fast}}$ current in SNr GABA neurons. B: the decay time constants of the tail current or the deactivation time constant of $I_{DR\text{-fast}}$ were plotted against membrane potential, clearly indicating that the deactivation becomes faster upon hyperpolarization.

Fig. 10. Activation and inactivation of $I_{DR\text{-slow}}$ $K_V$ currents sensitive to 20 mM TEA. A, B: Representative traces of $K_V$ currents in nucleated membrane patch isolated from GABA neuron (A) and DA neuron (B), respectively, evoked by the activation protocol described in Fig. 5A1. The traces were obtained by subtraction method described in Fig. 7. C: Activation curves for GABA neurons (square, $n = 6$) and DA neurons (circle, $n = 7$). The parameters (midpoint voltage $V_{1/2}$ and slope factor $K$) of the two Boltzmann curves were not statistically different. D: Steady-state inactivation curves for GABA neurons (square, $n = 6$) and DA neurons (circle, $n = 7$). The parameters (midpoint voltage $V_{1/2}$ and slope factor $K$) of the two Boltzmann curves were not statistically different.

Fig. 11. Voltage-dependent activation and steady-state inactivation of $I_A$ resistant to 20 mM TEA in SNr GABA neurons and DA neurons. A, A1: Activation voltage protocol. Holding potential was $-100$ mV. Step increment was 10 mV. A2, A3: Representative $K_V$ current traces evoked by the activation protocol in nucleated membrane patches isolated from a GABA neuron (A2) and DA neuron (A3), in the presence of 20 mM TEA. A4: Activation curves for GABA neurons ($n = 6$) and DA neurons ($n = 7$). The parameters (midpoint voltage $V_{1/2}$ and slope factor $K$) of the two Boltzmann curves were not statistically different. B, B1: Steady-state inactivation voltage protocol. Holding potential was $-90$ mV. The conditioning
prepulse was 10 s. Step increment was 10 mV. Testing potential was at 20 mV for
100 ms. B2, B3: Representative traces of 20 mM TEA resistant currents evoked
by the steady-state inactivation protocol in nucleated membrane patches isolated
from a GABA neuron (B2) and DA neuron (B3). B4: Steady-state inactivation
curves for GABA neurons (n = 6) and DA neurons (n = 6). The parameters
(midpoint voltage $V_{1/2}$ and slope factor K) of the two Boltzmann curves were not
statistically different.

**Fig. 12.** Inhibition of Kv3 channel-mediated $I_{DR-fast}$ by 1 mM TEA impairs sustained
high frequency firing in SNr GABA neurons. A1-A8 were obtained in normal 2.5
mM Ca$^{2+}$-containing extracellular solution whereas B1-B8 show that our
conclusion is still valid in 0 mM Ca$^{2+}$ extracellular solution. A1: SNr GABA neurons
were able to fire sustained high frequency (around 40 Hz) fast spikes upon
injection of 300 pA current for 10 s. A2: in the presence of 1 mM TEA that blocks
$I_{DR-fast}$, the same 10 s 300 pA injection induced spike firing only for about 3 s or
less. Note the spike amplitude decreased progressively. A3: the SNr neuron
regained its sustained high frequency firing capability after washing out 1 mM
TEA. A5: action potential waveforms under control condition and during 1 mM
TEA. In the presence of 1 mM TEA, the spike duration was much prolonged and
the fAHP was blocked. A6: pooled instantaneous firing rate in 5 SNr GABA
neurons, binned over 1 s, during the 10 s period of 300 pA injection. During control
recording and after washing out TEA, these SNr GABA neurons maintained their
firing frequency during the 10 s period. Upon 1 mM TEA application, these
neurons were able to fire spikes of decreasing amplitude only within the first 3 s.
A7: pooled maximum spike rise rate from 5 SNr GABA neurons, binned over 1 s,
during the 10 s period of 300 pA injection. During control recording and after
washing out TEA, these SNr GABA neurons maintained their maximum rise rate
during the 10 s period. Upon 1 mM TEA application, the maximum rise rate
decreased quickly. A8: quantification of the averaged or mean firing frequency
upon different current injection under control condition and 1 mM TEA. The mean
firing frequency was calculated by counting all the spikes evoked by the 10 s current pulse and then divided by 10 s.

