Characterization of Ca$^{2+}$ Channels in Rat Subthalamic Nucleus Neurons

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Song, Wen-Jie, Yosuke Baba, Takeshi Otsuka, and Fujio Murakami. Characterization of Ca$^{2+}$ channels in rat subthalamic nucleus neurons. J Neurophysiol 84: 2630–2637, 2000. The subthalamic nucleus (STN) plays a key role in motor control. Although previous studies have suggested that Ca$^{2+}$ conductances may be involved in regulating the activity of STN neurons, Ca$^{2+}$ channels in this region have not yet been characterized. We have therefore investigated the subtypes and functional characteristics of Ca$^{2+}$ conductances in STN neurons, in both acutely isolated and slice preparations. Acutely isolated STN cells were identified by retrograde filling with the fluorescent dye, Fluoro-Gold. In acutely isolated STN neurons, Cd$^{2+}$-sensitive, depolarization-activated Ba$^{2+}$ currents were observed in all cells studied. The current-voltage relationship and current kinetics were characteristic of high-voltage-activated Ca$^{2+}$ channels. The steady-state voltage-dependent activation curves and inactivation curves could both be fitted with a single Boltzmann function. Currents evoked with a prolonged pulse, however, inactivated with multiple time constants, suggesting either the presence of more than one Ca$^{2+}$ channel subtype or multiple inactivation processes with a single channel type in STN neurons. Experiments using organic Ca$^{2+}$ channel blockers revealed that on average, 21% of the current was nifedipine sensitive, 52% was sensitive to α-conotoxin GVIA, 16% was blocked by a high concentration of α-agatoxin IVA (200 nM), and the remainder of the current (9%) was resistant to the co-application of all blockers. These currents had similar voltage dependencies, but the nifedipine-sensitive current and the resistant current activated at slightly lower voltages.

The subthalamic nucleus (STN), the only excitatory nucleus in the basal ganglia, directly excites both output structures of the basal ganglia: the substantia nigra reticulata and the internal globus pallidus (Kita et al. 1983; Kitai and Kita 1987, 1991; Van der Kooy and Hattori 1980; see Kitai and Kita 1987 for review). Since the discovery of the association of hemiballism with pathologic changes in the STN (Whittier 1947), the STN has been recognized as playing a vital role in voluntary movement control (see Mink and Thach 1993; Wichmann and DeLong 1996 for reviews). This notion is strongly supported by findings from animal experiments in which blockade of the activity of STN neurons induces severe motor disorders (Hamada and Hasegawa 1996; Wichmann et al. 1994).

Given the importance of the STN in motor control, it is of general interest to understand how the activity of STN neurons is regulated. The electrical activity of a neuron is driven by its synaptic inputs and shaped by the intrinsic properties of the cytoplasmic membrane. It has been shown that STN neurons receive excitatory inputs from the cerebral cortex (Bevan et al. 1995; Fujimoto and Kita 1993; Hartmann-von Monakow et al. 1978; Kitai and Deniau 1981; Nambu et al. 1996) and the thalamus (Bevan et al. 1995; Feger et al. 1994; Mouroux and Feger 1993), and inhibitory inputs from the globus pallidus (Groenewegen and Berendse 1990; Moriiizumi and Hattori 1992). Several studies have examined the membrane properties of STN neurons. In a pioneering study, Nakaniishi et al. (1987) studied the response of STN neurons to current injections in an acutely prepared slice preparation. Several other studies thereafter also examined the response properties of STN neurons in acutely prepared slices (Beurrier et al. 1999; Bevan and Wilson 1999; Overton and Greenfield 1995; Song et al. 1998) and in cultured slices (Plenz and Kitai 1999). In all these works, the importance of Ca$^{2+}$ conductances in the regulation of STN neuron activity was invariably noticed. Ca$^{2+}$ conductances were suggested to be involved in the generation of a plateau-like potential (Beurrier et al. 1999; Nakaniishi et al. 1987; Song et al. 1998), in the generation of rebound activities of STN neurons (Beurrier et al. 1999; Overton and Greenfield 1995; Plenz et al. 1997; Song et al. 1998), and in the regulation of Ca$^{2+}$-dependent conductances (Beurrier et al. 1999; Bevan and...
Wilson 1999; Nakanishi et al. 1987; Song et al. 1998). Nevertheless, because Ca\(^{2+}\) conductances in STN neurons have not been fully characterized, it remains unknown how Ca\(^{2+}\) channels are related to these functions. Ca\(^{2+}\) channels are currently classified into a low-voltage–activated (low-threshold) subtype (T-type) and several high-voltage–activated (high-threshold) subtypes (L-, N-, P-, Q-, and R-type) (Birnbaumer et al. 1994; Randall and Tsien 1995). Although it has recently been suggested that STN neurons express L-type (Beurrier et al. 1999) and T-type (Beurrier et al. 1999; Overton and Greenfield 1995; Plenz et al. 1997; Song et al. 1998) currents, the functional characteristics of these currents and their subcellular localization remain unknown. It is also unknown whether STN neurons express other subtypes of Ca\(^{2+}\) channels.

