Subthreshold Sodium Currents and Pacemaking of Subthalamic Neurons: Modulation by Slow Inactivation

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Summary

Neurons of the subthalamic nucleus (STN) are spontaneously active. By voltage clamping dissociated rat STN neurons with their own firing patterns, we found that pacemaking is driven by two kinds of subthreshold sodium current: a steady-state "persistent" sodium current and a dynamic "resurgent" sodium current, which promotes rapid firing by flowing immediately after a spike. These currents are strongly regulated by a process of slow inactivation that is active at physiological firing frequencies. Slow inactivation of the pacemaking sodium currents promotes a constant frequency of tonic firing in the face of small, steady changes in input and constitutes a form of adaptation at the single-cell level. Driving cells at a high rate (75 Hz) produced pronounced slow inactivation (60%–70%) of resurgent, persistent, and transient components of sodium current. This inactivation is likely to contribute to effects of clinical deep-brain stimulation on STN excitability.

Introduction

The subthalamic nucleus (STN) is the only source of excitation that is intrinsic to the basal ganglia, exciting output structures that in turn inhibit thalamic motor areas. STN neurons have intrinsic pacemaking properties, since the spontaneous firing seen in vivo (Bergman et al., 1994) is maintained in brain slices (Nakanishi et al., 1987) and persists when fast synaptic transmission is blocked (Bevan and Wilson, 1999; Beurrer et al., 2000). Reciprocal connections between the STN and the external segment of the globus pallidus (GPe) give rise to network rhythmicity (Plenz and Kitai, 1999) that is probably important for normal movements and may be pathologically strong in movement disorders (reviewed by Bevan et al., 2002b). Electrical high-frequency stimulation (HFS) of the STN can alleviate movement disorders in patients with Parkinson’s disease and in parkinsonian animal models (reviewed by Benazzouz and Hallett, 2000; Vitek, 2002). The mechanism of HFS is unknown. Because the effects resemble those of STN lesions (Bergman et al., 1990), one possibility is that HFS silences STN neurons through overstimulation or depolarization block, perhaps involving inactivation of sodium channels (Beurrer et al., 2001).

The best-known mechanism of pacemaking of excitable cells involves dynamic activation of the hyperpolarization-activated current Ih during the interval between spikes (Pape, 1996; Robinson and Siegelbaum, 2003). However, blocking Ih does not generally stop spontaneous firing of STN neurons, and it has been proposed that pacemaking of these neurons relies instead on a slowly inactivating, TTX-sensitive “persistent” sodium current flowing at subthreshold voltages (Bevan and Wilson, 1999; Beurrer et al., 2000). Only limited characterization of subthreshold sodium current is possible in brain slice experiments, and properties such as its kinetics during the pacemaking cycle and its relationship with conventional transient sodium current remain to be explored.

We have developed a preparation of acutely dissociated STN neurons that allows direct recording of individual ionic currents during the pacemaking cycle. We find that in addition to persistent sodium current, STN neurons possess a dynamic “resurgent” sodium current, previously described in cerebellar Purkinje neurons (Raman and Bean, 1997), which flows immediately after spikes and promotes rapid firing. A process of slow inactivation, which regulates resurgent current as well as persistent and transient sodium currents, serves as a feedback element regulating the frequency of tonic firing and may contribute to the effects of clinical deep-brain stimulation of the STN.

Results

Defining the ionic currents that drive pacemaking requires accurate voltage clamp and fast solution changes, both facilitated by the use of dissociated neurons. The STN is an ideal structure for dissociation (Sidach and Mintz, 2000; Song et al., 2000) because it comprises a highly homogeneous cell population (Afsharpoor, 1985). In initial experiments, STN neurons were identified by retrograde transport of fluorescent beads injected into the entopeduncular nucleus, the rat equivalent of the GPI (Figure 1A; Song et al., 2000). Labeled STN neurons were polygonal, pear shaped, or round, with somata about 10–25 μm in the long axis (Figure 1B). When studied in brain slices, labeled neurons were spontaneously active, as expected (Nakanishi et al., 1987), and spontaneous activity continued and was highly regular when fast synaptic transmission was blocked by CNQX, APV, and picrotoxin (Bevan and Wilson, 1999; Beurrer et al., 2000). Dissociated labeled neurons also fired tonically (13 of 13 cells), confirming that pacemaking is an intrinsic membrane property and does not require synaptic input.

In later experiments, when the location of the STN had become familiar, STN neurons could be clearly identified, even without labeling. Unlabeled neurons had the same morphology and characteristic electrophysiological properties, including spontaneous rhythmic firing and narrow action potentials, but were more robust for long-lasting recordings. Virtually all dissociated STN neurons were spontaneously active in a highly regular manner, firing at 25 ± 10 Hz (n = 49). This was faster than spontaneous firing of cells in brain slices at the
same temperature of 23°C, studied with blockers of fast synaptic transmission (10 ± 8 Hz, n = 16). Pacemaking is evidently driven by currents originating in the soma, since many dissociated cells had no obvious dendritic or axonal remnants. The slower pacemaking of intact cells is expected if dendrites act as a resistive and capacitative load, and input resistance was higher for dissociated cells (1356 ± 443 MΩ, n = 19) than for intact cells in brain slice with synaptic blockers present (187 ± 55 MΩ, n = 8).

