

# Human Subthalamic Neuron Spiking Exhibits Subtle Responses to Sedatives

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## ABSTRACT

**Background:** During deep brain stimulation implant surgery, microelectrode recordings are used to map the location of targeted neurons. The effects produced by propofol or remifentanyl on discharge activity of subthalamic neurons were studied intraoperatively to determine whether they alter neuronal activity.

**Methods:** Microelectrode recordings from 11 neurons, each from individual patients, were discriminated and analyzed before and after administration of either propofol or remifentanyl. Subthalamic neurons in rat brain slices were recorded in patch-clamp to investigate cellular level effects.

**Results:** Neurons discharged at  $42 \pm 9$  spikes/s (mean  $\pm$  SD) and showed a common pattern of inhibition that lasted 4.3 ms. Unique discharge profiles were evident for each neuron, seen using joint-interval analysis. Propofol (intravenous bolus 0.3 mg/kg) produced sedation, with minor effects on discharge activity (less than 2.0% change in frequency). A prolongation of recurrent inhibition was evident from joint-interval analysis, and propofol's effect peaked within 2 min, with recovery evident at 10 min. Subthalamic neurons recorded in rat brain slices exhibited inhibitory synaptic currents that were prolonged by propofol (155%) but appeared to lack tonic inhibitory currents. Propofol did not alter membrane potential, membrane resistance, current-evoked discharge, or holding current during voltage clamp. Remifentanyl (0.05 mg/kg) had little effect on overall subthalamic neuron discharge activity and did not prolong recurrent inhibition.

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## What We Already Know about This Topic

- During deep brain stimulation implant surgery, microelectrode recordings are used to map the location of targeted neurons; anesthetic agents have the potential to alter neuronal activity.

## What This Article Tells Us That Is New

- Using human microelectrode recordings from human neurons and from rat brain slices, electrophysiologic events were analyzed before and after administration of either propofol or remifentanyl. Neither propofol nor remifentanyl produce only clinically relevant alterations of subthalamic neuron discharge activity, suggesting minimal interference with deep brain stimulation implant surgery.

**Conclusions:** These results help to characterize the circuit properties and feedback inhibition of subthalamic neurons and demonstrate that both propofol and remifentanyl produce only minor alterations of subthalamic neuron discharge activity that should not interfere with deep brain stimulation implant surgery.

**D**EEP brain stimulation (DBS) has proven successful for the treatment of movement disorders in patients with Parkinson disease<sup>1–5</sup> and is gaining ground for the treatment of other brain disorders, including major depression<sup>6</sup> and obsessive-compulsive disorder.<sup>7</sup> Many centers use microelectrode recording to map the target site before implantation of the permanent stimulation electrodes. Mapping involves assessment of single-unit neuronal discharge responses to peripheral stimuli such as joint movement or tactile sensation. The use of sedatives during the microelectrode recording mapping procedure has usually been avoided because of the belief that neuronal firing patterns and/or responsiveness to peripheral stimuli could be adversely affected.<sup>8</sup> However, the anxiety and pain associated with the surgical procedure can lead to an increase in blood pressure, which leads to an increase in the risk of intracerebral hemorrhage.<sup>9,10</sup> It would be advantageous to use anxiolytic or sedative medications during DBS surgery if possible. The objective of our study was to provide a systematic characterization of the effects produced by propofol and remifentanyl on discharge responses of subthalamic nucleus (STN) neurons during DBS implantation surgery. An ideal sedative for DBS implant surgery would not interfere with microelectrode

mapping, *i.e.*, would not substantially alter STN discharge activity.

Only a few previous studies have reported the use of sedatives during DBS implantation surgery,<sup>11–13</sup> and none provided a systematic investigation of sedative effects produced on neuron discharge. A recent study of propofol effects on population activity in the STN suggested that the sedative appeared to interfere with microelectrode recording identification; however, single-unit recordings were not undertaken.<sup>14</sup> If sedatives are to be useful for DBS surgeries, then it will be essential to characterize the effects that are produced on single-unit discharge activity. The current study addressed this situation by comparing effects produced by propofol and an active control, remifentanyl, on STN neurons recorded from patients undergoing DBS implantation surgery. In addition, we studied the effects produced by propofol on STN neurons recorded from rat brain slice preparations to characterize the cellular and synaptic actions that underlie effects seen on human STN neurons.

## Materials and Methods

### Patients

We studied 23 patients undergoing DBS electrode implant surgery for the treatment of refractory Parkinson disease, from September 2005 until August 2009, at the Stanford University Medical Center, in Palo Alto, California. All patients were fully informed of our study objectives and procedures and gave their consent to be included in the study before surgery. Our experimental protocol was approved by the Stanford University human subjects Institutional Review Board (Stanford, Santa Clara, California) and conformed to ethical guidelines of the Society for Neuroscience and American Society of Anesthesiologists. Of these 23 patients, 11 provided stable, discriminated single-unit neuron recordings that lasted for the duration of each experiment (12 to 15 min) and were included in our analysis. Two female patients and 9 males were studied. Age, weight, disease stage, and other identifying information was blinded to conform with the Health Information Portability and Accountability Act.