To understand how Ca\(^{2+}\) channels are involved in the variety of functions of STN neurons, the aim of the present study was to identify the subtypes and functional characteristics of Ca\(^{2+}\) conductances in STN neurons. To this end, we first studied Ca\(^{2+}\) channels in acutely dissociated, retrogradely labeled STN neurons. Acute dissociation trims off dendritc processes and thus improves space clamp, at the expense of dendritic information. To gain information on Ca\(^{2+}\) conductances in dendrites, we also performed current-clamp recordings from STN neurons in thin slices. Our results from acutely dissociated cells revealed the absence of low-threshold Ca\(^{2+}\) channels and the presence of four subtypes (N-, L-, Q-, and R-type) of high-threshold Ca\(^{2+}\) channels. A low-threshold Ca\(^{2+}\) spike, however, was observed in STN neurons in slice. We conclude that STN neurons express low-threshold Ca\(^{2+}\) channels and several subtypes of high-threshold channels, with the possibility of a preferential distribution of the low-threshold channel to the dendritic processes. Part of these results has been fully characterized, it remains unknown how Ca\(^{2+}\) channels are related to these functions.

## Methods

### Retrograde labeling

To identify STN neurons after dissociation, STN neurons were retrogradely labeled by injecting a fluorescent dye, Fluoro-Gold, into the globus pallidus, before dissociation. Sprague-Dawley rats at the age of postnatal day 19 (P19) to P24 were anesthetized with intraperitoneal injections of ketamine (60 mg/kg) and xylazine (7.5 mg/kg). The adequacy of the anesthesia was judged by the absence of reflex to ear pinches. All experiments were conducted in compliance with the Guidelines for Use of Laboratory Animals of Osaka University. Fluoro-Gold (Fluorochrome, Englewood, CO; 3% in saline; 0.3 ~ 0.8 μl) was injected bilaterally into the globus pallidus (AP = −0.2, L = 2.2 ~ 2.4, D = 5.6 ~ 5.9), with a glass micropipette attached to a Hamilton syringe.

### Acute dissociation

Five to 7 days later, STN neurons were acutely dissociated using procedures similar to those described previously (Song and Surmeier 1996). The rats that had received Fluoro-Gold injection, were anesthetized at P26–30 with ethylether and decapitated; brains were quickly removed, iced, and then blocked for slicing. The blocked midbrain region was cut into 400-μm-thick slices in horizontal plane with a Microlicer (Dosaka, Kyoto, Japan) while bathed in a low Ca\(^{2+}\) (100 μM), N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES)–buffered salt solution (in mM; 140 Na isethionate, 2 KCl, 4 MgCl\(_2\), 0.1 CaCl\(_2\), 23 glucose, and 15 HEPES; pH 7.4, 300–305 mOsm/l). Retrograde labeling was then examined under an epifluorescent microscope (Olympus, Tokyo), to make sure that the STN was labeled, but that the zona incerta was not (Fig. 1, A–C). Slices were then incubated for 1–6 h at room temperature (20–22°C) in NaHCO\(_3\)-buffered saline bubbled with 95% O\(_2\)-5% CO\(_2\) (in mM; 126 NaCl, 2.5 KCl, 2 CaCl\(_2\), 2 MgCl\(_2\), 26 NaHCO\(_3\), 1.25 NaH\(_2\)PO\(_4\), 1 pyruvic acid, 0.2 ascorbic acid, 0.1 N-nitro-l-arginine, 1 kynurenic acid, and 10 glucose; pH 7.4 with NaOH, 300–305 mOsm/l). Slices were then removed into the low Ca\(^{2+}\) buffer, and, with the aid of a dissecting microscope, regions of the STN were dissected with a pair of thin tungsten needles. Dissection at the medial side of the STN was done at a distance <1 mm from the lateral border. The dissected STN regions were placed in an oxygenated beaker containing pronase (1–3 mg/ml) in HEPES-buffered Hank’s balanced salt solution (in mM; 140 NaCl, 2 KCl, 1 CaCl\(_2\), 2 MgCl\(_2\), 10 glucose, and 15 HEPES; pH 7.4 with NaOH, 300–305 mOsm/l) at 35°C.

