Differences in Na\(^+\) Conductance Density and Na\(^+\) Channel Functional Properties Between Dopamine and GABA Neurons of the Rat Substantia Nigra

Vincent Seutin and Dominique Engel

GIGA Neurosciences, Electrophysiology Unit, University of Liege, Sart Tilman, Liege, Belgium

Submitted 12 June 2009; accepted in final form 26 March 2010

Seutin V, Engel D. Differences in Na\(^+\) conductance density and Na\(^+\) channel functional properties between dopamine and GABA neurons of the rat substantia nigra. J Neurophysiol 103: 3099–3114, 2010. First published March 31, 2010; doi:10.1152/jn.00513.2009. Dopamine (DA) neurons and GABA neurons of the substantia nigra (SN) promote distinct functions in the control of movement and have different firing properties and action potential (AP) waveforms. APs recorded from DA and GABA neurons differed in amplitude, maximal rate of rise, and duration. In addition, the threshold potential for APs was higher in DA neurons than in GABA neurons. The activation of voltage-gated Na\(^+\) channels accounts largely for these differences as the application of a low concentration of the voltage-gated Na\(^+\) channel blocker TTX had an effect on all of these parameters. We have examined functional properties of somatic Na\(^+\) channels in nucleated patches isolated from DA and GABA neurons. Peak amplitudes of macroscopic Na\(^+\) currents were smaller in DA neurons in comparison to those in GABA neurons. The mean peak Na\(^+\) conductance density was 24.5 pS \(\mu\)m\(^2\) in DA neurons and almost twice as large, 41.6 pS \(\mu\)m\(^2\), in GABA neurons. The voltage dependence of Na\(^+\) channel activation was not different between the two types of SN neurons. Na\(^+\) channels in DA and GABA neurons, however, differed in the voltage dependence of inactivation, the mean mid-point potential of steady-state inactivation curve being more positive in DA neurons than in GABA neurons. The results suggest that specific Na\(^+\) channel gating properties and Na\(^+\) conductance densities in the somatic membrane of SN neurons may have consequences on synaptic signal integration in the soma of both types of neurons and on somatodendritic release of dopamine by DA neurons.

INTRODUCTION

The substantia nigra (SN) pars reticulata is composed of a majority of projecting GABA neurons and participates in the control of voluntary movement by being one of the output regions of the basal ganglia (Hikosaka et al. 2000). The SN pars compacta contains a majority of dopamine (DA) neurons and is implicated in the modulation of motor control and in the processing of rewarding stimuli (Schultz et al. 1997). GABA neurons are also interneurons modulating the activity of DA neurons via axon collaterals (Tepper and Lee 2007). Dysfunctions of SN neurons have been linked to several disorders related to movement perturbations, in particular to the symptoms of Parkinson’s disease (Riederer and Wuketich 1976). DA neurons and GABA neurons, which subserve distinct functions, are spontaneously active and differ markedly in their electrophysiological properties (Hausser et al. 1995; Richards et al. 1997; Yung et al. 1991). The spontaneous firing frequencies are lower in DA neurons than in GABA neurons, and the action potential (AP) is longer in duration and slower in maximal rate of rise in DA neurons in comparison to GABA neurons. AP amplitudes are slightly but not significantly different between DA and GABA neurons (Richards et al. 1997; Yung et al. 1991).

The tonic AP firing in GABA neurons is important because the absence of firing is responsible for the disinhibition of postsynaptic neurons, an important mechanism for the basal ganglia to control movement (Hikosaka et al. 2000). DA neurons—with their lower firing frequencies and longer AP—exert modulatory effects on their postsynaptic targets by the release of dopamine from presynaptic axonal boutons and locally in the SN by the somatodendritic release of dopamine.

The underlying causes of these different active properties in DA and GABA neurons are not fully understood. Different ensembles of voltage-gated and calcium-dependent ion channels may contribute to the above-mentioned differences and may be distributed nonhomogenously in the different compartments of the neuron. Moreover, passive membrane properties and cellular architecture may also influence the AP shape (Grace and Bunney 1983). A particular feature of SN neurons is the origin of the axon located along a dendrite (Hausser et al. 1995). The position of the emergence of the axon is variable along the somatodendritic compartment of SN neurons and determines whether APs invade first the soma or the dendrites (Hausser et al. 1995). Long distances between the axon initial segment and the soma may isolate electrophysiologically the soma from the site of AP generation (Grace and Bunney 1983) or modify AP waveform. Electrophysiological hallmarks of DA neurons are the presence of a hyperpolarization-activated cation current \(I_h\) (Mercuri et al. 1995; Neuhoff et al. 2002; Silva et al. 1990) and small-conductance calcium activated K\(^+\) currents (Aumann et al. 2008; Silva et al. 1990). A fast inactivating A-type conductance and a slow inactivating delayed rectifier conductance are also expressed in DA neurons (Liss et al. 1999; Segev and Korngreen 2007; Silva et al. 1990). The A-type conductance has been shown to regulate AP height and width and the delayed rectifier AP repolarization in DA neurons (Segev and Korngreen 2007). Whether these conductances are present in GABA neurons and how they may influence AP waveform remains largely unknown. Mean AP maximal rate of rise has been shown to be faster in GABA neurons in comparison to DA neurons (Yung et al. 1991). The rising phase of the AP in SN neurons may be primarily supported by the activation of voltage-gated Na\(^+\) conduc-
tances. The presence and the functional characteristics of voltage-gated Na⁺ channels in SN neurons have not been explored yet and their contribution to AP shape remains to be addressed. Na⁺ channel characteristics might differ between DA and GABA neurons and from those in other neurons of the nervous system.

Spontaneous and evoked APs were recorded in substantia nigra DA and GABA neurons to describe their time course. APs recorded from SN neuron somata differed in amplitude, half-duration, maximal rate of rise, and in the voltage threshold for APs. In complementary experiments, somatic Na⁺ currents were recorded from DA and GABA neurons using nucleated patches to compare the parameters of these currents between the two types of neurons. DA and GABA neurons differed in Na⁺ current rise time, maximal amplitude, Na⁺ conductance density and steady-state inactivation. To support these results, AP waveform-evoked Na⁺ currents during a train of APs had different amplitudes in DA neurons and in GABA neurons.

METHODS

Experimental procedures described in this article followed the rules of the Institutional Animal Care and Use Committee (IACUC) of the University of Liege under supervision of the Belgian Ministry of Health (division animal welfare), the national legal rules concerning animal experimentation (Décrets royaux of the 23.12.1998 and 13.09.2004), and the EU guidelines (N.86/609/CEE).

Slice preparation and cell identification

Twelve- to 19-day-old Wistar rats were killed by rapid decapitation. The brain was quickly removed and submerged in oxygenated, ice-cold physiological saline solution. Coronal 250 μm thick slices were cut using a vibratome (DTK-1000, Dosaka, Kyoto, Japan) in the same saline solution. Slices containing the substantia nigra were caudal to the medial mammillary nucleus (German and Manaye 1993). Brain slices were incubated at 35°C for 30–60 min prior to experiments and then stored at room temperature.

In the substantia nigra, neurons were visualized using infrared differential interference contrast (IR-DIC) videomicroscopy (Stuart et al. 1993) with a CCD camera (C7500-51, Hamamatsu, Hamamatsu City, Japan) and an infrared filter (RG 9, Schott, Mainz, Germany). DA and GABA neurons were identified based on their electrophysiological signature, namely the presence of a hyperpolarization-activated inward current (Ih) and the AP firing pattern (Richards et al. 1997; Yung et al. 1991). Immediately after rupturing the membrane, the recording mode was switched to the current-clamp mode (I-clamp fast position on the Axopatch 200 B), and a hyperpolarizing current pulse of 200 pA and of 1 s duration was applied to all cells to determine the presence of a sag in the membrane potential (Radnikow and Misgeld 1998). Depolarizing current pulses were also applied to visualize the AP pattern (Fig. 1, E and F). In voltage clamp, a test pulse to evoke an Ih current was applied consisting of a hyperpolarizing pulse of 60 mV amplitude and of 1.5 s duration from a holding potential of −60 mV. An Ih current ratio was calculated for each neuron by measuring the amplitude of the current at the end of the capacitive transient over the amplitude of the current at the end of the voltage pulse.