### Surgery

DBS procedures were performed using the Nexframe targeting platform (Medtronic, Inc., Minneapolis, MN) as previously described.<sup>15,16</sup> Briefly, magnetic resonance imaging was used without fiducial markers in place to minimize image distortion. On the day before surgery, skull-mounted fiducial markers were placed and volumetric computed tomography was done. These images were reconstructed using the Framelink software package (Medtronic, Inc.) and targets were chosen in the subthalamic nucleus bilaterally at 11–12 mm lateral to the midline, 5 mm inferior to the anterior commissure–posterior commissure plane, and 3 mm posterior to the midpoint. On the day of surgery, Nexframe

targeting platforms were placed bilaterally, and microelectrode recording was carried out to map the target region.

### Microelectrode Recording

As previously described,<sup>16</sup> a platinum-iridium microelectrode (D-ZAP, FHC Inc., Bowdoinham, MA) was advanced continuously starting from a point 15 mm above the expected target point. Signals were processed through an Axon Guideline 3000 system (Axon Instruments, Sunnyvale, CA) and fed to individual headphones through a Sennheiser wireless transmission system (Sennheiser, Old Lyme, CT). Each member of the surgical team was thus provided with audible feedback during electrode advancement and mapping. Recording electrode signals were amplified (10,000 times) and conditioned by filtering 0.1–10,000 Hz and with direct current offset, before being digitized at 10°K samples per second (Axon Instruments or Cambridge Electronic Design microelectrode recording systems, Cambridge, United Kingdom). Data were stored on a computer disk for subsequent analysis.

In general, one to three recording passes were made, and neuronal responses exhibiting high signal-to-noise ratio and good stability (less than 10% variation in spike frequency) were chosen for additional study. After stable unit recordings were obtained from the STN for 2 min and consistent with our Institutional Review Board approval, 11 patients undergoing STN-DBS were given bolus injections of either propofol (0.3 mg/kg) (N = 7) or remifentanyl (0.8 g/kg) (N = 4), and STN action potential discharge activity was recorded for an additional 10 or more min after administration of the drug. No adverse drug effects were noted and none of the patients reported pain on injection.

### Postexperimental Surgery

After our experiments and any subsequent mapping, DBS electrodes were introduced to the target area and test stimulation was carried out to confirm therapeutic effect and ensure that there were no stimulation related side effects. After surgery, computed tomography was performed to further evaluate lead position, assess postoperative pneumocephalus, and rule out asymptomatic hemorrhage in all patients. The patients had their internal pulse generators implanted in a separate outpatient procedure the week after DBS lead placement.

### Signal Analysis

Single-unit action potential discharges were discriminated using amplitude and time window thresholds set to a tolerance of  $\pm 10\%$  of a manually predetermined spike template for each recording session, using Neuromatic software running under IgorPro (Wavemetrics, Lake Oswego, OR). This inevitably led to a loss of some data, especially for recording sites that exhibited a large cardiobalistic artifact, because some unit discharges would fall below threshold with each

heartbeat (see results), but at least 90% of unit discharge activity was captured for each neuron. Interspike discharge intervals were calculated from the discriminated unit data using IgorPro. Joint-interval analysis<sup>17,18</sup> was performed using the interspike interval data, also in IgorPro.

### Brain Slice Recordings

After approval by the Stanford University Institutional Review Board (Santa Clara, California), male Sprague-Dawley rats (approximately 200 gm; 21 days old) were deeply anesthetized with isoflurane, brains were removed, and 400- $\mu$ m coronal slices were prepared and maintained as previously described.<sup>19</sup> The protocol adhered to the best-practice guidelines of the Society for Neuroscience, and the “Guide for the Care and Use of Laboratory Animals” from the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (Washington, DC). Whole cell recordings of STN neurons were made under visual guidance. Electrode signals were amplified (1,000 times), filtered (0–20,000 Hz) and conditioned with voltage offset (Axon Instruments, MultiClamp 700A) before being digitized and recorded using IgorPro software (10°K samples/s) and stored on computer disk for subsequent analysis. Current clamp signals were analyzed using Neuromatic running under IgorPro; voltage clamp, especially spontaneous inhibitory postsynaptic currents (IPSCs), were analyzed using Minifit, also running under IgorPro.

### Materials

Propofol in an emulsion (Diprivan) was obtained from AstraZeneca (Waltham, MA) for patient use during surgery. Pure propofol for brain slice studies was obtained from AstraZeneca, and stock solutions were prepared in solvent. All chemicals for physiologic solutions were reagent grade or better and obtained from Sigma Chemical Company (St. Louis, MO). Physiologic and drug containing solutions were prepared fresh before each experiment.

### Statistical Analysis

Drug effects were compared with control responses using ANOVA with a post-ANOVA, Newman-Keuls test, running under IgorPro. A criterion of *P* less than 0.05 was taken to be representative of measures that were statistically significant. For all comparisons we used actual values, nonnormalized and not indexed to baseline. Because each patient (or brain slice) had an internal control before drug application to compare to drug effects, we are confident that the small sample sizes we used can provide meaningful comparisons in a two-tailed, repeated measures analysis.