After 30–35 min of enzyme digestion, tissue was rinsed three times in the low Ca\(^{2+}\). HEPES-buffered saline and mechanically dissociated with a graded series of fire-polished Pasteur pipettes. The cell suspension was then plated into a 35-mm Lux Petri dish mounted on the stage of an inverted microscope.

### Whole cell recordings from dissociated cells

Whole cell recordings employed standard techniques (Hamill et al. 1981). The internal solution consisted of (in mM) 170 N-methyl-D-glucamine (NMG), 4 HEPES, 4 MgCl\(_2\), 0.1 bis-(o-aminophenoxo)-N,N,N‘,N‘-tetraacetic acid (BAPTA), 12 phosphocreatine, 2 Na\(_2\)ATP, 0.2 Li\(_2\)GTP, and 0.1 leupeptin; pH 7.2–3 with H\(_2\)SO\(_4\), 265–270 mOsm/l. The external solution consisted of (in mM) 127 NaCl, 20 CsCl, 1 MgCl\(_2\), 10 HEPES, 0.0005 TTX, 5 BaCl\(_2\), and 10 glucose; pH 7.4 with NaOH, 300–305 mOsm/l.

Nifedipine and (-)-Bay K 8644 (RBI, Natick, MA) were made up as concentrated stock solutions in 95% ethanol and diluted immediately before use. These solutions were protected from ambient light. Final ethanol concentrations never exceeded 0.05% (vol/vol; equal solvent concentrations were added to all control solutions). α-Conotoxin GVIA (α-CgTx) and α-agatoxin IVA (α-AgTx; all from Peptide Institute, Osaka, Japan) were made up as concentrated stock solutions in water, aliquoted, and frozen; aliquots were thawed and diluted on the day of use. Final dilutions were made in external media containing 0.1% cytochrome C. Drugs were applied through a gravity-fed manifold system. Solution changes were effected by electronic valves controlling the inflow to a manifold feeding a single outlet capillary. The application capillary (~500 μm ID) was positioned about 1 mm from the cell under study.

Recordings were obtained using an Axon Instruments 200B patch-clamp amplifier and controlled and monitored with a Pentium PC running pCLAMP (v. 6.0) with a 125-kHz interface (Axon Instruments, Foster City, CA). Electode resistances were typically 3–6 MΩ in the bath. After seal rupture, series resistance (7–15 MΩ) was compensated (80–90%) and periodically monitored. The adequacy of voltage control was assessed by examining the tail currents following strong depolarizations. Cells in which tail currents were broad or unstable at subthreshold potentials were excluded from the analyses. Potentials were not corrected for the liquid junction potential. Recordings were made only from retrogradely labeled neurons.

### Whole cell recordings in slice

Sprague-Dawley rats at the age of P21–27 were used. The rats were anesthetized with ethyl ether and decapitated. Brains were quickly removed into an ice-cold Ringer solution and kept in the solution for at least 5 min. The solution consisted of (in mM) 126 NaCl, 2.5 KCl, 1 MgSO\(_4\), 2 CaCl\(_2\), 1.25 KH\(_2\)PO\(_4\), 26 NaHCO\(_3\), and 10 glucose, pH 7.4 with HCl, 300–305 mOsm/l. The region of the brain stem containing the STN was cut into 200-μm-thick slices, in a horizontal plane. The slices were then kept in the Ringer solution at room
temperature for at least 1 h, before being transferred to a recording chamber mounted on an upright microscope (Olympus, Tokyo). The slice was continuously perfused with the saline during recording.