When tonic firing was stopped by hyperpolarization to between −80 mV to −90 mV, some dissociated neurons (12/49) produced intermittent spontaneous bursting (Figure 1C), a behavior also seen in a similar fraction (4/16) of STN neurons in brain slices. Many dissociated neurons produced rebound bursts following hyperpolarization, and some had plateau potentials with overriding spikes that outlasted a pulse of depolarizing current, as in brain slices (Nakanishi et al., 1987; Beurrier et al., 1999; Otsuka et al., 2001, Bevan et al., 2002a). Thus, dissociated STN neurons retain intrinsic membrane properties characteristic of more intact neurons.

Pacemaking and Subthreshold Sodium Current
To examine what ionic currents are involved in generating the spontaneous depolarization between spikes, we recorded a cell’s own spontaneous spike train, replayed it to that cell as a command in voltage clamp, then used ionic substitutions and blockers to isolate individual currents flowing during pacemaking (Figure 2A). Resolution was improved by signal averaging the currents over multiple pacemaking cycles.

We focused on three likely candidates for driving pacemaking: I_h, calcium current, and voltage-dependent sodium current. We targeted I_h with 3 mM external Cs⁺ (Pape, 1996; Beurrier et al., 2000). The current blocked by 3 mM Cs⁺ was consistently either zero or slightly outward in the interspike interval between −70 mV and −50 mV (five of five cells; Figures 2B and 2C), suggesting that little or no I_h flows during the spontaneous depolarization between spikes. There was an outward Cs⁺-sensitive current during the falling phase of the spikes, probably reflecting a weak block of potassium channels.

Total calcium current was isolated by isomolar substitution of Co²⁺ for Ca²⁺, with TEA present in both solutions at high concentration (160 mM, replacing sodium) to block Ca²⁺-activated potassium current. A large calcium current was present during the spike, as expected (Sidach and Mintz, 2000; Song et al., 2000), reaching a peak during the falling phase. However, calcium current contributed little or no current during the interspike interval (Figure 2B). Cobalt-sensitive current was ±0.1 ± 3 pA at −70 mV, −0.1 ± 3 pA at −60 mV, and −1.5 ± 2.7 pA at −50 mV (n = 7). Thus, calcium current does not appear to contribute to the spontaneous depolarization during the pacemaking cycle.

In contrast to I_h and calcium current, TTX-sensitive
TTX-sensitive current also flowed at all times during the interspike interval, even at the most hyperpolarized voltage following spikes (15 of 15 cells, Figure 2B). The average interspike sodium current was $-6.8 \pm 3.8$ pA at $-70\text{mV}$, $-14.2 \pm 7.3$ pA at $-60\text{mV}$, and $-56.2 \pm 32.7$ pA at $-50\text{mV}$ ($n = 15$, Figure 2C). The sodium current flowing during the early interspike interval was nearly equal to net ionic current, calculated from $C_m\frac{dV}{dt}$ (Hodgkin and Huxley, 1952), being $-6.8 \pm 3.8$ pA and $-7.1 \pm 9.8$ pA at $-70\text{mV}$, respectively, and $-7.4 \pm 3.8$ pA and $-7.7 \pm 7.4$ pA at $-64\text{mV}$. Thus, TTX-sensitive current is sufficient to account for the spontaneous depolarization during the early interspike interval. Later in the interspike interval, TTX-sensitive sodium current was larger than net ionic current. TTX-sensitive current and net ionic currents were $-10.6 \pm 6.0$ pA and $-8.1 \pm 6.4$ pA at $-62\text{mV}$, and $-23.6 \pm 15$ pA and $-12.3 \pm 9.9$ pA at $-56\text{mV}$ ($n = 15$). This divergence suggests activation of substantial subthreshold potassium currents.