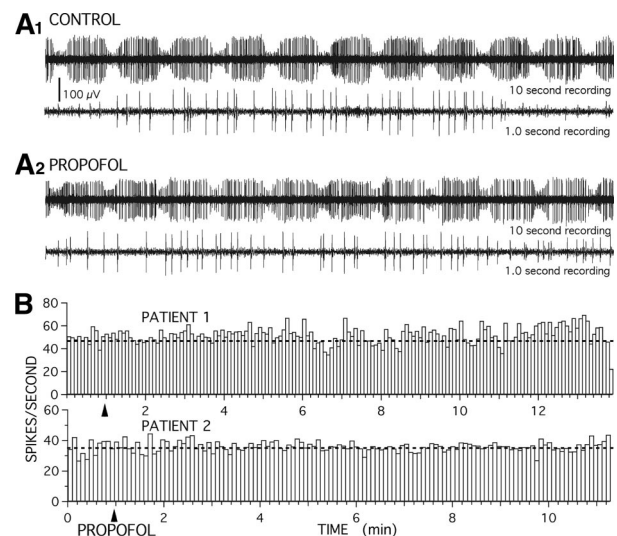
## Results

### Human Subthalamic Neuron Discharge Activity

During control recording periods, STN neurons discharged continuously at approximately 40 Hz (range = 32–67 Hz;

42  $\pm$  9 (mean  $\pm$  SD) for 11 neurons, each recorded from individual patients). Action potential wave forms were similar for all STN neurons recorded and consisted of a multiphasic, small positive, large sharp negative, large prolonged positive, and delayed negative/positive response, all lasting approximately 2.5 ms. Discharge patterns were highly variable, with mixed high and low frequency activity seen that ranged between 1.0 and 300 Hz. Often the highest frequency activity was grouped into short bursts of two to four spikes/burst.

A prominent cardiobalistic artifact was evident for some recording sites (5 of 11), such as the one shown in figure 1. The 10-s long recording shown at the top of this figure (*A*<sub>1</sub>), during the predrug recording of baseline activity (CONTROL), exhibits a cardiobalistic effect producing “burst” discharges occurring at approximately 1.0 Hz. This corresponds to the heart rate, *i.e.*, on each pulse the electrode moves away from the recorded cell and action potential amplitude decreases. Note that after administration of the drug (*A*<sub>2</sub>; PROPOFOL), the pulse artifact decreases secondary to the patient relaxing, with a decrease in their blood pressure. This cardiobalistic artifact makes accurate discrimination of single units impossible, because some spikes will inevitably be lost to analysis when their amplitude falls below criteria ( $\pm$  10% peak-to-peak amplitude,  $\pm$  0.5% peak to valley timing), using template matching. However, the result of



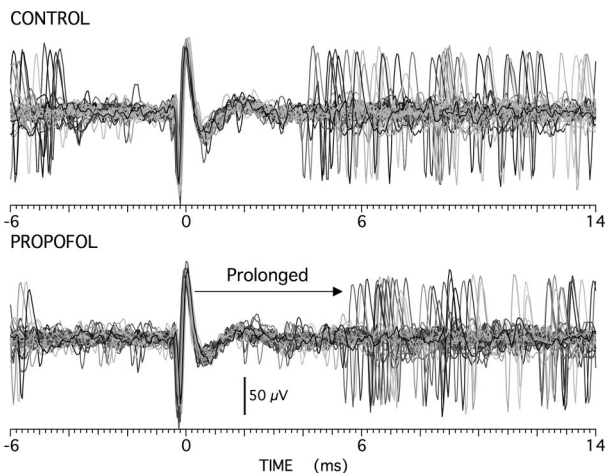
**Fig. 1.** An intravenous bolus dose of propofol (0.3 mg/kg) that produced sedation did not alter the overall discharge activity of subthalamic nucleus (STN). Recordings at the top show the discharge pattern of an STN neuron before (*A*<sub>1</sub> control; top two recordings at a slow and fast timebase) and after propofol administration (*A*<sub>2</sub> lower two recordings). Rate meter graphs on the bottom (*B*) show little effect after propofol administration in two individual patients. Rate meters demonstrate the relatively stable discharge activity of STN neurons, averaged over 5-s epochs, throughout the recording period. A single bolus injection of propofol was given at the time indicated by the arrows (0.3 mg/kg intravenously delivered within 10 s) to awake patients.

this artifact is easily seen and contributes only minimally (and predictably) to subsequent analysis. It should also be noted that mean discharge rates (approximately 40 Hz) calculated using our discrimination techniques were comparable to those reported in previous studies<sup>20,21</sup> of patients with Parkinson disease and in normal monkey STN neurons.