Whole cell recordings from slices employed standard techniques (Edwards et al. 1989). The STN was visually identified as an cellular dense structure anterior to the substantia nigra and surrounded by the internal capsule from the rostral, lateral, and caudal sides. For voltage-clamp recordings, the internal consisted of (in mM) 120 KCl, 3 MgCl₂, 10 HEPES, 0.2 NaOH, 300 mOsm/l. The external solution consisted of (in mM) 140 NMG, 15 NaCl, 4 MgCl₂, 40 HEPES, 0.1 BAPTA, 12 phosphocreatine, 2 Na₂ATP, 0.2 Li₃GTP, and 0.1 leupeptin, pH 7.2–3 with H₂SO₄, 265–270 mOsm/l; the external solution consisted of (in mM) 150 TEACl, 2 MgCl₂, 5 HEPES, 0.001 TTX, 5 BaCl₂, and 20 glucose; pH 7.4 with NaOH, 300 ± 5 mOsm/l. For current-clamp recordings, the internal solution consisted of (in mM) 120 KCl, 3 MgCl₂, 10 HEPES, 0.2 EGTA, 12 phosphocreatine, 2 Na₂ATP, 0.2 Li₃GTP, and 0.1 leupeptin, pH 7.2–3 with H₂SO₄, 265–270 mOsm/l; the external solution consisted of (in mM) 126 NaCl, 2.5 KCl, 1 MgSO₄, 2 CaCl₂, 1.25 KH₂PO₄, 26 NaHCO₃, and 10 glucose, pH 7.4 with NaOH, 300–305 mOsm/l.

Recordings were obtained with an EPC-7 patch-clamp amplifier (List Instruments, Germany) and controlled and monitored with a Pentium PC running pCLAMP (v. 6.0) with a 125-kHz interface (Axon Instruments, Foster City, CA).

**Statistical methods**

Sample statistics are given either as medians or as means with standard error of the mean. Box plots were used for graphic presentation of the data because of the small sample sizes (Tukey 1977). Statistical methods

In some experiments, membrane permeability was estimated as a function of membrane potential using the Goldman-Hodgkin-Katz constant current equation (Hille 1992; Song and Surmeier 1996)

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I(V_m) = g(V_m)P(V_m), \quad g(V_m) = z^2(V_mF^2/RT)\left[\frac{[Ca^++] - [Ca^{2+}]}{\exp(-zV_mF/RT)/[1 - \exp(-zV_mF/RT)]}\right]: \quad I(V_m) \text{ is the measured membrane current density (A/cm}^2); \quad V_m \text{ is the membrane potential (mV); } z = 2; \quad \text{and } F, R, \text{ and } T \text{ have their usual meanings. Membrane area was calculated from whole cell capacitance assuming 1 \mu F/cm}^2. \quad [Ca^+] \text{ and } [Ca^{2+}] \text{ are Ca}^{2+} \text{ concentrations inside and outside the cell, respectively. [Ca] was assumed to be 100 nM. The external Ba}^{2+} \text{ concentration was taken as } [Ca]_o.

**RESULTS**

*Cell identification after dissociation*

The STN is located anterior to the substantia nigra, medial to the internal capsule, and lateral to the zona incerta. Because axon bundles are less transparent under translucent illumination compared with soma-rich regions, the STN, under a dissecting microscope, can be identified as a brighter region surrounded by the darker internal capsule in a horizontal section (Fig. 1A). In this way, the caudal, rostral, and lateral edges of the nucleus could be identified, but not the mediolateral edge (Fig. 1B). Thus dissecting the STN from untreated slices is problematic, as contamination from the zona incerta, which lies immediately medial to the STN, is unavoidable. To circumvent this problem, we labeled STN neurons retrogradely by injecting Fluoro-Gold into the globus pallidus. Shown in Fig. 1, A: a photomicrograph of the STN region in a horizontal slice 400 μm thick. The STN can be recognized as the structure invading the internal capsule (IC), rostral to the substantia nigra (SN). The STN was dissected out approximately at the level marked by the straight lines. The border on the medial side, however, is not clear. Dissection was made at a distance <0.1 mm medial to the IC. B: the same view showing the Fluoro-Gold fluorescence in STN and SN. Fluoro-Gold had been injected into the globus pallidus. C: the STN region in B at a higher magnification. D: a photomicrograph of an acutely dissociated cell. E: the same cell under ultraviolet (UV) illumination gave rise to Fluoro-Gold fluorescence. All recordings were made from such retrogradely labeled neurons. Bar in A, 0.5 mm for A and B; bar in C, 0.1 mm and in D, 10 μm for D and E.
fluorescent illumination, respectively. In most experiments, only one or two cells could be identified and recorded. All recordings were made in retrogradely labeled neurons. These neurons had whole cell capacitances of 4–8 pF.