In recordings with K⁺-based solution (KMeSO₄) in the pipette, the Ih ratio for DA neurons was 0.24 ± 0.02 (n = 22; mean ± SE), and for GABA neurons, the Ih ratio was 0.71 ± 0.03 (n = 25; P < 0.001; Wilcoxon rank sum test). In recordings with Cs⁺-based pipette solution, the Ih ratio for DA neurons was 0.35 ± 0.02 (n = 22), and for GABA neurons, the Ih ratio was 0.82 ± 0.02 (n = 38; P < 0.001). Neurons recorded with KCl in the pipette were classified using the same Ih current ratio criteria (data not shown).

Patch pipettes were pulled from borosilicate glass tubing (2.0 mm OD, 0.5 mm wall thickness; Hilgenberg, Malsfeld, Germany) with a P-87 or a P-97 puller (Sutter Instruments, Novato, CA), and heat polished before use. The resistance of patch pipettes was 2–5 MΩ when filled with internal solution. The seal resistance was >1 GΩ.

AP recordings from substantia nigra neurons

APs were recorded from DA and GABA neurons using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA) in the I-clamp fast mode, filtered at 10 kHz using the internal 4-pole low-pass Bessel filter and sampled at 50–100 kHz using a Digidata 1320A interface (Molecular Devices). Stimulus generation and data acquisition were performed with pClamp version 8.2 or 10.2 (Molecular Devices). As the majority of DA and GABA neurons were spontaneously active, resting membrane potential could not be determined. Series resistance was 6.2–15.3 MΩ; it was compensated (>80% with lag ~10 μs) with the compensation circuit of the amplifier and carefully monitored during experiments. Holding potential was set to −60 mV for AP evoked with current injection by applying constant negative or positive current (< ±200 pA). No correction for liquid junction potentials was made.

Na⁺ current recordings in nucleated patches

Nucleated patches were extracted from the soma by applying negative pressure to the pipette (130–200 mbar) and by retracting the pipette out of the slice (Sather et al. 1992). Care was taken to keep the nucleated patch far from the slice and close to the surface of the solution to ensure complete isolation of the patch. Following the extraction of the nucleated patch, the pressure was reduced to 30–40 mbar. Nucleated patches had an almost spherical shape, and their diameter was measured from video printout images using a video printer (UP897MD, Sony, Shinagawa, Japan). Nucleated patches from DA neurons had a diameter of 9.8 ± 0.3 μm (n = 15), and those from GABA neurons had a slightly but not significantly smaller diameter (8.7 ± 0.4 μm; n = 17; P > 0.05). The corresponding mean surface area was 304 ± 16 μm² for DA neurons (n = 15) and 247 ± 21 μm² for GABA neurons (n = 17).

Currents were recorded with an Axopatch 200B amplifier, filtered at 10 kHz, and sampled at 50 kHz (100 kHz for deactivation).

Nucleated patches were held at −80 mV for the characterization of Na⁺ currents with rectangular pulses. A 50 ms potential pulse to −120 mV was systematically applied before each test pulse to obtain complete recovery from fast inactivation. Na⁺ currents were isolated pharmacologically by blocking K⁺ channels with internal Cs⁺ and external TEA and 4-aminopyridine (4-AP) and by blocking Ca²⁺ channels with Cd²⁺. Leakage and capacitive currents were subtracted on-line using the pipette capacitance compensation circuit of the amplifier and with a P−4 protocol. TTX (1 μM) was applied at the end of the experiments. Alternatively, Na⁺ currents were isolated off-line by subtracting the current recorded without TTX from currents in the presence of TTX. In some recordings, a residual outward current resistant to external TEA, 4-AP, and Cd²⁺ and internal Cs⁺ was observed. Experiments showing Na⁺ current traces with residual outward current for which TTX subtraction has not been obtained were discarded from the analysis. Some traces in the figures were filtered digitally (Gaussian characteristics, 3 or 5 kHz). Test pulses were applied every 4–5 s. Patches were not used for further analysis.
when during the experiments the amplitude of the Na⁺ current was not stable within ±20% of the initial value. In the recordings obtained from one DA neuron and two GABA neurons, no voltage-activated current was observed. For experiments in Fig. 7, A–D, depolarizing stimuli were scaled trains of action potentials previously recorded from DA and GABA neurons in current clamp without current injection. For comparative purposes, the same train of action potentials recorded from a DA neuron (from a GABA neuron) was used for all DA neurons (for all GABA neurons, respectively). Leakage and capacitive currents were subtracted on-line using the pipette capacitance compensation circuit of the amplifier and with a P/4 protocol. Current traces in Fig. 7, A and B, are averages of 25 and 43 single

FIG. 1. Action potential (AP) characteristics in dopamine (DA) and GABA neurons. Substantia nigra (SN) neurons fired spontaneous action potentials on whole cell breakthrough. A and C: patterns of action potential firing in a DA neuron (A) and in a GABA neuron (C). Traces were recorded without current injection and were filtered off-line at 1 kHz. B and D: traces represent APs at a higher time resolution from A and C, respectively. Traces were filtered on-line at 10 kHz. E and F: voltages traces evoked by a series of 1,000 ms current pulses (from −200 pA to −40 pA, increment 40 pA; and a current pulse to +40 pA) in a typical DA neuron (E) and in a GABA neuron (F). Potentials were filtered off-line at 1 kHz. Insets: traces represent currents evoked by 2 potential pulses (~60 and −10 mV) of 1,500 ms duration. G: APs recorded from a DA neuron (trace in gray) and from a GABA neuron (black trace). APs were evoked in both cases with a 40 pA current injection step of 1,000 ms in duration. Gray and black dots represent AP threshold in a DA neuron and in a GABA neuron (respectively) determined at the point where dV/dt = 20 V s⁻¹.
sweeps, respectively. No prepulse was applied before the AP train waveform. No correction for liquid junction potentials was made. Recordings were carried out at room temperature (24.0 ± 0.3°C). The data included in this study were obtained from 75 nucleated patches (29 DA patches and 46 GABA patches).

**Solutions and chemicals**

During experiments, slices were continuously superfused with physiological saline solution of the following composition: (in mM) 125 NaCl, 25 NaHCO₃, 25 glucose, 2.5 KCl, 1.25 NaH₂PO₄, 2 CaCl₂, and 1 MgCl₂ (pH 7.4 with 95% O₂-5% CO₂ gas mixture, 310 mosmol kg⁻¹). The same solution was used for dissection and storage of slices. For action potential recordings (in Figs. 1, 2, A–E, and 7), pipettes were filled with a K⁺ rich solution containing (in mM) 125 KMeSO₄, 12 KCl, 4 MgCl₂, 4 K₂ATP, 0.5 Na₂GTP, 5 Na₂-phosphocreatine, 0.1 EGTA, and 10 HEPEs (pH adjusted to 7.3 with KOH, 310 mosmol kg⁻¹). Spontaneous synaptic activity was blocked with 10 μM 6-cyano-7-nitroquinoline-2,3-dione (CNQX), 10 μM bicuculline methiodide, and 20 μM N-2-amino-5-phosphonopentanoic acid (AP-5).

For Na⁺ current recordings (Figs. 2F and G, Fig. 3–6), pipettes were filled with a Cs⁺ rich solution, containing (in mM) 145 CsCl, 2 MgCl₂, 2 Na₂ATP, 0.5 Na₂GTP, 5 Na₂-phosphocreatine, 0.1 EGTA, and 10 HEPEs (pH adjusted to 7.3 with CsOH, 320 mosmol kg⁻¹). The discrimination of DA from GABA neurons based on the sag in the membrane potential in current-clamp or based on the I₉ ratio in voltage clamp was still possible with Cs⁺ solution-filled electrodes (Merceri et al. 1995; Rudikin and Misgeld 1998). For Na⁺ currents in Fig. 7, E and F, pipettes were filled with a solution containing (in mM) 155 KCl, 2 MgCl₂, 2 Na₂ATP, 0.5 Na₂GTP, 5 Na₂-phosphocreatine, 0.1 EGTA, and 10 HEPEs (pH adjusted to 7.3 with CsOH, 320 mosmol kg⁻¹). TEA (20 mM), 3 mM 4-AP, and 200 μM CaCl₂ were added to the bath solution to block voltage-gated K⁺ channels and Ca²⁺ channels. Chemicals were either from Sigma or from Tocris. KMeSO₄ was from ICN Biomedicals.