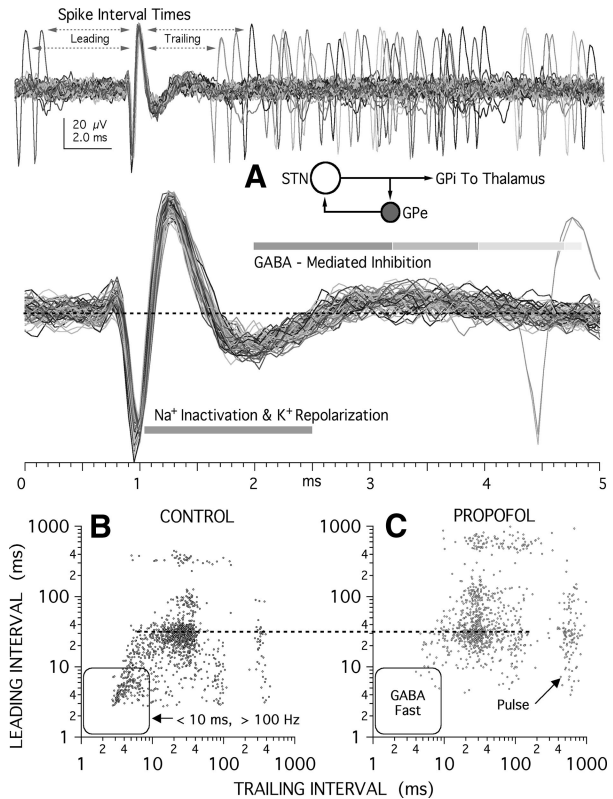
**Propofol Has Little Effect on Neuronal Discharge**

After administration of a bolus intravenous (IV) injection of propofol (0.3 mg/kg), very little effect on discharge activity was seen (fig. 1B). When spike data were accumulated into 5-s bins for rate meter graphs, virtually no effect on discharge frequency was apparent (less than 2.0%), nor did spike discharge variability appear to change appreciably after propofol administration (fig. 1, A, B). Each rate meter graph is from separate recordings in individual patients. Thus, propofol appears to produce sedation with little alteration of STN neuronal discharge activity.

Upon closer inspection of STN action potential discharge (fig. 2), it was clear that propofol had no effect on action potential wave form characteristics; however, the highest frequency discharge activity seen in control recordings was shifted, resulting in a prolonged postdischarge refractory period. The refractory period was prolonged from approximately 4.0 ms in control to 5.5 ms in the presence of propofol (fig. 2;  $4.34 \pm 0.32$  to  $5.58 \pm 0.24$  ms;  $n = 11$ ;  $P < 0.001$ ). In addition, in most neurons (five of seven), propofol increased the background noise slightly, possibly by activating a population of as-yet uncharacterized small amplitude



**Fig. 2.** Propofol (0.3 mg/kg intravenously) prolonged the refractory period of subthalamic nucleus neuron discharge from approximately 4 s to more than 5 s, without changing the spike amplitude, rise time, or decay time, or after polarization. Single-action potential spikes were isolated, aligned to within a one tenth ms of their peak negativity, and superimposed. This selective effect on the highest frequency discharge activity was not evident in rate meters (e.g., fig. 1B) because the same number of spikes still occurred in each 5.0 s period used for analysis.



**Fig. 3.** The propofol-induced prolongation of refractory period is consistent with a propofol-induced increase in the time period of inhibition ( $\gamma$ -aminobutyric acid [GABA]-mediated) from globus pallidus (A; Gpi and Gpe) to subthalamic nucleus (STN). This effect is best seen using joint-interval analysis (bottom graphs), which plot the leading and trailing spike interval times for each action potential in control conditions (B) and after propofol administration (C). Note the reduction in joint intervals less than 10 ms (boxes) produced by propofol, indicating a depression of high frequency (more than 100 Hz) discharge activity. This represents an increased time course for inhibition. Note also that the mean discharge frequency (dashed line) was not altered by propofol. The slow discharge activity indicated by the right arrow for intervals of 500–800 ms is caused by the pulse artifact that was particularly prevalent in this neuron, caused by small movements of the recording electrode relative to the recorded unit on each heart beat (cardiobalistic effect).

interneurons or by synchronizing STN activity. This is consistent with findings from a recent study using multiunit recordings from STN neurons.<sup>13</sup>

To better characterize this subtle propofol effect on high-frequency discharge responses and to look at drug effects on the overall discharge pattern of each neuron, we used joint-interval analysis.<sup>17,18</sup>

**Propofol Alters Neuronal Discharge Seen Using Joint-Interval Analysis**

For joint-interval analysis, the time interval since the last discharge (leading interval) and the interval until the next action potential discharge occurred (trailing interval; fig. 3)

were measured for each action potential in a 1-min recording. These measures were used to construct joint-interval analysis graphs that provide a useful way to visualize discharge activity of each neuron. This analysis is particularly good at characterizing different firing modes that are associated with different components of the circuit that control STN neuronal discharge (fig. 3A). Note the recurrent feedback inhibition from the globus pallidus to STN in the circuit contributes to the refractory period after each action potential (2.5–10 ms). In the joint-interval graphs shown in figure 3, B and C this short latency (short interval) discharge activity can be seen as discharges that occur within 10 ms after the previous action potential (box in lower left; 1–10 ms).