Channel activation and inactivation properties

When the membrane potential was depolarized from a holding potential of $-80$ mV, inward currents were evoked (Fig. 2A). These currents could be blocked by Cd$^{2+}$ in a dose-dependent manner, with an IC$_{50}$ near 1 mM (average of $0.9$ mM in 3 cells; data not shown). The amplitude of the currents decreased when the concentration of extracellular Ba$^{2+}$ was changed from 5 to 2 mM ($n = 5$, data not shown). These results suggest that STN neurons express Ca$^{2+}$ channels, and that the currents recorded are Ba$^{2+}$ currents through Ca$^{2+}$ channels.

The amplitude of the current was voltage dependent. Shown in Fig. 2B is the relationship between voltage and the amplitude of the current 5 ms after initiation of the depolarizing pulse (Fig. 2A, arrow). Current-voltage curves exhibited an inverted bell shape, with the peak at either 0 or $-10$ mV when examined with voltages changing by a 10-mV step (Fig. 2B; $n = 25$). Currents began to appear at approximately $-50$ mV. This was also true when the holding potential was shifted to $-100$ mV ($n = 9$). These results suggest the absence of T-type channels in dissociated STN neurons. The kinetics of the current also showed some voltage dependence. Currents activated progressively faster with stronger depolarization (Fig. 2A). Some inactivation occurred at voltages more positive than $-20$ mV during the 30-ms pulse, but no inactivation was noticed for currents evoked at lower voltages (Fig. 2A).

To examine the voltage dependence of the Ca$^{2+}$ conductances, tail current amplitudes were normalized and plotted against voltage (Fig. 2C). This plot should reflect the steady-state activation of Ca$^{2+}$ channels in STN neurons, with a minor error caused by the moderate inactivation occurred during the 30-ms pulse. The data could be well fitted with a single Boltzmann function, with a half-activation voltage of $-13.6$ mV and a slope factor of 6.8 mV (Fig. 2C). In a sample of 12 cells, the average half-activation voltage was $-12.6 \pm 1.3$ (SE) mV, and the average slope factor was $7.0 \pm 0.6$ mV. These results suggest that most, if not all, of the Ca$^{2+}$ channels in acutely dissociated STN neurons are of the high-voltage-activated subtype.

The fact that the voltage dependence of activation could be described by a single Boltzmann function suggests that there is either a single subtype of channel in STN neurons or there are multiple subtypes having similar voltage dependence. To test these possibilities, we first examined the voltage dependence of inactivation of the channels, using a conventional prepulse protocol. As shown in Fig. 3A, prepulses of 3 s suppressed the amplitude of the current evoked by a subsequent pulse to 0 mV, in a voltage-dependent manner. The prepulse only partially inactivated the current, as shown in Fig. 3B (60.8 $\pm$ 0.1%, mean $\pm$ SE, $n = 8$). The voltage-dependent inactivation could be approximated with a single Boltzmann function, with a constant term expressing the residual current. The average half-inactivation voltage was $-30.0 \pm 2.1$ mV ($n = 8$), and the slope factor was $8.1 \pm 0.9$ mV. Because the voltage dependence of both activation and inactivation of the currents did not show clear heterogeneity, we next examined the kinetics of inactivation. For this, currents evoked by a pulse to 0 mV for 10 s were recorded (Fig. 3C). In agreement with the prepulse experiments, on average 38.3% of the current ($n = 6$) did not inactivate during the pulse. Of interest is the decay phase of the current. Shown in Fig. 3D is the log plot of the current against time. It is clear from the figure that the current decayed with more than one time constant: three time constants could be dissected in all cases tested ($n = 6$), with the longest being 41.7 $\pm$ 15.3 s, the middle being 1.9 $\pm$ 0.3 s, and the shortest being 217.6 $\pm$ 62.0 ms (mean $\pm$ SE; Fig. 3D). These results raise the possibility that STN neurons have either multiple subtypes of high-threshold Ca$^{2+}$ channels or multiple inactivation processes with a single channel type.