In current-clamp experiments using dissociated cells (data not shown), spontaneous firing continued (eight of eight cells) when $I_h$ was blocked by 3 mM external Cs$^+$. Firing frequency generally slowed with an application of Cs$^+$, from $30 \pm 19$ Hz in control to $22 \pm 16$ Hz with Cs$^+$ ($p = 0.01$, Wilcoxon matched pairs test). This slowing was accompanied by a depolarization of the interspike voltage, with the peak afterhyperpolarization changing from $-79\text{mV} \pm 5\text{mV}$ in control to $-76\text{mV} \pm 6\text{mV}$ ($p = 0.04$) and maximal spike upstroke velocity changing from $94\text{mV}/\text{ms} \pm 93\text{mV}/\text{ms}$ to $68\text{mV}/\text{ms} \pm 40\text{mV}/\text{ms}$ ($p = 0.03$). Therefore, the effects of Cs$^+$ are probably not due to the block of $I_h$ but rather to a reduction in potassium conductances activated during the action potential, leading to a reduced AHP and increased inactivation of sodium channels. With application of the organic blocker ZD7288 (10 $\mu$M), two of the six cells tested stopped firing. However, cessation of firing was due to depolarization and not hyperpolarization. In the four of six cells in which firing continued, the frequency of firing increased slightly, from $18 \pm 11$ to $22 \pm 13$ Hz ($p = 0.1$). Thus, when there were effects of Cs$^+$ or ZD7288, they were due to depolarization rather than hyperpolarization and probably reflect small effects of both blockers on currents other than $I_h$.

When Ca$^{2+}$ was replaced by Co$^{2+}$, spontaneous firing generally continued (eight of nine cells) but became irregular, with single spikes or clusters of spikes (lasting $\sim 1 \text{s}$) interspersed with periods of subthreshold oscillations. Since Ca$^{2+}$ currents did not flow during interspike intervals, these changes probably reflect the block of Ca$^{2+}$-activated potassium current, consistent with the interpretation of similar results in brain slice (Bevan and Wilson, 1999). The application of TTX halted spontaneous activity completely (three of three cells), as for STN neurons in brain slice (Beurrier et al., 1999, 2000; Bevan and Wilson, 1999), producing a stable resting potential of $-59\text{mV} \pm 1\text{mV}$.

Characterization of Subthreshold Sodium Current These results are consistent with previous proposals that TTX-sensitive persistent sodium current underlies the pacemaking of STN neurons (Bevan and Wilson, 1999; Beurrier et al., 2000). To test whether the sodium...
current during the interspike interval represents a steady-state persistent current, we compared the sodium current elicited by an action potential to that elicited by the same waveform slowed down by a factor of 50 or 100. The membrane potential changed so slowly during this waveform that maximal fast inactivation should be reached at each voltage during the cycle, leaving only steady-state current. The voltage protocol (Figure 3A) consisted of four spikes, the first pair on the natural time scale and the second pair on a time scale 100 times slower. The current elicited by the slowed spikes had virtually identical magnitude and voltage dependence to that elicited by slow voltage ramps (20 mV/s), the usual way of defining steady-state persistent sodium current (Figure 3B).

By plotting the current elicited by the slowed spikes on a 100-fold compressed time base, we could compare steady-state persistent sodium current with additional components of sodium current that may depend on dynamic changes in voltage. The biggest difference was the large transient sodium current flowing during the upstroke of the action potential (Figure 3C). However, there was also a dynamic current during the repolarization and early interspike interval (five of five cells). This dynamic current is reminiscent of resurgent sodium current previously seen in cerebellar Purkinje neurons (Raman and Bean, 1997), which flows during repolarization following depolarizations that are long enough to produce fast inactivation and which appears to originate from sodium channels that recover from inactivation through the open state. Indeed, when we tested STN neurons with suitable voltage protocols (e.g., Figure 4A), 101 of 104 STN neurons displayed clear resurgent sodium current. The voltage dependence and kinetics of resurgent current in STN neurons were very similar to resurgent current in Purkinje neurons (Raman and Bean, 1997, 2001). With repolarization to −40 mV, where the magnitude was generally maximal, peak current was $-348 \pm 337$ pA, was reached 3.8 ± 1.2 ms after repolarization, and decayed with a time constant of 18 ± 6 ms. At −70 mV, the magnitude was $-133 \pm 84$ pA, time to peak was 0.6 ± 0.5 ms, and the decay time constant was 6.8 ± 8.4 ms (n = 17, Figure 4A).

Figure 4B illustrates an experiment that distinguished resurgent current from tail current, which might flow through channels that do not inactivate completely during a spike. We compared the TTX-sensitive sodium current elicited by the spike waveform (Figure 4B, black trace) with that elicited by a hybrid step/waveform protocol in which the falling phase of the action potential was preceded by a 5 ms step to +15 mV, the peak of the action potential (blue trace). The 5 ms step produces maximal inactivation and thus eliminates almost all tail current that might otherwise flow. Also plotted is the steady-state persistent sodium current elicited by the 100-fold slowed spike waveforms (red trace). Inactivation is indeed incomplete at the peak of the natural spike (Figures 2 and 4), and significant tail current does flow during the early part of the repolarization. But by the time of the afterhyperpolarization, the total sodium current elicited by the natural spike was comparable to the current elicited by the hybrid protocol, which had a clear rising phase. Thus, the dynamic current flowing at the end of the action potential is almost entirely resurgent current. Resurgent current flows before persistent current has activated and continues well into the interspike interval. The same result was obtained in each of five cells tested with this protocol.
Figure 4. Resurgent Sodium Current Activated by Voltage Steps and during the Interspike Interval