**B1**, an example SNr GABA neuron firing sustained high frequency (around 40 Hz) fast spikes upon injection of 300 pA current for 10 s. **B2**: removing extracellular Ca\(^{2+}\) increased spike firing. **B3**: after addition of 1 mM TEA, the neuron became unable to fire spikes in a sustainable manner. The firing stopped after about 3 s. Note the spike amplitude decreased progressively. **B4**: the neuron regained its fast spiking ability after washing out TEA. **B5**: action potential waveforms under control condition, 0 mM Ca\(^{2+}\), and during 1 mM TEA. 0 mM Ca\(^{2+}\), only modestly prolonged spike duration whereas 1 mM TEA drastically prolonged it. **B6**: pooled instantaneous firing rate from 5 SNr GABA neurons, binned over 1 s, during the 10 s period of 300 pA injection. During control recording and after washing out TEA, these SNr GABA neurons maintained their firing frequency during the 10 s period. Upon 1 mM TEA application, these neurons fired spikes of decreasing amplitude only within the first 3 s. **B7**: pooled maximum spike rise rate from 5 SNr GABA neurons, binned over 1 s, during the 10 s period of 300 pA injection. During control recording and after washing out TEA, these SNr GABA neurons maintained their maximum rise rate during the 10 s period. Upon 1 mM TEA application, the maximum rise rate decreased quickly. **B8**: quantification of the averaged firing frequency upon different current injection under control condition, 0 mM Ca\(^{2+}\), and 1 mM TEA and 0 mM Ca\(^{2+}\).

### Table legend

**Table 1.** Summary of parameters of component Kv currents in SNr GABA neurons and nigral DA neurons

The numbers in the table have been described in the text and are listed here for easy comparison.
A GABA neuron
A1 Raw traces
Control: 1
1 mM TEA: 2
20 mM TEA: 3

A2 1 mM TEA-sensitive current ($i_{DR-1mm}$)
1-2

A3 20 mM TEA-sensitive current ($i_{DR-20mm}$)
2-3

A4 20 mM TEA-resistant $i_A$
3 500 pA 30 ms

B DA neuron
B1 Raw traces
Control: 1
1 mM TEA: 2
20 mM TEA: 3

B2 1 mM TEA-sensitive current ($i_{DR-1mm}$)
1-2

B3 20 mM TEA-sensitive current ($i_{DR-20mm}$)
2-3

B4 20 mM TEA-resistant $i_A$
3 750 pA 30 ms
A $I_{DR-fast}$ in GABA neuron
A1 composite $K_v$  A2 1 mM TEA  A3 Wash  A4 1 mM TEA-sensitive $I_{DR-fast}$ (A1–A2)

B $I_{DR-fast}$ in DA neuron
B1 composite $K_v$  B2 1 mM TEA  B3 Wash  B4 1 mM TEA-sensitive $I_{DR-fast}$ (B1–B2)

C Pooled data on the isolated $I_{DR-fast}$
C1 Activation
\[
\frac{G}{G_{max}} = \frac{1}{1 + \frac{V_{cmd}}{V_{1/2}}} 
\]
Command potential (mV)

C2 Steady-state inactivation
\[
\frac{I}{I_{max}} = \frac{1}{1 + \left( \frac{V_{pre}}{V_{1/2}} \right)^{n}} 
\]
Pre-pulse (mV)
A  Decay of 1 mM TEA-sensitive tail current

B  Voltage-dependence of the tail current decay
A $I_A$ current activation

A1 Voltage waveform

50 mV

-100 mV

A2 GABA neuron

Current (nA)

Time (ms)

A3 DA neuron

Current (nA)

Time (ms)

A4 Pooled data

G/G$_{max}$

Command potential (mV)

B $I_A$ current steady-state inactivation

B1 Voltage waveform

0 mV

20 mV

10 s prepulse

B2 GABA neuron

Current (nA)

Time (s)

prepulse

B3 DA neuron

Current (nA)

Time (s)

prepulse

B4 Pooled data

$I/I_{max}$

Prepulse (mV)
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<th></th>
<th>GABA neuron</th>
<th>DA neuron</th>
<th>p</th>
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<tr>
<td><strong>Composite peak Kv conductance density</strong></td>
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<tr>
<td>At +40 mV</td>
<td>5.5±0.7 nS/pF</td>
<td>5.1±0.8 nS/pF</td>
<td>&gt;0.05</td>
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<td><strong>Fast delayed rectifier (I_{f,fast})</strong></td>
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<td>Contribution to peak at +20 mV</td>
<td>58.8±5.3 %</td>
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<td>Midpoint voltage</td>
<td>-8.5±1.6 mV</td>
<td>-6.2±1.2 mV</td>
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<tr>
<td>Slope factor K</td>
<td>8.9±0.6</td>
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<tr>
<td>20-80 % rise time at +40 mV</td>
<td>0.41±0.03 ms</td>
<td>0.47±0.06 ms</td>
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<tr>
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<td>11.2±1.1</td>
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<td><strong>Slow delayed rectifier (I_{f,slow})</strong></td>
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