**Data analysis**

For APs recorded during spontaneous activity, the AP amplitude was measured from a short segment of potential immediately preceding spike initiation using Mathematica 6.0.3 (Wolfram Research, Champaign, IL). For APs recorded with a short or a long current pulse, AP amplitude was measured from the holding potential (−60 mV) preceding the current pulse. AP threshold was determined from the first AP generated after the onset of a 1-s-long somatic current injection (40–160 pA for DA neurons, 0–40 pA for GABA neurons). Voltage threshold for APs was defined as the membrane potential at which the rate of rise corresponds to 20 V s⁻¹.

In nucleated patch recordings, measurements of current amplitudes were performed with Stimfit 0.8.14 (Christoph Schmidt-Hieber, Physiology Department, University of Freiburg). The maximal Na⁺ conductance (gNa “bar”) was determined as Iₚeak/(V – ENa) × Popen × A, where Iₚeak is the peak current at 0 mV, ENa is the Na⁺ current reversal potential, Popen is the maximal open probability (Popen = 0.5; (Engel and Jonas 2005; Fenwick et al. 1982), and A is the calculated patch area. As Na⁺ conductance achieved at 0 mV was ~70% of maximal, gNa “bar” was corrected for 100% maximal conductance. Curve fitting was performed with Stimfit or Mathematica 6. The time course of Na⁺ channel deactivation was fitted with either a single exponential or the sum of two exponential functions. Na⁺ channel deactivation was generally biexponential at potentials ≥ −50 mV, and the fast decay time constant was taken as the deactivation time constant (Oxford 1981). The activation time constant was fitted with an exponential function with delayed onset I(t) = I₀[1 − \exp{−(t − τₐ)/τₐ}] for t ≥ 0 and t < 0, where I₀ is the amplitude, τₐ the activation time constant, and δ the delay (Engel and Jonas 2005; Martina and Jonas 1997). Activation and inactivation data of each patch were fitted with a Boltzmann function of the form f(V) = [1 + exp (Vmid – V/k)]⁻¹, where Vmid is the midpoint potential and k is the slope factor. Values of Vmid and k given in the text are means of results from individual experiments. The mean peak Na⁺ current-voltage (I-V) relation was fitted with a Boltzmann function of the form f(V) = a(V – ENa)[1 + exp (Vmid – V/k)]⁻¹, where a corresponds to the peak conductance. The dose-response relation for the effects of different concentrations of TTX on Na⁺ current was fitted with a sigmoidal function of the form: y = 100/[1 + 10^((IC₅₀ - x)*n)] where IC₅₀ is the concentration of TTX for blocking 50% of the Na⁺ current amplitude and n is the slope factor.

All numerical values given in the text and the symbols and error bars in the figures indicate means ± SE. Differences in AP parameters and in Na⁺ channel parameters between DA neurons and GABA neurons were tested for statistical significance using a nonparametric Wilcoxon rank sum test (or a Wilcoxon signed-rank test for paired data) at the significance level (P < 0.05). Data were analyzed with Genumeric and GNU R (Windows).

**RESULTS**

Recordings were obtained from neurons localized in the pars compacta and pars reticulata of the SN under visual guidance. Putative DA neurons were differentiated from putative GABA neurons by the presence of a hyperpolarization-activated current (I₉ current) corresponding to the presence of a “sag” in the membrane potential, by checking their firing pattern and by their AP characteristics (see Methods and Fig. 1).

**Characteristics of action potentials in SN neurons**

Current-clamp recordings were made from the soma of SN DA and GABA neuron in the presence of the synaptic blockers CNQX, AP5, and bicuculline (10, 20, and 10 μM, respectively) to determine differences in APs waveform between the two types of neurons. On breakthrough into whole cell configuration, spontaneous APs were observed in almost all SN neurons, without the requirement for current injection (Fig. 1, A and C). The mean frequency of APs in GABA neurons was significantly higher (6.6 ± 1.2 Hz, n = 12) than in DA neurons (0.4 ± 0.1 Hz, n = 7, P < 0.001, Wilcoxon rank sum test). In GABA neurons, the mean amplitude of spontaneous APs was 76.0 ± 6.4 mV, the mean maximal rate of rise dV/drmax 186.5 ± 17.2 V s⁻¹, and the half-duration 1.2 ± 0.1 ms (n = 12). In comparison, DA neurons had a significantly smaller mean AP amplitude (64.1 ± 3.2 mV, P < 0.05) and dV/drmax (55.6 ± 13.6 V s⁻¹, P < 0.01) and a longer AP half-duration (3.2 ± 0.4 ms, n = 7, P < 0.01; Fig. 1, B and D). As the potential history preceding APs may influence their waveform, APs were evoked by current injection from a holding potential of −60 mV. Application of short current pulses (0.5 ms; 2.4 – 3.6 nA for DA neurons, 1.6–2.4 nA for GABA neurons) elicited APs with distinct characteristics (Fig. 2, A and B). Evoked APs in DA neurons had significantly smaller amplitudes (86.7 ± 2.8 mV; n = 8) than evoked APs in GABA neurons (107.7 ± 1.3 mV; n = 10, P < 0.001, Wilcoxon rank sum test, Fig. 2C). A marked difference was also observed in half-amplitude duration of APs. The duration at half-maximal amplitude of APs was significantly longer in DA neurons (1.42 ± 0.08 ms; n = 8) in comparison to AP duration in GABA neurons (0.98 ± 0.07 ms; n = 10, P < 0.01; Fig. 2D). Moreover, the mean maximal rate of rise (dV/drmax) of APs was approximately...
FIG. 2. Effects of TTX on action potentials and Na⁺ currents in DA and GABA neurons. A and B, top: traces of APs \( V(t) \) evoked by a 500 \( \mu \)s current pulse of 2.6 nA in a DA neuron (A) and of 1.8 nA in a GABA neuron (B). A and B, bottom: differentiated voltage signals \( dV/dt(t) \) of the corresponding APs. B, top: trace in black represents an AP recorded from a GABA neuron in control conditions (in the presence of synaptic blockers), trace in red represents an AP recorded in a GABA neuron in the presence of 20 nM TTX and trace in green represents an AP recorded after the washout of TTX. B, bottom: traces represents the corresponding differentiated signal in control (black), in the presence of 20 nM TTX (red) and after TTX washout (green). Traces in A and in Fig. 1E, and inset of E, are from the same DA neuron. Traces from B and Fig. 1F, and inset of F, are from the same GABA neuron. C–E: summary bar graphs comparing AP amplitudes, AP durations at half-maximal amplitude and AP maximal rates of rise (\( dV/dt_{\max} \)) in 8 DA neurons (DN, \( \bullet \)), in 10 GABA neurons (GN, \( \circ \)), in GABA neurons in the presence of 20 nM TTX (GN + TTX, \( \circ \), \( n = 10 \)) and in GABA neurons after the washout of TTX (GN wash, \( \circ \), \( n = 7 \)). AP parameters are reported in Table 1. F: Na⁺ currents recorded in nucleated patches isolated from GABA neurons. The pulse protocol is in inset and consists of a 50 ms prepulse to −120 mV from a holding potential of \( V_h = −80 \) mV followed by 30 ms voltage pulse to 0 mV and a step back to −80 mV. The Na⁺ current trace in control represent averages from 34 single sweeps. The Na⁺ current traces in 2, 20, and 200 nM TTX are averages from 30, 17, and 5 single sweeps, respectively. G: effects of 3 different concentrations of TTX on Na⁺ current amplitude. Dots represent the effect of TTX in percentage of control (without TTX), plotted against the concentration of TTX in nM using a logarithmic scale. Data points were fitted with a sigmoidal function (black curve) giving an IC₅₀ of 7.2 nM and a slope of 0.8. The control black dot (0 nM TTX) set to 100% is from 17 nucleated patches and the dots for 2, 20, and 200 nM TTX are averages from 8, 9, and 7 patches, respectively.
three times slower in DA neurons (103.7 ± 7.2 V s⁻¹; n = 8) in comparison with GABA neurons (320.5 ± 28.3 V s⁻¹; n = 10; P < 0.001; Fig. 2, A, B, bottom, and E). The difference in the duration at half-maximal amplitude of APs in SN neurons may be explained by the interplay between voltage-gated Na⁺ channels and different voltage-gated K⁺ channels. However, amplitude and maximal rate of rise may be more specifically associated to the availability of voltage-gated Na⁺ channels.