(A) Voltage protocol for characterizing resurgent current (top) and TTX-sensitive current flowing in response to the protocol (bottom). A step to produce maximal fast inactivation (−30 mV, 5 ms) preceded a family of test steps (−80 mV to −20 mV in 10 mV increments for 200 ms). Break in time axis represents 100 ms. Three seconds at −90 mV separated the end of one sweep and the start of the next. NMDG-phosphate internal solution, TEA/Cs/Cd Tyrode’s external solution. was applied, pacemaking continued in four of seven cells. NMDG-phosphate internal solution, TEA/Cs/Cd Tyrode’s external solution. was applied, pacemaking continued in four of seven neurons, without a change in average ages. In principle, I_h might be more important in intact neurons than in intact cell bodies (cf. Magee, 1999). Also, the longer interspike interval of intact neurons might allow more time for activation of I_h, which would be faster at physiological temperatures. Therefore, we examined the role of I_h in pacemaking of intact STN neurons at 35°C.

Although I_h was difficult to resolve in dissociated STN neurons, it was large in intact neurons (Figure 5A, 15/15 cells), as previously reported (Beurrier et al., 2000). However, activation of I_h required hyperpolarizations negative to −70 mV, while during pacemaking of STN neurons in brain slice at 35°C, the most negative voltage reached (the AHP following a spike) averaged −65 mV ± 5 mV (n = 41). It is therefore unlikely that there is significant dynamic activation of I_h during the physiological pacemaking cycle, especially since activation occurred relatively slowly, with a time constant of about 600 ms at −80 mV (Figure 5A).

In voltage-clamp experiments, I_h was blocked nearly completely by 1–3 mM Cs⁺ (n = 7) or 10 μM ZD7288 (n = 7), but these blockers had little effect on firing. The frequency of firing in slices at 35°C with synaptic blockers was 19 ± 14 Hz (n = 41). When external Cs⁺ was applied, pacemaking continued in four of seven cells tested. In these four cells, the spike frequency increased from 16 ± 7 Hz to 25 ± 7 Hz (p = 0.14). In the three cells in which firing stopped, the cessation of firing was due to depolarization block, and firing could be recovered by injection of steady hyperpolarizing current. When 10 μM ZD7288 was applied, firing continued in six of seven neurons, without a change in average firing frequency (18.2 ± 7.4 Hz in control, and 18.1 ± 7.0 Hz with ZD7288). The cessation of firing in the remaining cell was due to depolarization and reversed with steady hyperpolarizing current. These effects of Cs⁺ or ZD7288 were opposite to those expected if I_h served as a pacemaker current. The results suggest significant nonspecific effects of ZD7288 as well as Cs⁺, and we found that 10 μM ZD7288 substantially inhibited persistent potassium currents activated by step depolarizations (data not shown).

The waveform of pacemaking reached less negative voltages in intact cells at 35°C (troughs of −65 mV ± 5 mV, n = 41) than in dissociated cells at 23°C (−76 mV ± 6 mV, n = 50) and had narrower spikes (460 ± 156 μs, n = 41 versus 2.5 ± 1.8 ms, n = 50). To determine whether subthreshold sodium current was effectively activated during the pacemaking cycle under physiological conditions, we used a segment of spontaneous activity from an intact cell at 35°C as a voltage command
for dissociated cells studied at 35°C. This experiment defines the kinetics of sodium current during the physiological waveform at physiological temperature (Figure 6). TTX-sensitive sodium current was substantial during the interspike interval, being $-20 \pm 18$ pA at $-65$mV, $-23 \pm 17$ pA at $-60$mV, and $-77 \pm 30$ pA at $-50$mV ($n = 6$). This interspike sodium current is larger than that of dissociated neurons clamped with their own pacemaking (Figure 2), probably due to three factors: pacemaking of cells in slice is slower, which might generate a lower level of inactivation; there is more depolarized AHPs, which would activate a larger and longer-lasting persistent sodium current; and a more depolarized interspike interval, which would activate a greater amount of persistent current. These experiments are consistent with subthreshold sodium current being the principal engine of pacemaking in intact STN neurons as well as dissociated neurons.

**Slow Inactivation of Pacemaking Sodium Currents**

After a hyperpolarizing current injection or a train of IPSPs that halts firing, STN neurons display a period of heightened firing (Bevan et al., 2002a) that can last several seconds (e.g., Figures 1C and 7C). When we released dissociated STN neurons from a 5 s hyperpolarization to between $-80$mV and $-90$mV, the pacemaking frequency was initially elevated by 47% $\pm$ 13% and then relaxed to baseline in 4–9 s ($n = 8$).