Propofol produced a marked reduction in this short-interval activity (from 48 spikes in control to 6 in propofol), without altering the overall discharge pattern of the neurons (compare fig. 3, B and C). Despite this large effect on short-interval discharge, there was no effect on the average discharge interval (dashed line connecting fig. 3, B and C) for most action potentials. Longer interval discharge activity (more than 200 ms), corresponding to the cardiobalistic pulse artifact, also shifted to longer times as the heart rate slowed in this patient after propofol administration. More spikes were counted in these longer interval clusters because the pulse artifact amplitude was diminished as blood pressure also decreased after propofol administration (compare fig. 3, B and C), thus fewer spikes fell below our threshold for spike detection, because electrode movement was decreased on each heartbeat. Although we did not quantitatively assess sedation, we did ask patients if they felt better or more relaxed. All patients reported that they were more relaxed, and none of the patients lost their ability to respond to questions. We also noted a general decrease in blood pressure and heart rate after the bolus administration in each patient.

Each STN neuron we recorded had a distinct discharge pattern, evident in both control and propofol recordings; *i.e.*, propofol did not dramatically alter the overall discharge activity of these neurons. The joint-interval graphs shown in figure 4A demonstrate these unique discharge patterns seen for four additional neurons, each recorded from individual patients. Note that propofol consistently reduced the short-interval discharge activity of each cell but did not alter the overall shape of each graph.

The time course for propofol's effect was evident from joint-interval graphs as the time to loss and recovery of short-interval discharge activity (fig. 4B). Propofol depressed short-interval activity within 1 min of IV administration. The peak effect was observed at approximately 2 min postinjection ( $123 \pm 8$  s;  $n = 11$ ) and recovery began within 3 min postinjection. Full recovery was evident by 10 min ( $589 \pm 64$  s;  $n = 7$  – four recordings were lost before full recovery).

Also note that propofol did not alter the overall shape of the joint-interval graph for the neuron shown in figure 4B.

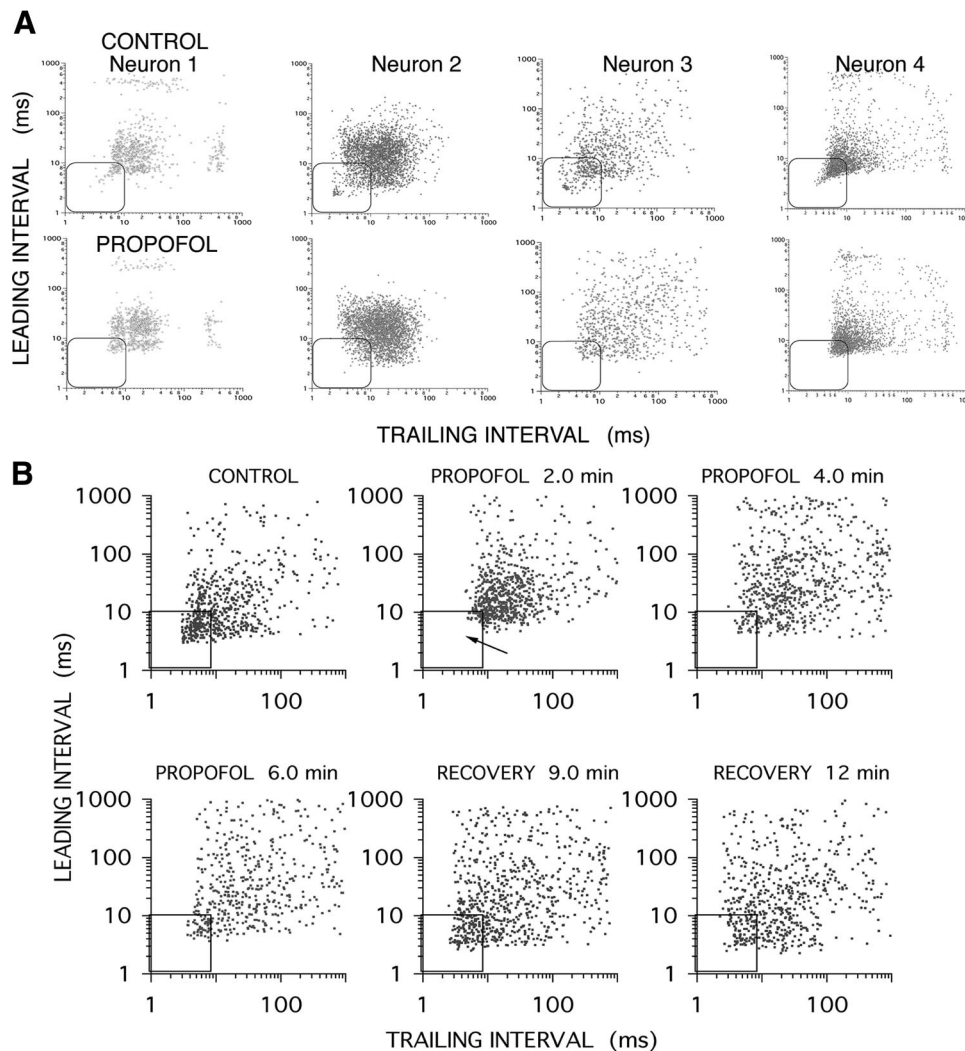
### Propofol Effects on STN Neuron Synaptic Currents and Cell Excitability

The propofol effect on short-interval discharge activity would be consistent with a prolongation of  $\gamma$ -aminobutyric acid (GABA)-mediated feedback inhibition from globus pallidus neurons (fig. 3A). The lack of effect observed on overall discharge frequency is consistent with a lack of effect on tonic GABA receptors in these STN neurons. To test whether these possibilities occur, we measured propofol effects on both tonic and synaptic GABA currents in STN neurons recorded in rat brain slices.