A higher maximal rate of rise in GABA neurons may correspond to a higher proportion of available Na⁺ channels in GABA neurons in comparison with DA neurons. To test this hypothesis, a low concentration of the specific Na⁺ channel blocker TTX was applied in the bath solution while recording APs in GABA neurons. Addition of 20 nM TTX decreased slightly but significantly the amplitude of APs in GABA neurons (in control: 107.7 ± 1.3 mV; in TTX: 95.0 ± 1.9 mV; n = 10; P < 0.01, Wilcoxon signed-rank test; Fig. 2C). Similarly, the application of TTX decreased significantly the mean dV/dtmax in GABA neurons (in control: 320.5 ± 28.3 V s⁻¹; in TTX: 177.1 ± 14.7 V s⁻¹; n = 10; P < 0.01; Fig. 2E).

The duration at half-maximal amplitude of APs was slightly but significantly augmented by TTX (in control: 0.98 ± 0.07 ms; in TTX: 1.15 ± 0.10 ms; n = 10; P < 0.01; Fig. 2D). The effects of TTX were largely reversible in 7 of 10 cases. Washout of TTX induced an increase in mean AP amplitude (in TTX: 95.0 ± 1.9 mV; after TTX washout: 103.5 ± 2.4 mV; n = 7; P < 0.05; Wilcoxon signed-rank test; Fig. 2C), an increase in mean dV/dtmax (in TTX: 177.1 ± 14.7 V s⁻¹; after TTX washout: 262.8 ± 27.3 V s⁻¹; n = 7; P < 0.05; Fig. 2E) and a decrease in mean duration at half-amplitude (in TTX: 1.15 ± 0.10 ms; after TTX washout: 1.02 ± 0.12 ms, n = 7, P < 0.05; Fig. 2D; Table 1). These results suggest that a higher proportion of available Na⁺ channels contribute to the higher amplitude and maximal rate of rise of APs in GABA neurons in comparison to DA neurons. In addition to these differences, the voltage threshold for AP differed between the two groups of SN neurons when APs were evoked with 1-s-long current injection steps. In DA neurons, AP threshold was significantly higher (−35.3 ± 2.4 mV, n = 11) in comparison to GABA neurons (−48.7 ± 1.6 mV, n = 8, P < 0.01, Wilcoxon rank sum test, Fig. 1G). Application of 20 nM TTX slightly but significantly increased AP threshold in GABA neurons (−43.5 ± 2.2 mV in TTX vs. −48.7 ± 1.6 mV in control, n = 8, P < 0.05, Wilcoxon signed-rank test, data not shown). This result indicates that the density of Na⁺ channels influence the setting of the voltage of AP threshold in SN neurons.

**Effects of the Na⁺ channel blocker TTX on Na⁺ currents in GABA neurons**

Different concentrations of TTX (2, 20, and 200) were tested on Na⁺ currents in nucleated patches isolated from GABA neurons to measure the sensitivity of Na⁺ channels to TTX. With 2 nM TTX, Na⁺ currents were reduced to 74 ± 2% of control (n = 8), to 29 ± 2% of control (n = 9) in 20 nM TTX, and to 11 ± 2% of control (n = 7) in 200 nM TTX (Fig. 2, F and G). The effects of different TTX concentrations on Na⁺ currents were plotted with a logarithmic scale and fitted with a sigmoidal function giving an IC₅₀ value of 7.2 nM and a slope factor of 0.8 (Fig. 2G). When assuming a mean Na⁺ current amplitude of 210.9 pA (0 mV) in GABA neurons and of 155 pA in DA neurons, the mean amplitude of Na⁺ current in DA neurons represents 73.5% of the mean current in GABA neurons, corresponding to a reduction of the Na⁺ current in GABA neurons by a TTX concentration of 1.9 nM.

For comparison, in GABA neurons, 20 nM TTX reduced the mean AP amplitude only to 90% of control, the dV/dtmax to 55% of control and increased the half-duration by 17%, whereas a large percentage of Na⁺ current (71%) was blocked in nucleated patches. These results indicate that the somatic Na⁺ current is proportionally more affected than the AP parameters by a concentration of TTX of 20 nM.

**Voltage activated Na⁺ currents and steady-state activation in SN neurons**

To examine whether somatic Na⁺ channels differ between DA neurons and GABA neurons, we recorded Na⁺ currents in the two classes of neurons using nucleated patches. In the presence of external TEA, 4-AP, Cd²⁺, and internal Cs⁺, Na⁺ currents in SN neurons were transient, with a fast exponential activation phase followed by a fast inactivation phase (Fig. 3, A and B). Currents were completely blocked by 1 μM TTX (Fig. 3C). Inactivation of Na⁺ channels in DA and GABA neurons was almost complete at the end of a 30 ms test pulse to 0 mV as the steady-state current measured at the end of the test pulse was ~1% of the peak current. Peak Na⁺ current amplitudes at 0 mV were significantly smaller in DA neurons (−155.0 ± 11.7 pA; n = 15) than in GABA neurons (−210.9 ± 17.8 pA; n = 17; P < 0.05; Fig. 3D, top). The mean peak Na⁺ conductance density, estimated using the calculated membrane area of the patch was 24.5 ± 1.7 pS μm⁻² in DA neurons (n = 15) and almost twice as large in GABA neurons, 41.6 ± 4.6 pS μm⁻² (n = 17; P < 0.001; Fig. 3D, bottom). The peak

**TABLE 1. Comparison showing differences in evoked action potential parameters in DA neurons and GABA neurons of the rat substantia nigra**

<table>
<thead>
<tr>
<th></th>
<th>DA Neurons</th>
<th>GABA Neurons</th>
<th>GABA Neurons in TTX</th>
<th>GABA Neurons After TTX Washout</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplitude, mV</strong></td>
<td>86.7 ± 2.8 (n = 8)</td>
<td>107.7 ± 1.3 (n = 10; P &lt; 0.001)</td>
<td>95.0 ± 1.9 (n = 10; P &lt; 0.01)</td>
<td>103.5 ± 2.4 (n = 7; P &lt; 0.05)</td>
</tr>
<tr>
<td><strong>Duration at half-maximal amplitude, ms</strong></td>
<td>1.42 ± 0.08 (n = 8)</td>
<td>0.98 ± 0.07 (n = 10; P &lt; 0.01)</td>
<td>1.15 ± 0.10 (n = 10; P &lt; 0.01)</td>
<td>1.02 ± 0.10 (n = 7; P &lt; 0.05)</td>
</tr>
<tr>
<td><strong>dV/dtmax, V s⁻¹</strong></td>
<td>103.7 ± 7.2 (n = 8)</td>
<td>320.5 ± 28.3 (n = 10; P &lt; 0.001)</td>
<td>177.1 ± 14.7 (n = 10; P &lt; 0.01)</td>
<td>262.8 ± 27.3 (n = 7; P &lt; 0.05)</td>
</tr>
</tbody>
</table>

Numerical values are given as means ± SE and total number of recordings in parentheses. Statistical significance was tested between dopamine (DA) and GABA neurons for action potential (AP) amplitude, duration at half amplitude, and maximal rate of rise with a Wilcoxon rank sum test; between GABA neurons in control and GABA neurons in presence of TTX for the same parameters and between GABA neurons in the presence of TTX and after the washout of TTX with a Wilcoxon signed-rank test.
Na\(^+\) current-voltage (I-V) relation was studied for both classes of neurons using test potentials ranging between ~90 and +80 mV (preceded by a 50 ms pulse to ~120 mV) and was fitted adequately in both cases by the product of a Boltzmann function and a linear relation to the data points (Fig. 3E). Na\(^+\) currents were detected at potentials at least ~50 mV, were maximal in amplitude at ~0 mV, decreased with increasing test potentials (>0 mV) and reversed at +60.5 ± 2.8 mV in DA neurons (n = 8) and +64.6 ± 2.7 mV in GABA neurons (n = 11; P > 0.05). Measured reversal potential values were close to the theoretically determined reversal potential of +59 mV for Na\(^+\) currents. To represent Na\(^+\) channel activation curves, the Na\(^+\) permeability (P_{Na}) at a given test potential was calculated from the respective peak current amplitude using the Goldman-Hodgkin-Katz equation (Hille 2001). Permeability was normalized to the maximal value and plotted against test potential (~90 to +30 mV). Activation curves for DA neurons and GABA neurons were fitted with Boltzmann functions and gave comparable values for both mid-point potentials (~9.6 ± 1.9 and ~12.6 ± 1.4 mV, respectively; n = 8 and 11, respectively; P > 0.1) and slope factors (12.0 ± 0.7 and 11.0 ± 0.5 mV; P > 0.2; Fig. 3F, Table 2). These results show that voltage dependence of activation did not differ between the two classes of neurons.