A number of ionic mechanisms might contribute to this regulation of firing frequency. Hyperpolarization would remove inactivation from T type calcium channels and activate $I_h$. However, while these currents are well suited for rebound bursts of several hundred ms (Bevan et al., 2002a), they would decay in less than a second over the voltage range of pacemaking and cannot account for slower relaxations of firing frequency. Given the importance of resurgent and persistent sodium current in tonic firing, we asked whether regulation of these currents might be involved. Neither persistent current nor resurgent current should be reduced by normal fast inactivation of sodium channels, since persistent current flows when fast inactivation is at steady state, and resurgent current flows most effectively with an initial condition of complete fast inactivation (Raman and Bean, 2001). However, with maintained or repeated depolarization, a fraction of sodium channels in many neurons enter inactivation states from which recovery is much slower than for normal fast inactivation (reviewed by Vilin and Ruben, 2001). Slow inactivation of sodium channels contributes to slow cumulative adaptation of spikes elicited by long current injections in neocortical pyramidal neurons (Fleidervish et al., 1996), attenuation of back-propagating action potentials in dendrites of hippocampal pyramidal neurons (Colbert et al., 1997; Jung et al., 1997), and variance adaptation in salamander retinal ganglion neurons (Kim and Rieke, 2003). In some cases, persistent sodium current is also susceptible to slow inactivation (Fleidervish et al., 1996). Whether resurgent sodium current is regulated by slow inactivation has not been explored.

The experiment in Figure 7A tested for slow inactivation of transient, resurgent, and persistent components of sodium current by long steady depolarizations. A 20 s depolarization to voltages from $-90$mV to $-40$mV was followed by 100 ms at $-90$mV, to allow recovery from fast inactivation, then by a test sequence consisting of a 10 ms step to $+15$mV, which elicits transient current (and also primes for resurgent current) and a 100 ms step to $-40$mV, which elicits resurgent current. Persistent current can be measured as the steady-state current at the end of the step to $-40$mV. All three components of sodium current were highly susceptible to slow inactivation (Figure 7B). Slow inactivation appeared saturated at $-40$mV, where transient, persistent, and resurgent currents were reduced to 45 $\pm$ 15, 32 $\pm$ 7, and 29% $\pm$ 20% of their control values, respectively ($p = 0.4$, Kruskal-Wallis H test, $n = 6$; Figure 7B). The steady-state voltage dependence of slow inactivation could be fit well by Boltzmann functions, with midpoints of $-62$mV $\pm$ 7mV, $-59$mV $\pm$ 13mV, and $-65$mV $\pm$ 7mV ($p = 0.6$) for transient, persistent, and resurgent currents, and slope factors of 8mV $\pm$ 4mV, 7mV $\pm$ 5mV, and 6mV $\pm$ 3mV ($p = 0.7$).

To ask if slow inactivation regulates pacemaking frequency, we used as a voltage command the spontaneous firing of a cell that was released from several seconds of hyperpolarization to $-90$mV (Figure 7C). In this...
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Figure 7. Slow Inactivation of Pacemaking Sodium Currents

(A) Slow inactivation of transient, persistent, and resurgent sodium currents. (Top) A 20 ms prepulse was followed by three test sequences, each comprising a 100 ms step to −90mV (to allow recovery from fast inactivation), 10 ms conditioning step to −15mV, 100 ms test step to −40mV, and 100 ms recovery step to −90mV. One sequence is shown. 10 s at −90mV preceded the next prepulse. (Bottom) TTX-sensitive current, averaged across three sequences per prepulse voltage, displayed for prepulses between −90mV and −40mV. Peak transient current is off scale.

(B) Slow inactivation of transient current (circles, defined as peak minus steady-state current during the conditioning step to −15mV), persistent current (squares, steady state during test step to −40mV minus baseline), and resurgent current (triangles, peak minus steady state during the test step). Mean conductances are plotted as a function of prepulse voltage and fit with Boltzmann functions, (1 − P)/(1 + exp(V − Vh)/k)), where V is the prepulse voltage, Vh is the voltage of half inactivation, k is the slope factor, and P is the pedestal (n = 6, error bars are SD). Boltzmann functions were fit to G-V relationships of transient, persistent, and resurgent for each cell, yielding mean voltages of half inactivation of −62mV ± 7mV, −59mV ± 13mV, and −65mV ± 7mV, respectively, and mean slope factors of 8mV ± 4mV, 7mV ± 5mV, and 6mV ± 3mV. Diamonds: Voltage dependence of fast inactivation, assessed with 200 ms prepulses from −65mV (n = 4) and fit with a Boltzmann function; average midpoint was −67mV ± 1mV and slope factor 6mV ± 1mV.