Channel subtypes

To test whether acutely dissociated STN neurons have multiple subtypes of channels, organic Ca$^{2+}$ channel blockers were applied at saturating concentrations (Randall and Tsien 1995). Currents here were evoked by a slow voltage ramp, to estimate at the same time the voltage dependence of channel activation (Bargas et al. 1994). Ramps with a rate of 0.3 mV/ms were examined.
found to produce current-voltage curves similar to those produced with voltage steps \((n = 6); \) data not shown and were therefore used throughout the experiments. As shown in Fig. 4, A and B, application of the L-type channel blocker, nifedipine \((5 \mu M)\), partially blocked the current. Bay K 8644 \((1 \mu M)\) increased the current amplitude and slowed down the tail current decay \((n = 5); \) data not shown. Application of \(\omega\)-CgTx \((1 \mu M)\) in the presence of nifedipine greatly reduced the current \((\text{Fig. 4A})\). \(\omega\)-AgTX had no effect on the current at a concentration of 20 nM \((n = 14)\), but blocked part of the current at 200 nM \((\text{Fig. 4A})\). Co-application of all drugs did not completely block the current \((\text{Fig. 4A})\). In a sample of six neurons from six animals, the residual current, or R-type current, consisted 8.7 ± 3.1% of the total current; \(\omega\)-CgTx–sensitive current, or N-type current, was the major \(\text{Ca}^{2+}\) current, comprising 52.1 ± 2.4% of the total; currents sensitive to high concentrations of \(\omega\)-AgTX, defined as Q-type current \((\text{Randall and Tsien 1995})\), comprised 16.3 ± 4.1%, while L-type current was 21.0 ± 2.9% of the total \((\text{Fig. 4B, inset})\).

Currents of each subtype were isolated by subtraction and are shown in Fig. 4C. In agreement with the results shown in Fig. 2, the current-voltage relationship of the subtypes had similar shapes. However, some differences in channel voltage dependence were also noticed. Shown in Fig. 4D are the voltage dependencies of channel activation curves, calculated from the currents shown in Fig. 4C, using the Goldman-Hodgkin-Katz current equation \((\text{see METHODS})\). The activation of each current subtype could be well fitted with a single Boltzmann function. For clarity, the fitted Boltzmann curves are shown in the inset of Fig. 4D. Although each subtype had similar voltage dependencies, R-type current had the lowest threshold, and the voltage dependence of L-type current was shifted ~5 mV toward hyperpolarization, as compared with that of the Q- and N-types. Similar observations were obtained in all cells tested \((n = 6)\). The median half-activation voltage was ~19.8 mV for L-type, ~16.3 mV for N-type, ~15.9 mV for Q-type, and ~18.6 mV for R-type current. The half-activation voltage of L-type current was significantly lower than both N- and Q-type \((P < 0.05)\), Wilcoxon’s signed-ranks test).

Low-threshold \(\text{Ca}^{2+}\) spikes in a slice preparation

The absence of T-type channels in acutely dissociated STN neurons seems to be at odds with previous reports that suggest the presence of T-type channels in STN neurons in slice \((\text{Beurrier et al. 1999; Overton and Greenfield 1995})\) and in culture \((\text{Plenz et al. 1997})\). Results obtained from acutely isolated STN neurons, however, should primarily reflect the channel subtype composition in the somatic membrane. To gain information on \(\text{Ca}^{2+}\) channels in dendritic processes, we studied \(\text{Ca}^{2+}\) channel expression in STN neurons in a slice preparation, focusing on low-threshold channels. When the membrane potential was depolarized from ~80 to ~50 mV in voltage-clamp mode, a transient current was observed with a delay. This delay was voltage dependent, indicating a lack of space clamp \((n = 3); \) data not shown. The current thus could not be characterized in voltage-clamp mode. Because T-type
channels give rise to a characteristic slow spike potential in current-clamp mode (Bal and McCormick 1996; Deschenes et al. 1982; Llinàs and Yarom 1981), we did current clamp recordings from STN slices. As shown in Fig. 5A, in response to a hyperpolarizing current injection, the membrane potential hyperpolarized and then exhibited a slow return to the resting potential; on termination of the current pulse, a rebound action potential was observed. Application of the Na$^+$ channel blocker, tetrodotoxin (TTX, 1 $\mu$M) blocked the action potential, revealing an underlying slow spike (arrow). C: Ni$^{2+}$ (40 $\mu$M) blocked the spike, suggesting it to be a low-threshold Ca$^{2+}$ spike. D: 3 mM Cs$^+$ removed the voltage sag, indicating the presence of an H current.