**Steady-state inactivation of Na\(^+\) channels**

Inactivation curves give an indication about the channel availability for a given potential. Inactivation curves were constructed by measuring Na\(^+\) current amplitudes obtained with a 30 ms pulse potential to 0 mV preceded by prepulses...
TABLE 2. Comparison between the functional properties of Na⁺ channels in DA neurons and GABA neurons of the rat substantia nigra

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DA Neurons</th>
<th>GABA Neurons</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Na⁺ conductance density, pS µm²</td>
<td>24.5 ± 1.7 (15)</td>
<td>41.6 ± 4.6 (17)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Vₘₐₓ of activation, mV</td>
<td>−9.6 ± 1.9 (8)</td>
<td>−12.6 ± 1.4 (7)</td>
<td>—</td>
</tr>
<tr>
<td>k of activation, mV</td>
<td>12.0 ± 0.7</td>
<td>11.0 ± 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Vₘₐₓ of inactivation, mV</td>
<td>−48.9 ± 1.4 (11)</td>
<td>−56.1 ± 1.8 (15)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>k of inactivation, mV</td>
<td>−10.5 ± 0.7</td>
<td>−9.9 ± 0.6</td>
<td>—</td>
</tr>
<tr>
<td>Activation time constant τₐ, µs</td>
<td>474 ± 84 s at −30 mV (9)</td>
<td>246 ± 48 s at −30 mV (10)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Deactivation time constant τₚ, µs</td>
<td>161 ± 20 s at −40 mV (5)</td>
<td>160 ± 11 s at −40 mV (9)</td>
<td>—</td>
</tr>
<tr>
<td>Inactivation time constant τᵦ, ms</td>
<td>1.8 ± 0.2 s at −20 mV (6)</td>
<td>1.3 ± 0.2 s at −20 mV (6)</td>
<td>—</td>
</tr>
<tr>
<td>τ of onset of inactivation at −55 mV, ms</td>
<td>45.0 ± 8.0 (60 ± 2%)</td>
<td>25.8 ± 4.0 (74 ± 6%)</td>
<td>—</td>
</tr>
<tr>
<td>τ of recovery from inactivation at −120 mV, ms</td>
<td>1.4 ± 0.1 (77 ± 3%)</td>
<td>1.2 ± 0.2 (73 ± 2%)</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>84.8 ± 29.3 (9)</td>
<td>107.6 ± 21.9 (7)</td>
<td>—</td>
</tr>
</tbody>
</table>

Numerical values are given as means ± SE, and total number of nucleated patches in parentheses. The values given in parentheses for the time constants of recovery from inactivation indicate the amplitude contribution of the fast recovery (expressed as percentage). The values given in parentheses for the onset of inactivation at −55 mV indicate the amplitude proportion of the remaining Na⁺ current amplitude at the end of the 190 ms pulse. Statistical significance is given by P value and — represent (P > 0.05).

ranging between −120 and −10 mV. Data points were calculated by normalizing peak Na⁺ current amplitudes obtained for different prepulses with the peak Na⁺ current amplitude obtained with a prepulse of −120 mV. Na⁺ current amplitude declined with a prepulse potential more positive than −80 mV, and no current could be elicited from prepulse potentials more positive than −10 mV (Fig. 4, A and B). In both types of neurons, Boltzmann functions fitted well the data points (Fig. 4C). A pronounced difference was observed in the voltage dependence of steady-state inactivation between DA and GABA neurons. In DA patches, the mid-point potential was significantly more depolarized (−48.9 ± 1.4 mV; n = 11) than in GABA neurons (−56.1 ± 1.8 mV; n = 15; P < 0.01, Fig. 4D, Table 2). The slope factor was similar for the two types of neurons (−10.5 ± 0.7 mV for DA neurons and −9.9 ± 0.6 mV for GABA neurons, P > 0.1).

FIG. 4. Inactivation of Na⁺ channels in SN neurons. A and B: voltage-dependent steady-state inactivation of Na⁺ currents in DA neurons (A) and GABA neurons (B). Protocol: holding potential, Vₜᵦ = −80 mV, 50 ms prepulses to potentials between −120 to −10 mV (increment 10 mV) followed by a 30 ms potential pulse to 0 mV, step back to Vₜᵦ = −80 mV (protocol is in inset). Currents were filtered off-line at 3 kHz. C: Na⁺ channel inactivation curves in SN neurons. Na⁺ peak current amplitudes were normalized to the Na⁺ current obtained with the first prepulse (−120 mV). (●) are mean values from 11 DA neurons; (○) mean values from 15 GABA neurons. Data points were fitted with Boltzmann functions (black curves). Parameters are reported in Table 2. D: histogram shows the difference in mid-point potential for steady-state inactivation between DA neurons and GABA neurons. Bar charts represent mean values from 11 DA neurons and 15 GABA neurons. (●, ○) are single values from DA neurons and GABA neurons, respectively, and error bars are SE values.
Activation and deactivation time courses of Na\(^+\) channels

Activation kinetics of Na\(^+\) channels were investigated with test pulses ranging between −30 and +40 mV. Activation time constants were measured by fitting the rise of Na\(^+\) currents at different test pulses to a monoeXponential function with delayed onset (see METHODS; Fig. 5, A and B). In both DA and GABA neurons, mean time constants of activation decreased with increasing potential pulses (Fig. 5D). Mean activation time constant at −30 mV was approximately two times larger in DA neurons (474 ± 84 μs; n = 9) in comparison to GABA neurons (246 ± 48 μs; n = 10; P < 0.05; Table 2). At −20 mV, the apparent difference was not significant (332 ± 85 vs. 279 ± 49 μs, respectively, P > 0.1). At −10 mV, the mean activation time constant of Na\(^+\) currents was also not different in the two types of SN neurons (DA neurons: 270 ± 33 μs; GABA neurons: 183 ± 23 μs, P > 0.05).

Deactivation time constants of Na\(^+\) channels were obtained with a 300 μs potential pulse to 0 mV followed by steps to potentials between −110 and −30 mV. The Na\(^+\) tail current was fitted with an exponential function (Fig. 5C). Mean deactivation time constants increased with increasing potential pulses and the values were not different in the two types of neurons over the range of potentials tested (−110 to −30 mV, Fig. 5D). Deactivation time constant at −40 mV was 161 ± 20 μs (n = 5) in DA neurons and 160 ± 11 μs in GABA neurons (n = 9; P > 0.1; Table 2).

Time course of onset of inactivation of SN Na\(^+\) channels

We applied voltage pulses between −30 and +30 mV to investigate the time course of the onset of inactivation. The time course was well fitted with a monoeXponential function in all the patches and over the whole range of potential pulses investigated. In both DA and GABA neurons, the time constants decreased with increasing potential pulse (Fig. 5E), but the values were not different between the two classes of neurons. At −20 mV, the inactivation time constant was 1.8 ± 0.2 ms in DA neurons (n = 6) and 1.3 ± 0.2 ms in GABA neurons (n = 7, P > 0.1; Fig. 5F; Table 2).