STN neurons were recorded using visual guidance and appeared as a homogenous population of round-shaped cells approximately 15–20  $\mu\text{m}$  in diameter. They were readily identified electrophysiologically because they exhibited spontaneous discharge activity (1.0–5.0 Hz), as previously noted.<sup>22,23</sup> Propofol (2.0  $\mu\text{M}$ ) did not alter the discharge activity of STN neurons recorded from rat brain slices. When cells were artificially depolarized with injected current (fig. 5A), no effect on spike threshold or discharge frequency was seen. Similarly, no effect on hyperpolarizing responses to injected current was evident. We chose a propofol concentration of 2.0  $\mu\text{M}$  to produce an effect site concentration of 0.4  $\mu\text{M}$  at a depth of 0.2 mm (the recording depth in brain slices) based on previous experimental and diffusion modeling results.<sup>24</sup> A concentration of 0.4  $\mu\text{M}$  is thought to correspond to an effect site concentration achieved by the sedative dose administered to patients.<sup>24</sup>

Voltage clamp recordings revealed spontaneous inhibitory postsynaptic currents occurring at  $4.2 \pm 0.7$  Hz ( $n = 1,640$  events in 12 cells recorded from 5 slices; each slice from a different animal). These synaptic currents could be completely abolished using the GABA receptor/chloride channel blocker, picrotoxin (100  $\mu\text{M}$ , fig. 5B), or the competitive GABA antagonist, bicuculline (10  $\mu\text{M}$ , not shown). These currents exhibited fast rise times ( $0.9 \pm 0.2$  ms) and decay times ( $11.3 \pm 4$  ms) with amplitudes of  $23.2 \pm 11$  pA for the inhibitory postsynaptic currents (IPSC) responses recorded from 12 cells.

Propofol did not significantly change IPSC frequency ( $3.9 \pm 8$  Hz;  $n = 12$ ;  $P > 0.1$  compared with control) nor did this sedative alter the rise time ( $0.9 \pm 0.1$  ms). Similarly, there did not appear to be any change in the need for holding currents to voltage-clamp STN neurons at  $-60$  mV. Propofol did significantly prolong the decay time constant of IPSCs ( $17.8 \pm 6$  ms;  $P < 0.01$  compared with control; fig. 5C). Summary data for propofol effects on resting membrane potential, membrane resistance, evoked spike discharge frequency, IPSC frequency, IPSC amplitude, IPSC decay time ( $\tau$ ) and holding current ( $I_m$ ) have been normalized and are presented in figure 5D. Note that only effects