(C) Slow inactivation of sodium current during basal spontaneous activity. (Top) Waveform of spontaneous firing taken from a cell that was released from hyperpolarization to −90mV and applied as a voltage command to other cells. The command waveform is preceded by a probe (10 ms step to −15mV and a 100 ms step to −30mV) to evoke the three components of sodium current. Each presentation of the waveform contained the same probe, preceded by 100 ms at −90mV to remove fast inactivation, in a different position to monitor the development of slow inactivation. Three seconds at −90mV separated each presentation. (Inset) The probe voltage protocol, with currents elicited when the probe was presented either before the spike conditioning waveform (larger resurgent current) or 9 s into the waveform (smaller resurgent current). Peak transient current is off scale. (Bottom) Time course of slow inactivation of transient, persistent, and resurgent currents during spontaneous firing, expressed as ratio of test and reference sodium currents (n = 7). Plotted on the right axis (continuous trace) is the relative frequency of spiking in the command waveform. NMDG-phosphate internal solution, TEA/Cs/Cd Tyrode’s external solution. Experiments performed near 23°C.

cell, firing occurred at 79 Hz immediately after release from hyperpolarization and then slowly declined to a steady value of 40 Hz. A test protocol to measure transient, persistent, and resurgent currents was delivered before this waveform, and the same step protocol was embedded in the waveform itself, at a different time point for each presentation. As illustrated in Figure 7C, slow inactivation develops during spontaneous firing after release from hyperpolarization. After 9 s of spontaneous firing, transient current was reduced to 62% ± 5%, persistent current to 77% ± 8%, and resurgent current to 55% ± 9% of their initial values (n = 7). The development of slow inactivation accompanied the slow decline in firing frequency after the release from hyperpolarization.

This result shows that even under basal conditions of spontaneous firing, slow inactivation of sodium channels is substantial and likely influences the frequency of pacemaking. We next explored higher rates of firing driven by exogenous stimulation. These experiments were also done at room temperature, since cell lifetime was too short at 35°C. To roughly match HFS frequency with that of clinical deep-brain stimulation (100–200 Hz), we used a stimulation frequency of 75 Hz, corresponding to a Q10 of about two, similar to the Q10 of pacemaking frequency (Bevan and Wilson, 1999). Of ten cells tested with 75 Hz stimulation for one min, seven fired faster throughout the period of HFS. Three cells followed the stimulation faithfully and resumed pacemaking immediately afterward with little change in frequency (e.g., Figure 8A, top). One cell followed HFS faithfully and resumed firing at a slowed rate that returned to control over several seconds. Three cells followed HFS partially (firing on roughly 40% of the stimuli) and showed a short post-HFS period of silence (between 200 ms and several seconds) before resuming tonic firing that returned to the control rate over 2–10 s (e.g., Figure 8A, bottom). The three remaining cells stopped firing during stimulation and never recovered robust pacemaking afterward, resting near −50mV and firing sporadic single spikes. Overall, these results suggest that, at the single-cell level, HFS induces changes in excitability that generally
outlast the period of stimulation by only a few seconds. However, even in cells that fired on each stimulating pulse, the elicited spikes became progressively smaller and broader, showing a decrease in underlying excitability during the period of HFS.

To examine how sodium current was affected by driven firing, we used the current-clamp recording from a typical cell stimulated at 75 Hz as a conditioning train to other neurons studied under voltage clamp and examined the effect on the various components of sodium current (Figure 8B). This conditioning train reduced transient, persistent, and resurgent sodium currents to 36% ± 5%, 28% ± 16%, and 37% ± 13% of their control values, respectively (n = 6). Recovery from these effects (at a holding potential of −80 mV) occurred in a biphasic manner that was similar for the three components of current (Figure 8C). Each recovered to about 60%–80% of its control value over the first 20 s, followed by a smaller and slower component. The time to 65% recovery was 5 ± 1, 7 ± 4, and 5 ± 3 s for transient, persistent, and resurgent sodium currents (n = 6, Figure 8C). We performed a separate series of experiments to assess recovery at −65 mV (n = 7) and found similar kinetics as at −80 mV, suggesting little voltage dependence to recovery kinetics, similar to previous observations in hippocampal granule neurons (Ellerkmann et al., 2001).

Discussion

Prominent resurgent sodium current has been described previously in only one other cell type, cerebellar Purkinje neurons (Raman and Bean, 1997). Glutamatergic STN neurons and GABAergic Purkinje neurons serve very different functional roles, but they are similar in firing spontaneously at relatively high frequencies (Häusser and Clark, 1997; Raman and Bean, 1997; Beurrier et al., 1999; Bevan and Wilson, 1999). Even without resurgent current, the decay of potassium currents and the influence of background currents would probably depolarize the cell sufficiently for activation of persistent current, which could return the cell to spike threshold. However, pacemaking based on persistent current alone (cf. Taddese and Bean, 2002) is likely to be slower than observed for Purkinje and subthalamic neurons. Two properties of resurgent current are important for driving rapid firing. The most obvious is the injection of inward current immediately after a spike. But, in addition, resurgent current is associated with unusually rapid recovery from inactivation, so that the sodium channels producing resurgent current also reprimed rapidly and are quickly available to pass transient current to fire another action potential (Raman and Bean, 1997, 2001). Both properties promote rapid firing of a subsequent action potential, opposite to the refractory period associated with conventional sodium channels that recover from inactivation without passing current. Modeling studies support the idea that the presence of a resurgent current accelerates both spontaneous rhythmic firing (Khalil et al., 2003) and burst firing during stimulation (D’Angelo et al., 2001).