We also investigated the time course of onset of inactivation at −55 mV using prepulses of increasing duration (Fig. 6, A and B). In the majority of DA neurons, the onset of inactivation was described by a monoeXponential function (6 of 9 cells). Mean time constants were τ = 45.0 ± 8.0 ms (range: 22.3−79.4 ms) in DA neurons. These values were close to the inactivation time constant value obtained for CA1 neuron dendrites (37 ms) (Pan and Colbert 2001). In three DA neurons, the time course of onset of inactivation was better described with two exponential functions. These three patches were not included in the analysis. In all GABA neurons (7 of 7 cells), the time course of onset of inactivation was described with a single exponential function giving a mean time constant of τ = 25.8 ± 4.0 ms (range: 11.9−39.7 ms). Mean time course of the onset of inactivation at −55 mV was slightly longer in DA neurons in comparison to GABA neurons, but mean values were not significantly different (P > 0.05). At the end of the 190 ms voltage pulse, 50 ± 2% of Na\(^+\) channels remained available in DA neurons (n = 6) and 43 ± 6% in GABA neurons (n = 7; P > 0.1; Table 2). The results indicate that almost the same proportion of Na\(^+\) channels remained avail-

able at subthreshold potentials at the end of a 190 ms prepulse in the two types of SN neurons.

Recovery from inactivation

The time course of recovery of Na\(^+\) channels from inactivation was studied with a double-pulse protocol, consisting of two pulses of 30 ms to 0 mV separated by a pulse to −120 mV for different segments of time ranging between 1 and 290 ms (Fig. 6, C and D). The time course of recovery was biexponential in both groups of neurons. In DA neurons, time constants were τ\(_1\) = 1.4 ± 0.1 and τ\(_2\) = 84.8 ± 29.3 ms (n = 9). Time constants were not significantly different in GABA neurons (τ\(_1\) = 1.2 ± 0.2 ms and τ\(_2\) = 107.6 ± 21.9 ms, n = 7, P > 0.1 for rapid and slow time constants). These values were similar to those observed in CA1 pyramidal neurons (Martina and Jonas 1997). The amplitude contribution of the fast time course was 77 ± 3% in DA neurons (n = 9) and 73 ± 2% in GABA neurons (n = 7; P > 0.1; Table 2).

Perisomatic Na\(^+\) currents evoked during a train of action potentials

To determine the Na\(^+\) channel availability in DA and GABA neurons during their own activity, Na\(^+\) currents were evoked with a voltage command consisting of a train of APs in nucleated patches (Fig. 7, A and B). These trains of APs were recorded previously in DA and GABA neurons and applied to neurons of respectively the same identity (Bean 2007). Na\(^+\) currents evoked after >2 s of activity by the APs with an asterisk are represented in Fig. 7, C and D (bottom traces). In both types of neurons, the amplitude of Na\(^+\) currents was relatively small. Na\(^+\) currents started to activate near the voltage at which dV/dt = 20 V s\(^{-1}\). They reached maximal amplitude during the depolarizing phase of the AP. Mean Na\(^+\) current amplitude in DA neurons (8.0 ± 1.0 pA, n = 7) was significantly smaller than the mean Na\(^+\) current amplitude in GABA neurons (14.6 ± 2.0 pA, n = 8, P < 0.01, Wilcoxon rank sum test). For control, Na\(^+\) currents were also evoked with a standard rectangular stimulus (Fig. 7, E and F) in the same nucleated patches. Note the larger Na\(^+\) current amplitude obtained with a rectangular voltage command in both types of SN neurons despite the fact that the protocols are not directly comparable. These data reveal that during typical physiological activity, somatic Na\(^+\) currents underlying APs are active and are larger in GABA neurons in comparison to those in DA neurons. In addition, these results show that somatic Na\(^+\) currents underlying an AP during normal activity are relatively small in both types of SN neurons.

Discussion

We described AP properties in DA and GABA neurons of the rat SN. In DA neurons, spontaneous APs and APs evoked with current injection were longer in duration and had a slower maximal rate of rise (dV/dt\(_{\text{max}}\)) in comparison to APs in GABA neurons, confirming data from previous studies (Richards et al. 1997; Yung et al. 1991). APs were also significantly smaller in amplitude in DA neurons than in GABA neurons, and the voltage threshold for APs was more depolarized in DA neurons than in GABA neurons.
Using the nucleated patch configuration, we have investigated the functional properties of somatic voltage-gated Na\(^+\) channels in SN neurons in almost ideal voltage-clamp conditions. This study is the first describing detailed Na\(^+\) channel gating properties in DA neurons and in GABA neurons of the SN. Our results show that somatic Na\(^+\) channels differed between DA and GABA neurons in peak Na\(^+\) current amplitude, in peak Na\(^+\) conductance density, activation kinetics and voltage dependence of steady-state inactivation. In comparison to other central neurons, Na\(^+\) channels in DA and GABA neurons showed voltage-dependent activation curves shifted toward more positive potentials (Martina and Jonas 1997).
FIG. 6. Inactivation onset at −55 mV and recovery from inactivation of Na\(^+\) channels. A: time course of the onset of inactivation at a potential of −55 mV in a DA neuron (top current traces) and in GABA neuron (bottom current traces). Pulse protocol: \(V_h = −80\) mV, 50 ms pulse to −120 mV, prepulse to −55 mV of variable duration, and a 30 ms test pulse to 0 mV (protocol is in inset). The first trace is without prepulse. Traces were filtered off-line at 3 kHz. B: Na\(^+\) peak current amplitude, normalized to the current evoked without prepulse, and plotted against prepulse duration in 7 DA neurons (○) and 7 GABA neurons (○). Black curves represent monoexponential fits to data points. Constants for the onset of inactivation at −55 mV are reported in Table 2. C: time course of recovery from inactivation in a GABA neuron. Pulse protocol: \(V_h = −80\) mV, 50 ms pulse to −120 mV, 30 ms pulse to 0 mV (test pulse) and step back to \(V_h\). Current traces were filtered off-line at 3 kHz and truncated to the 1st 20 ms. D: ratio of peak Na\(^+\) current amplitude calculated by dividing currents obtained at different test pulse by currents obtained with the conditioning pulse and plotted against the duration of the interpulse interval. Black curves represent biexponential fits to DA neuron data points (○, 9 patches) and to GABA neurons (○, 7 patches). Parameters are reported in Table 2.

AP parameters in SN DA and GABA neurons

APs in SN DA neurons in comparison to APs in GABA neurons were longer in their half-maximal amplitude duration as shown previously (Richards et al. 1997; Yung et al. 1991). The differences in the duration at half-maximal amplitude may be due to the contribution of distinct ensembles of K\(^+\) channels or to different K\(^+\) conductance densities between the two types of SN neurons. DA neurons have been shown to express a rapidly inactivating A-type K\(^+\) current, which is very small or absent in GABA neurons (Liss et al. 1999). This current has been shown to influence the height and the width of APs in DA neurons (Segev and Korngreen 2007). Spontaneous and electrically evoked APs in DA neurons had also an approximately three to four times slower AP d\(V/\text{d}t_{\text{max}}\) in comparison to APs