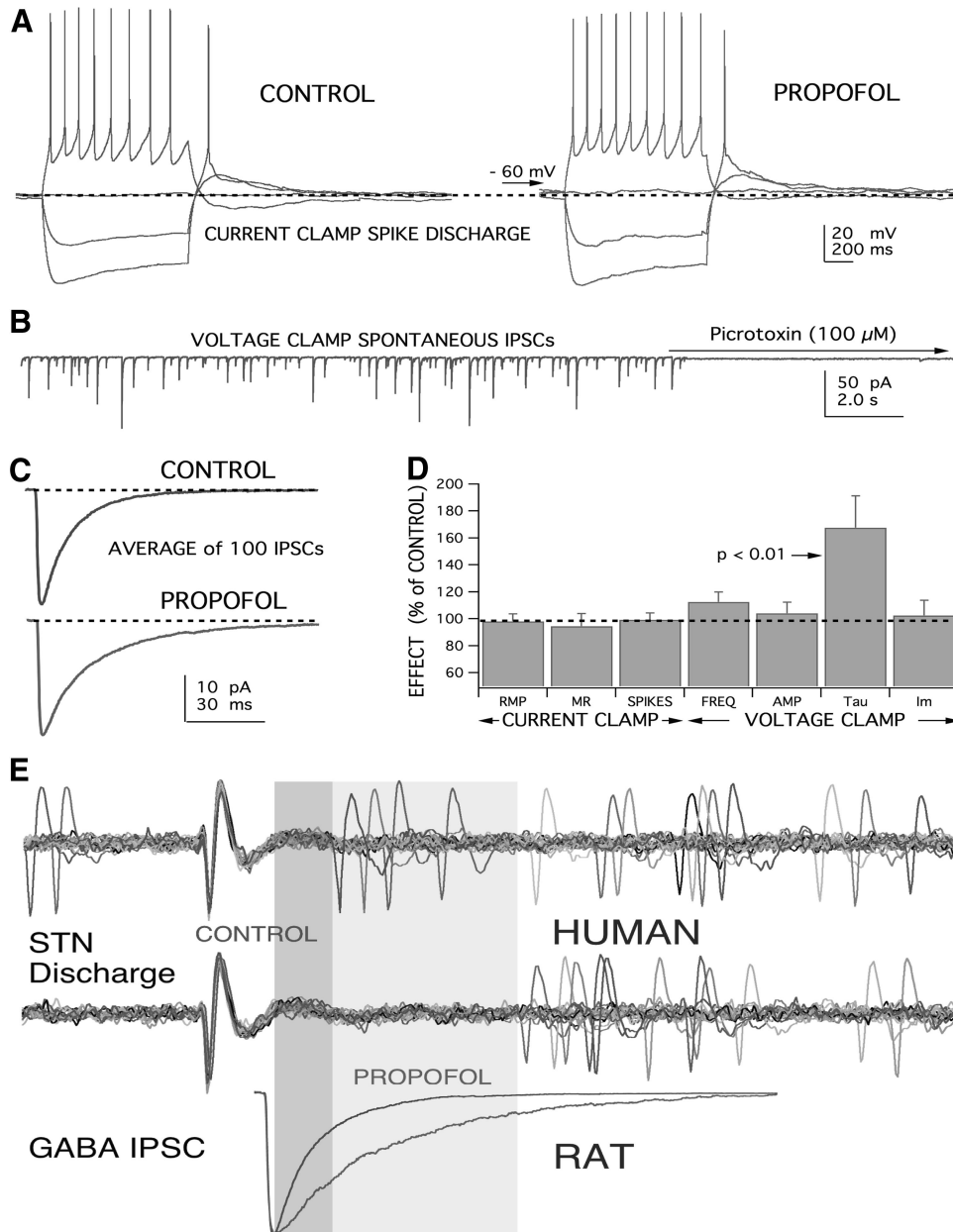
**Fig. 4.** (A) Joint-interval graphs for four subthalamic neurons demonstrating the consistent depression of short interval discharge activity (less than 10 ms; boxes). Also note that each neuron exhibited a unique discharge pattern, although mean discharge frequencies were very similar for each cell. Propofol did not appear to alter the overall discharge pattern of any of the neurons, except for the selective depression of high frequency discharge. The neurons exhibited various degrees of cardiobalistic pulse artifact. (B) Joint-interval graphs showing the time course of effects on a subthalamic neuron. Short latency spike intervals (arrow;  $< 10\text{ ms}$ ; boxes) were depressed after a bolus administration of propofol (0.3 mg/kg intravenously). Indicated times are from the start of the bolus. The sedative had a rapid onset, peaking at 2.0 min and a gradual recovery that required approximately 10 min after administration.

on the decay time constant of IPSCs were significant ( $P < 0.01$ ). The prolongation of IPSC decay time mapped nicely onto the increase in refractory period produced by propofol on human STN neuron discharge activity (fig. 5E), when time course was corrected for temperature differences (scale factor = 1.8; based on comparing IPSC responses recorded at  $37^{\circ}\text{C}$  vs.  $22^{\circ}\text{C}$ ).<sup>25,26</sup>

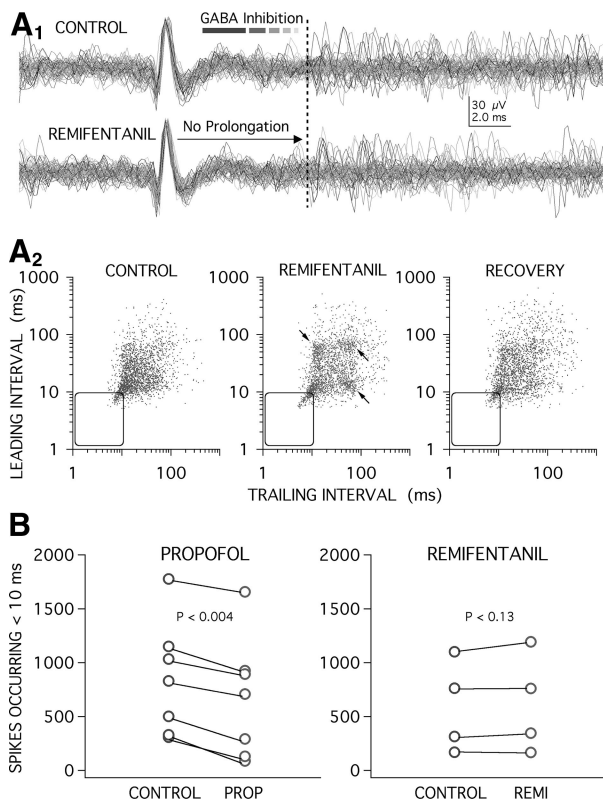
#### Remifentanyl was Used as an Active Control Sedative to Compare with Propofol