**DISCUSSION**

By recording from both acutely dissociated STN neurons and STN neurons in slice, we found that rat STN neurons express both low- and high-voltage-activated Ca$^{2+}$ currents. All known high-voltage-activated channels were found in STN neurons except the P-type. We also obtained evidence suggesting the possibility that low-threshold, or T-type, channels are preferentially distributed to dendritic processes.

**Cell identification**

These conclusions clearly depend on reliable identification of the neurons recorded. Acutely dissociated cells provide a good preparation for patch-clamp recording, but dissociation also makes it difficult to identify the neuronal phenotype. Cell identification is especially important here, as the border between STN and the zona incerta is not discernible. Our method of identification of the STN, using a combination of landmark identification and retrograde labeling, leaves little ambiguity. Our identification method also limits the neurons analyzed in this study to STN neurons that project to the globus pallidus. Such neurons, however, should represent the majority of STN neurons, because it has been shown that more than 90% of STN neurons project to the globus pallidus (Van der Kooy and Hattori 1980).

**Channel subtypes expressed in STN neurons**

Although the presence of L-type Ca$^{2+}$ conductance in STN neurons has been reported recently (Beurrier et al. 1999), expression of other subtypes has remain unknown. Here we have shown that all high-voltage-activated subtypes, except P-type, are expressed at different levels in STN neurons. It is well established that the L-type current can be identified by its sensitivity to dihydropyridines and Q-type current can be identified by its sensitivity to high concentrations of $\omega$-AgTx (Randall and Tsien 1995). But the identification of R-type current can be problematic, as it is only defined by its resistance to all known organic blockers. The presence of an R-type current in STN neurons is based on the fact that we used saturating concentrations of blockers to other subtypes of current. Nevertheless, the proportion of R-type current might have been overestimated, because of the slow time course of $\omega$-AgTx block. The presence of R-type current in STN neurons is also evidenced by the biophysical differences between R- and Q-type currents shown in this study. Furthermore, class E $\alpha$1 subunit mRNA, which is suggested to encode R-type channels (Zhang et al. 1993), has been shown to be expressed in STN neurons (Yokoyama et al. 1995).

Because currents recorded from acutely dissociated cells began to activate at around $-50$ mV, Ca$^{2+}$ channels in acutely dissociated STN neurons are exclusively of the high-voltage-activated variety. The absence of low-threshold channels in dissociated cells is also evidenced by the inactivation kinetics of the current. In addition to the low threshold of activation (approximately $-60$ mV), fast inactivation is another characteristic of T-type current (Carbone and Lux 1984). T current inactivates with a time constant of 10–25 ms at room temperature (Mouginot et al. 1997; Tarasenko et al. 1998). The fact that the shortest inactivation time constant in STN neurons was longer than 200 ms (see Fig. 3D) supports the view that dissociated STN neurons has little, if any, T-type current.

The TTX-resistant broad spike observed on the rebound potential in slice is likely to be a low-threshold Ca$^{2+}$ spike (Llinàs and Yarom 1981). First, the spike occurred at a low membrane potential close to $-60$ mV. Second, the shape of the spike resembled that of low-threshold Ca$^{2+}$ spikes reported in other neuron types (Bal and McCormick 1996; Deschenes et al. 1982). And third, the spike could be blocked by micromolar concentration of Ni$^{2+}$ (Fox et al. 1987). Previous electrophysiological studies have also suggested the presence of a low-threshold Ca$^{2+}$ spike in STN neurons (Beurrier et al. 1999; Overton and Greenfield 1995; Plenz et al. 1997). These observations are consistent with the recent demonstration that STN neurons express mRNAs coding for T-type channels (Perez-Reyes et al. 1998; Talley et al. 1999). However, T-type channels were not observed in acutely dissociated STN neurons. This discrepancy might be attributable to the age difference in the slices used.