FIG. 5. Activation, deactivation, and inactivation kinetics of Na\(^+\) channels in DA neurons and GABA neurons. A and B: time courses of activation onset in a DA neuron (A) and in a GABA neuron (B). Current rising phases were fitted with an exponential function with delayed onset (red). The protocol is depicted in inset and consists of 3 30-ms potential pulses to −30, −10, and 10 mV preceded by a 50 ms prepulse to −120 mV, \(V_h = −80\) mV. Traces were filtered on-line at 10 kHz. C: time courses of deactivation in a DA neuron. Voltage protocol: \(V_h = −80\) mV, 50 ms pulse to −120 mV, 300 μs prepulse to 0 mV, 50 ms pulse to −110, −40, and −30 mV and back to \(V_h\). The decay of the tail currents was fitted with a monoexponential function (red curves) for −110 and −40 mV and with a biexponential function (green curve) for −30 mV. D: plot of activation \(\tau_a\) for 9 DA neurons (○) and 10 GABA neurons (○), and deactivation \(\tau_d\) for 5 DA neurons (○) and 9 GABA neurons (○) against voltage. The fastest deactivation time constant was used for analysis when the tail current was fitted with a biexponential function (Oxford 1981). E: time courses of inactivation onset in a GABA neuron. Voltage protocol: \(V_h = −80\) mV, 50 ms to −120 mV, 30 ms test pulses to −30, 0, and 30 mV, back to \(V_h\). The decay phase of the current was fitted with a monoexponential function (red traces). Traces were filtered at 3 kHz. F: plot of the onset of inactivation \(\tau_a\) for 6 DA neurons (○) and 6 GABA neurons (○).
in GABA neurons, confirming a comparable difference in AP $dV/dt_{\text{max}}$ in SN neurons reported previously (Yung et al. 1991). We observed however a higher AP amplitude in GABA neurons when comparing to DA neurons, a difference that was not significant in previous reports (Richards et al. 1997; Yung et al. 1991). These divergent observations might be explained by the use of synaptic blockers in our study. In GABA neurons, the application of a low concentration (20 nM) of TTX decreased AP amplitude and $dV/dt_{\text{max}}$ to values slightly above those observed for APs in DA neurons, suggesting a higher Na$^+$ channel availability in GABA neurons in comparison to DA neurons. Moreover, the AP threshold was higher in DA neurons than in GABA neurons. Despite a tendency of the voltage threshold for APs to be different in SN neurons reported previously (Nedergaard and Greenfield 1992; Richards et al. 1997), a large significant difference in voltage threshold for APs has not been reported so far between SN DA and GABA neurons. Mean voltage threshold value measured in DA neurons ($-35.3 \text{ mV}$) is comparable to values of previous reports (more than $-40 \text{ mV}$) (Liss et al. 1999; Nedergaard 1999; Nedergaard and Greenfield 1992), and mean threshold value measured in GABA neurons ($-48.7 \text{ mV}$) is also similar to

![Figure 7](http://jn.physiology.org/)

**FIG. 7.** Action potential evoked Na$^+$ currents during a train in SN neurons. A and B, top: voltage traces (red) recorded from a DA neuron (A) and a GABA neuron (B) of $-4$ and $-2$ s duration, respectively. These voltage traces were used as a voltage command in voltage-clamp to evoke Na$^+$ currents in nucleated patches. A and B, bottom: Na$^+$ current traces (black) are averages of 25 and 43 single sweeps, respectively, filtered off-line at 3 kHz. C and D, top: traces represent APs at a higher time resolution from A and B, respectively, and correspond to the APs with an asterisk. Values and black dots at the top of APs represent AP maximal potential value. Values and dots at the AP initiation phase represent the value at which the potential corresponds to $dV/dt = 20$. C and D, bottom: corresponding Na$^+$ currents (black). Note the presence of a small outward current (D) not observed with a rectangular voltage command (F). E: averaged Na$^+$ current trace obtained with a rectangular voltage step in the same nucleated patch (DA neuron) as in A. F: averaged Na$^+$ current trace obtained from the same patch (GABA neuron) as in B. The pulse protocol is in inset and consists of a 50 ms prepulse to $-120 \text{ mV}$ from a holding potential of $V_h = -80 \text{ mV}$ followed by a 30 ms voltage pulse to 0 mV and a step back to $-80 \text{ mV}$. Currents are averages of 5 and 14 single sweeps, respectively, and are filtered off-line at 5 kHz.
values from an other report (−43.3 mV) (Atherton and Bevan 2005). The reason for the difference in AP threshold remains unclear. It might partly be due to the fact that the initiation site of APs can be far from the soma in SN neurons, particularly in DA neurons (Hauser et al. 1995). The distance between the soma and the AP initiation site might contribute substantially to the setting of the value of AP threshold. Alternatively, the density and/or the functional properties of Na⁺ channels might differ between DA and GABA neurons at the site of AP initiation. The measure of AP threshold obtained from somatic recordings needs however to be used with precautions as it has been recently reported that an accurate measure of this value is only obtained from the site of AP initiation (Hu et al. 2009; Kole and Stuart 2008).

**Difference in peak somatic Na⁺ conductance density between DA and GABA neurons**

Nucleated patch recordings from DA and GABA neurons revealed several differences in somatic Na⁺ channel properties. Mean peak Na⁺ conductance density, calculated from peak Na⁺ current amplitude at 0 mV, was approximately two times smaller in DA neurons (24.5 pS µm⁻²) in comparison to GABA neurons (41.6 pS µm⁻²), indicating a higher somatic density of Na⁺ channels in GABA neurons than in DA neurons. These values were, however, smaller than those reported for neocortical pyramidal cells (80 pS µm⁻²; after correction for maximum open probability) (Stuart and Sakmann 1994) or hippocampal oriens-alveus interneurons (210 pS µm⁻²) (Martin et al. 2000). Furthermore, in the neocortex, Na⁺ conductance density values were not different between pyramidal and non pyramidal neurons (126 vs. 120 pS µm⁻²; after correction for maximum open probability; (Huguenard et al. 1988). Thus our report of a cell-specific difference in the Na⁺ conductance density in a specific brain region at a defined maturational stage is to our knowledge the first. When assuming a single-channel conductance of 12 pS (Engel and Jonas 2005; Hille 2001), the somatic density of Na⁺ channels in DA neurons is 2 and in GABA neurons 3 channels µm⁻². These values are comparable to the Na⁺ channel density calculated for bovine chromaffin cells (2.3 channels µm⁻²) (Fenwick et al. 1982). Whether the difference in somatic Na⁺ conductance density accounts for the difference in AP shape within SN neurons is not clear. Somatic Na⁺ channels may contribute to some extent to AP shape recorded in the soma; however, as reported very recently, the Na⁺ channels located at the axon initial segment play a more significant role in shaping the AP, at least in cortical neurons (Hu et al. 2009; Kole et al. 2008). Alternatively or additionally, AP shape might be modified while APs travel between the axon initial segment and the soma—in the case of the emergence of the axon from a dendrite—when long distances separate both compartments.

Applications of low doses of TTX on somatic Na⁺ current and AP recordings in GABA neurons showed that Na⁺ currents were more sensitive to TTX block than APs. A concentration of 20 nM TTX blocked 71% of the Na⁺ current and only 10% of the AP amplitude and 45% of the AP maximal rate of rise. These results are comparable to observations made in CA1 pyramidal neurons with whole cell recordings (Madeja et al. 2000). The percentage of Na⁺ current blocked by Madeja with 20 nM TTX (~70%) is very similar to the percentage of block obtained in nucleated patches. Values for the IC₅₀ (7.2 nM) and the slope factor (0.8) are similar to those by Madeja as well (6.4 nM and 0.9, respectively). However, the populations of Na⁺ channels activated during the Na⁺ currents in both studies are most probably different, as isolated neurons may retain a portion of the axon (see also Safronov et al. 1997).

**Functional properties of Na⁺ channels in SN neurons**

The mean midpoint potential of Na⁺ channel activation curve in GABA neurons (−12.6 mV) was slightly but not significantly more hyperpolarized in comparison to the mean midpoint potential in DA neurons (−9.6 mV). These values were however notably more depolarized in comparison to those observed in hippocampal neurons in identical recording conditions (−23.9 and −25.1 mV for basket cells and pyramidal cells, respectively (Martina and Jonas 1997). The reasons for the shift of activation curves in SN DA and GABA neurons toward more positive potentials are not clear. An explanation might be the direct influence of protein kinase C (PKC). In *Xenopus* oocytes, PKC has been shown to shift the voltage dependence of activation curve of Na⁺ channels toward more positive potentials to reduce the steepness of the slope of the activation curve and to reduce maximal conductance (Dascal and Lotan 1991). PKC is present in DA and GABA neurons of the SN (Tanaka and Nishizuka 1994) and might exert an influence on Na⁺ channels in these neurons. A marked difference between DA and GABA neurons is the voltage-dependent steady-state inactivation of Na⁺ channels. The mid-point potential of inactivation was significantly more depolarized in DA neurons (−48.9 mV) in comparison to GABA neurons (−56.1 mV). While the mid-point potential for GABA neurons is comparable to values observed in cortical neurons (−58.3 mV in hippocampal basket cells), the mid-point potential for DA neurons was −14 mV more depolarized in comparison to a hippocampal pyramidal neuron (−62.9 mV) (Martina and Jonas 1997). This indicates that the fraction of available Na⁺ channels for activation is smaller in GABA than in DA neurons at a given membrane potential. However, a more hyperpolarized membrane potential in GABA neurons, less than −60 mV (Atherton and Bevan 2005), may readjust the difference in Na⁺ channel availability between the two cell types.