No detectable Ih flows between spikes, and the effects of Cs+ or ZD7288 on firing were associated with depolarization, probably from effects on potassium channels.
These results support previous evidence against a primary role for $I_h$ in STN pacemaking (Bevan and Wilson, 1999; Beurrier et al., 2000). Although $I_h$ channels are evidently not dynamically activated during the normal pacemaking cycle, they may serve a secondary role by ensuring that cells do not become hyperpolarized beyond the range where resurgent and persistent sodium currents can drive pacemaking. Resurgent sodium current is a "rebound" current that requires a preceding spike, so pacemaking based exclusively on this current would not restart once stopped. Persistent sodium current does not require a preceding spike but does require depolarization positive to $-70$ mV. Hence, pacemaking based on resurgent and persistent sodium currents can be halted by hyperpolarization of just a few millivolts near $-70$ mV. Activation of $I_h$ at these potentials, even if slow and small, could act as a safety mechanism for the pacemaking of STN neurons, even if not usually needed (see also Beurrier et al., 2000). A similar role has been suggested for $I_h$ in cerebellar Purkinje neurons (Williams et al., 2002).

Transient, persistent, and resurgent components of sodium currents in STN neurons are all powerfully regulated by a process of slow inactivation, which can be produced effectively by repeated narrow action potentials as well as by long steady depolarizations. The ability of short depolarizations to produce slow inactivation is similar to other central neurons (Colbert et al., 1997; Jung et al., 1997; Ellerkmann et al., 2001; Taddese and Bean, 2002) but different from sodium channels in skeletal muscle, where short depolarizations are ineffective (Ong et al., 2000). Slow inactivation in STN neurons has an unusually steep voltage dependence, with a slope factor of $\sim 7$ mV, compared to slow inactivation of sodium current reported in other central neurons, with slope factors of $12$ mV–$17$ mV (Kuo and Bean, 1994; Fleidervish et al., 1999). The similarity of slow inactivation for all three components of sodium current suggests that they originate from the same, or at least overlapping, pools of sodium channels, consistent with previous evidence that a single type of sodium channel can contribute to resurgent, persistent, and transient components of current (Raman et al., 1997). However, the relative contribution to each component seems likely to vary among molecular subtypes of sodium channels.

Slow inactivation of the resurgent and persistent sodium currents that drive pacemaking has a negative feedback effect: slower firing rates or hyperpolarization decrease the level of slow inactivation and upregulate the pacemaking sodium currents, while faster firing rates or depolarization decrease these currents. Regulation of transient sodium current by slow inactivation is probably less important to firing rate, since the safety factor for spike formation is high (Madeja, 2000) but may also contribute somewhat by changing the threshold for spike firing.

The steep-voltage dependence of slow inactivation is positioned directly in the physiological operating range of STN neurons, with half-maximal voltages of $-65$ mV to $-59$ mV, typical of the interspike interval during pacemaking. STN neurons receive GABAergic afferents from the GPe, and the physiological reversal potential of the GABA$_A$ conductance is $-79$ mV (Bevan et al., 2000), where removal of slow inactivation is nearly complete (Figure 7B). During normal spontaneous firing, we find that resurgent current is reduced to $55\%$ by slow inactivation (Figure 7C), and faster firing from excitatory cortical and thalamic afferents would reduce it further. Slow inactivation is nearly maximal at $-50$ mV, a voltage reached for hundreds of milliseconds to several seconds during rebound firing (Bevan et al., 2002a), spontaneous bursting (Beurrier et al., 1999), and plateau potentials (Otsuka et al., 2001; Baufreton et al., 2003). Onset and removal of slow inactivation occur with predominant time constants of several seconds and will produce adaptation of excitability on this time scale.

Both resurgent sodium current and persistent sodium current help promote burst firing (Raman and Bean, 1997; D'Angelo et al., 1998; Brumberg et al., 2000) as well as pacemaking. Many STN cells fire rebound bursts following prolonged IPSPs (Bevan et al., 2002a), and some fire spontaneous bursts during a steady, small hyperpolarization (Beurrier et al., 1999). Resurgent current is larger and more prolonged with spike repolarization to the voltages near $-50$ that are typical of bursts than to more negative voltages, even though the driving force is less, and persistent current is nearly maximal at these voltages. Thus, both sodium currents are especially effective at driving high-frequency spiking on top of an underlying depolarization. With long-duration rebound bursts, there is often no sharp termination of the burst but, rather, a gradual deceleration of firing back toward the basal firing frequency (Bevan et al., 2002a). The onset of slow inactivation can account well for this gradual decline of firing frequency, which can take up to 5 to 10 s (Figure 7C).