![Image](http://jn.physiology.org/DownloadedFrom)
between animals used for the slice experiments (P21–27) and those used for dissociated cell experiments (P26–30), because T-type channels often disappear during maturation (Bargas et al. 1994; Chameau et al. 1999). In STN neurons, however, mRNAs coding for T-type channels are expressed even in adults (Perez-Reyes et al. 1998; Talley et al. 1999), although mRNA expression may not necessarily mean expression of functional proteins. Another possibility is that T-type channels may not survive the enzymatic treatment used for dissociation, but T-type currents have been successfully recorded in neostriatal neurons treated with the same enzyme as in the present experiment, under the same condition (Bargas et al. 1994). Thus the absence of detectable level of T-type currents in dissociated STN neurons may be attributable to loss of dendritic processes during dissociation, and suggests the possibility that STN neurons express T-type channels preferentially in dendrites. This notion, however, is based on the negative finding of T-type currents in dissociated neurons, and thus needs to be further tested.

Functional significance

Our results demonstrate that STN neurons express T-, L-, N-, Q-, and R-type Ca\(^{2+}\) channels. A question arises then of what is the functional significance of each subtype. This is especially puzzling for the high-voltage–activated subtypes, because they share similar biophysical properties.

The function of T-type current seems to be well established. With a sufficient channel density, T-type currents can generate a spike: T spike (Llinàs and Yarom 1981) (see Fig. 5B). This spike is broader than sodium spikes, because of the slower kinetics of the T-type channels, as compared with Na\(^+\) channels. Therefore on top of the T spike, a short train of action potentials is often elicited (Bal and McCormick 1996). Furthermore, by interacting with other ion channels, T-type channels are thought to be important in the generation of oscillatory activity (Bal and McCormick 1996). The functional significance of T-type channels in STN neurons, however, remains obscure. Although STN neurons express many key elements for oscillatory bursting, including T-type channels, H channels (Fig. 5) (Beurrier et al. 1999; Bevan and Wilson 1999; Plenz et al. 1997), and Ca\(^{2+}\)-dependent K\(^{+}\) channels (Beurrier et al. 1999; Bevan and Wilson 1999; Nakanishi et al. 1987), STN neurons do not appear to generate rhythmic bursting by themselves (Georgopoulos et al. 1983; Magill et al. 2000; but see Beurrier et al. 1999). Provided that T-type channels in STN neurons have a preferential distribution in dendritic processes, they may play a role in synaptic integration in STN neurons.

In the present experiment, among the high-voltage–activated subtypes, N-type current comprised more than half of the current. N-type current is expressed in most central neurons, but the proportion of this current to the total is often between 20 and 30% (Bargas et al. 1994; Cardozo and Bean 1995; Lorenzon and Foehring 1995). Because it is now well established that N-type current is subject to neuromodulation by a number of receptors coupled to trimeric guanine-binding proteins (Hille 1994), the predominance of N-type current in STN neurons would make Ca\(^{2+}\) entry into these neurons highly modifiable. A variety of neurotransmitters are known to be released within the STN, including GABA (Bevan and Bolam 1995), acetylcholine (Bevan and Bolam 1995), serotonin (Pompeiano et al. 1994), glutamate (Bevan and Bolam 1995; Mouroux and Feger 1993) and probably dopamine (Canteras et al. 1990). All these neurotransmitters may modulate Ca\(^{2+}\) entry through N-type channels into STN neurons, depending on the receptors expressed. It thus would be interesting to test how Ca\(^{2+}\) currents in STN neurons are modulated by these neurotransmitters. In addition to the difference in susceptibility to neuromodulation, different subtypes of the high-voltage–activated channels may be differentially coupled to other signaling mechanisms. For example, N-type channels, but not L-type channels, are coupled to Ca\(^{2+}\)-dependent K\(^{+}\) channels in motor neurons (Viana et al. 1993), while in hippocampal neurons L-type channels are known to be coupled to the K\(^{+}\) channel (Moyer et al. 1992). Modulation of Ca\(^{2+}\) currents in STN neurons may in turn change the activity of Ca\(^{2+}\)-dependent processes as well.

The biophysical differences between the high-voltage–activated channels, although small, may also have a significant impact on the contribution of each channel subtype to neuronal activity. The slightly lower activation voltage of the L- and R-type channels may result in them serving unique functions. A long-lasting plateau potential generated in STN neurons has been shown to be predominantly mediated by L-type currents (Beurrier et al. 1999; Song et al. 1998). Because the plateau is at a low voltage (approximately −40 mV) (Beurrier et al. 1999), it is likely that this unique function of L-type channels is attributable to its lower activation-voltage demonstrated in the present study.

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REFERENCES


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