A subthreshold, TTX-sensitive Na⁺ current has recently been described to participate in spontaneous firing of DA neurons (Puopolo et al. 2007). A noninactivating Na⁺ current component has, however, been noticed to be small in this study as the amplitude of the persistent component at the end of a 30 ms pulse to 0 mV was −1% of the peak current in DA and GABA neurons. This result is in accordance with previous reports obtained with nucleated patches isolated from hippocampal and spinal cord neurons (Martina and Jonas 1997; Safronov et al. 1997). The persistent Na⁺ current observed in dissociated mouse DA neurons (Puopolo et al. 2007) might be explained by its location in the axon initial segment (Astman et al. 2006). A resurgent Na⁺ current was also absent from nucleated patch recordings in this study.

AP waveforms during a train used as a voltage command revealed larger somatic Na⁺ currents in GABA neurons in comparison to those in DA neurons, implying a higher somatic Na⁺ channel availability in GABA neurons than in DA neurons during physiological conditions. Na⁺ currents evoked
with AP waveforms were relatively small in both types of SN neurons. Although the amplitude of Na⁺ currents evoked by a rectangular voltage step and Na⁺ current evoked by an AP waveform cannot be compared directly, the availability of Na⁺ channels is reduced when the current is evoked by an AP waveform in DA and GABA neurons. This is explained by the fact that the availability of Na⁺ channels is strongly reduced at the potential range preceding the spike and concomitantly a fraction of Na⁺ channels are not recovered from fast and slow inactivation.

**Regulation of Na⁺ channel activity in SN neurons**

The molecular basis underlying the differences in Na⁺ channel gating observed between the two types of SN neurons is not very clear. Na⁺ channels are composed of principal α-subunits (α1, α2, α3, α6) and regulatory β-subunits (β1 to 4), the latter probably affecting differentially the gating and the amount of expression of α-subunits (Isom et al. 1992). The activity of these subunits is also dependent on their phosphorylation/dephosphorylation state. Furthermore, dopamine, which has been shown to decrease Na⁺ current amplitude through phosphorylation of the α-subunit, may influence Na⁺ currents in DA and GABA neurons differently (Cantrrell and Catterall 2001; Cantrrell et al. 1997). In situ hybridization indicates moderate expression of mRNA coding for α1 and α2-subunits in the SN and a weak expression of α3-subunit mRNA (Furuyama et al. 1993). Recent immunohistochemical studies report high level of expression of Na⁺.1.1 in the SN (Gong et al. 1999; Jarnot and Corbett 2006), predominantly found in the soma. Somatic Na⁺ channels expressed in the SN are then likely to include Na⁺.1.1, possibly with Na⁺.1.2 and Na⁺.1.3.

Coexpression of α-subunit of Na⁺ channels with the β1 subunit in oocytes increases the peak amplitude of Na⁺ currents, shifts the voltage dependence of inactivation to more negative potentials, accelerates the kinetics of activation without altering the voltage dependence of activation and regulates the amount of expression of α-subunits (Isom et al. 1992; Patton et al. 1994). The effects due to the interaction between α and β1 might be compared with those observed for Na⁺ currents between DA and GABA neurons. The presence of β1 in GABA neurons might be a substrate to explain the difference in peak Na⁺ current and Na⁺ channel gating observed between DA and GABA neurons. However, whether the regulatory subunit β1 is specifically present in SN GABA neurons remains to be determined.

**Probable implications of different Na⁺ channel properties and Na⁺ conductance densities for SN functions**

The results from this study reveal that the somatic membrane of DA and GABA neurons is endowed with Na⁺ channels differing in conductance density, activation kinetics and steady-state inactivation. APs recorded in DA and GABA neurons differ markedly in their amplitude and maximal rate of rise (dV/dtmax). The effects of the Na⁺ channel blocker TTX on APs in GABA neurons indicate that Na⁺ channels are partly responsible for the differences in AP shape observed between DA and GABA neurons. Na⁺ channels from the axon initial segment—which are generally known to be implicated in AP generation—account probably to a larger extent for APs than those from the somatic compartment. The characteristics of Na⁺ channels in DA and GABA neurons may have several functional implications for the two types of SN neurons.

The activation of DA neurons during unexpected reward stimuli induces the release of dopamine from axonal presynaptic boutons but also from the somatodendritic domain (Beckstead et al. 2004). As dopamine release is TTX sensitive (Santiago et al. 1992), Na⁺ channel gating and density can influence the excitability of the somatodendritic domain and may impact on the amount of calcium current flowing through voltage-gated Ca⁺⁺ channels. Consequently, the amount of dopamine released from somatodendritic vesicular stores is closely dependent on the active properties of the soma and dendrites. Conversely, dopamine has been shown to decrease voltage-gated Na⁺ currents (Cantrrell et al. 1997) and affect AP backpropagation (Gentet and Williams 2007). Moreover, when dopamine transmission was potentiated by decreasing dopamine re-uptake by drugs of abuse such as cocaine, AP amplitude and Na⁺ current density were reduced by the presence of the drug and the Na⁺ channel activation curve was shifted to more positive potentials in the nucleus accumbens (Zhang et al. 1998). These observations underline the tight interaction between Na⁺ channel activity and extracellular dopamine concentrations in the functions of the SN neuronal network.

The propagation of APs along the somatodendritic axis of SN neurons has been shown to be robust and with little voltage amplitude attenuation (Hauser et al. 1995; Vetter et al. 2001). However, when APs are evoked by excitatory postsynaptic potentials, some of them may propagate with attenuations in their amplitude along the somatodendritic axis (Gentet and Williams 2007). Modalities of AP propagation may be complicated by different functional properties or densities of Na⁺ channels in the different subcellular compartments of the same neurons. A further major factor influencing AP propagation is the location of the emergence of the axon where the axon initial segment might be located. As the Na⁺ channel density is considered to be higher in axon than in soma and dendrites, the position of the axon emergence may determine the degree of AP attenuation along the somatodendritic axis and the compartment where the AP is initiated (Gentet et al. 2007). An AP generated at the axon initial segment located on the dendritic trunk (axon-bearing dendrite) at several tens of micrometers from the soma may need the recruitment of somatic Na⁺ channels to invade dendrites located at the opposite side of the soma (axon-lacking dendrites) and link the entire dendritic compartment electrically. Na⁺ channels present in the soma of SN neurons may consequently boost AP to propagate reliably along the whole somatodendritic axis.

In both DA and GABA neurons, somatic Na⁺ channels may also affect the synaptic integration in the soma (Scott et al. 2010; Stuart and Sakmann 1995) depending on their activation state and consequently influence the downstream AP output.

Finally, the modalities of AP propagation from its site of generation to the somatodendritic compartment and to axonal presynaptic boutons remain to be elucidated for both neuronal types of the SN. In addition, an estimation of the Na⁺ conductance density at the site of AP generation in both types of SN neurons would help to understand how Na⁺ channel activity shapes the initial phase of APs in these neurons. Solving these
questions will shed light on the link between AP coding and the control of movement and the processing of reward.

ACKNOWLEDGMENTS
We thank Drs. C.-C. Lien, C. Schmidt-Hieber, J. Bischöfberger, A. Draguhn, M. Kole, and D. Debanne for critically reading earlier versions of the manuscript. We are also grateful to Dr. B. Bean for sharing unpublished results.

GRANTS
This work was supported by a Belgium Fonds de la Recherche Scientifique-Fonds National de la Recherche Scientifique research associateship and by the F.R.S.-F.N.R.S. Grant 9.4560.03 to V. Seutin.

DISCLOSURES
No conflicts of interest are declared by the authors.

REFERENCES


Madeja M. Do neurons have a reserve of sodium channels for the generation of action potentials? A study on acutely isolated CA1 neurons from the guinea-pig hippocampus. Eur J Neurosci 12: 1–7, 2000.