Initial observations suggested that HFS delivered by deep-brain electrodes to treat parkinsonian motor conditions might silence STN neurons and remove tonic hyperexcitation of its target structures, the GPe and SNr (Benazzouz et al., 2000). However, glutamate release from STN afferents actually increases during HFS (Windels et al., 2000), and GPe and SNr neurons can be excited throughout a period of HFS applied to the STN (Hashimoto et al., 2003). In our experiments, even though HFS produced dramatic slow inactivation of sodium current, many cells still fired with each stimulating pulse throughout a period of HFS. Even when firing did not follow HFS faithfully, the average firing rate was generally higher than under basal conditions. Thus, depolarization block of excitability during HFS was the exception rather than the rule in our reduced preparation. Moreover, the slow inactivation induced by HFS was reversed by $80\%$–$70\%$ in a few seconds. This makes it seem unlikely that tonic firing of STN neurons would be silenced for more than a few seconds following termination of HFS, at least not from direct effects of HFS on sodium channels. Beurrier and colleagues (2001) found that extracellular HFS at very high frequencies ($>166$ Hz) could produce long-lasting silencing of tonic firing in brain slices; there could well be long-lasting effects within the STN of HFS applied in vivo or in brain slices that involve sodium channels less directly, including increases in extracellular potassium, release of modulatory neurotransmitters, or accumulation of intracellular calcium.

Although slow inactivation of sodium currents is unlikely to outlast a period of HFS for more than several
Preparation of Tissue

Long Evans rats (P13–18) were anesthetized with isoflurane and decapitated. One to three coronal slices of 300–400 μm were cut used an internal solution consisting of 108–126 mM KCH₃SO₃, 13.5 mM NaCl, 1.8 mM MgCl₂, 9 mM EGTA, 9 mM HEPES, 14 mM phosphocreatine (Tris salt), 4 mM MgATP, 0.3 mM GTP (Tris salt) (pH 7.2 with HPO₄). In later experiments, including all those in slice at 35°C, we switched to a solution with 90 mM EGTA to better approximate physiological calcium buffering. This solution (K-methanesulfonate solution) was 117 mM KCH₃SO₃, 13.5 mM NaCl, 1.8 mM MgCl₂, 0.09 mM EGTA, 9 mM HEPES, 14 mM phosphocreatine (Tris salt), 4 mM MgATP, 0.3 mM GTP (Tris salt) (pH 7.2 with KOH). The external solution for current-clamp recordings in slices was standard artificial cerebrospinal fluid (ACSF) consisting of 125 mM NaCl, 25 mM NaHCO₃, 2.5 mM KCl, 1.25 mM NaHPO₄, 2 mM CaCl₂, 1 mM MgCl₂, 25 mM glucose, equilibrated with 95% O₂/5% CO₂. To block fast synaptic transmission, this solution also contained 20 μM CNQX, 50 μM D,L-APV, and 100 μM picrotoxin. For the later experiments in slice at 35°C (Figures 5 and 6), the external solution was ACSF but with more physiological KCl (3.5 mM) and CaCl₂ (1.2 mM) and with 1–2 mM kynurenate substituting for CNQX and APV.

Junction potentials (K₃SO₄ solution, −8 mV; KPO₄ solution, −12 mV; 54 and 70 mM NMDGPO₄ solutions, −9 mV and −4 mV; NMDGCl solution, −9 mV), measured using a flowing KCl bridge (Neher, 1992), have been corrected.

In measuring slow inactivation, recordings were rejected if leak and compensated series resistance varied more than 20% or 0.1 MΩ, respectively, or if sodium current changed more than 20%, with an additional requirement in measuring recovery from slow inactivation that control sodium current was >50 pA so that changes could be accurately measured.

Data Analysis

 Signals were filtered at 10 kHz and sampled at 50 kHz or filtered at 5 kHz and sampled at 10 kHz. Some traces were also digitally filtered for display. Data were acquired and analyzed with pClamp 8.0 (Axon Instruments), Igor 3.14 (Wavemetrics, Lake Oswego, OR), DataAccess (Bruxton Corporation, Seattle, WA), and Excel (Microsoft Corporation, Seattle, WA). Statistics are presented as the mean ± SD. Tests of statistical significance employed the Mann-Whitney U test, the Wilcoxon matched pairs test, and the Kruskal-Wallis H test.

Acknowledgments

We thank Ekkehard Kaiser and Richard Born for help with retrograde labeling, and we thank Nathaniel Blair, Alexander Jackson,
Marco Martina, and Andrew Swensen for helpful discussions. Supported by NS36855 and NS38312. M.T.H.D. was supported by a predoctoral fellowship of the Howard Hughes Medical Institute.

Received: October 21, 2002
Revised: March 28, 2003
Accepted: May 16, 2003
Published: July 2, 2003

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