To determine whether the propofol-induced prolongation of refractory period was caused by a direct effect of this sedative on STN neuron GABA-mediated inhibitory currents, we used a non-GABA enhancing sedative, the

opioid remifentanyl, as an active control drug. When remifentanyl was administered as an intravenous bolus (0.5  $\mu\text{g}/\text{kg}$  in 5 s), similar to propofol, it also produced a rapid onset (approximately 1 min) and short-lasting period of sedation (less than 10 min). Unlike propofol, there was no change apparent for short-interval discharge activity of STN neurons (fig. 6A). In fact, either no change or a slight increase in short interval activity was noted in the four cells studied ( $1,880 \pm 560$  spikes in control,  $2,150 \pm 580$  in remifentanyl), but this change was not significant. Remifentanyl did not dramatically alter the shape of joint-interval graphs, but a tendency to produce burst discharge was seen in two of the recorded cells, and this can be seen as clusters in the joint-interval graphs (fig. 6, middle



**Fig. 5.** Propofol (2.0  $\mu$ M) increased the decay time constant of inhibitory postsynaptic currents (IPSCs) with little effect on the overall excitability of subthalamic nucleus (STN) cells. (A) STN neurons recorded in rat brain slice preparations exhibited prominent  $I_h$  currents and rebound discharge, similar to other types of thalamic neurons. Propofol did not alter the resting membrane properties nor discharge pattern of these cells in response to hyperpolarizing or depolarizing current injection. (B) Spontaneous IPSCs recorded from STN neurons were very homogenous, exhibiting fast rise times (less than 1.0 ms) and monoexponential decay times of  $10 \pm 2.4$  ms. They were completely eliminated using the chloride channel blocker, picrotoxin. (C) Propofol increased the decay time constant of these IPSCs recorded from STN neurons by approximately 70%. (D) Bar graphs summarizing the lack of propofol effect on current clamp responses, including resting membrane potential (RMP), membrane input resistance (MR), and spike discharge responses (SPIKES). Similarly, propofol had little effect on voltage clamp responses, including IPSC frequency (FREQ), IPSC amplitude (AMP), and holding current ( $I_m$ ) needed to clamp neurons at their initial resting potential; however, the decay time constants for IPSCs (Tau) were significantly increased. (E) The propofol-induced increase in  $\gamma$ -aminobutyric acid (GABA) IPSC decay times recorded from rat STN neurons were well correlated with the increase in refractory period observed for human STN discharge activity. The time course of IPSCs have been temperature compensated from 22°C (brain slice recording temperature) to 37°C to allow a direct comparison of human and rat response times. Thus, the major effect produced by propofol on spike discharge activity recorded from humans is consistent with a prolongation of GABA-mediated inhibition.



**Fig. 6.** We used an active control drug, remifentanil, to produce a comparable degree of sedation, but *via* a mechanism that does not involve inhibitory synapses. (*A*<sub>1</sub>) Remifentanil did not prolong the refractory time for subthalamic nucleus (STN) neuron discharge as did propofol. (*A*<sub>2</sub>) Joint-interval graphs demonstrate the remifentanil did not depress short latency discharge (less than 10 ms; boxes) as did propofol; however, the opioid sedative appeared to alter the discharge pattern of STN neurons in a manner different from propofol. The altered discharge pattern can be seen in the middle graph (arrows), where clusters of spikes appeared in the presence of the opioid, indicating an increased tendency to fire bursts of action potentials. The effect fully recovered after approximately 10 min. (*B*) To quantify and compare the effects produced by both sedatives, the number of spikes occurring with intervals of less than 10 ms were counted (from the boxed region in joint-interval graphs). Propofol produced a consistent and significant reduction in short-interval discharge activity recorded from seven neurons, each from individual patients. Remifentanil did not significantly alter the number of short interval spikes seen in four patients, although a trend toward higher frequency activity was evident, consistent with the opioid-induced burst discharge patterns seen in joint-interval graphs.

graph, arrows). Summary data for seven propofol and four remifentanil experiments are shown in figure 6B, and it is clear that the two sedatives produced significantly different effects on STN neuron short-interval discharge activity: propofol consistently reduced this activity ( $P$  less than 0.004), whereas remifentanil did not.

# STANPUMP program. Available at: <http://www.opentcl.org>. Accessed May 7, 2010.

## Discussion

### Sedatives Do Not Dramatically Alter STN Neuron Discharge Activity

This is thought to be the first study to systematically investigate sedative effects on STN neuron discharge activity recorded intraoperatively from humans. The results indicate that both propofol and remifentanil have only minimal effects on action potential discharge activity at the doses studied, *i.e.*, discharge frequencies changed less than 2.0% with either sedative. Our findings agree with a growing body of literature that indicates that sedation can be used in patients undergoing DBS electrode implant surgeries,<sup>1,12,13</sup> with little disruptive effect on neuronal discharge responses. Previous studies did not compare sedative effects on the same neurons before and after drug administration, so they lacked the internal control we had for each experiment. Similarly, previous work did not analyze single-unit discharge activities before and after drug administration, but focused on multi-unit or local field potential responses. That said, our results agree with previous findings indicating that sedatives have little effect on STN discharge. We targeted drug levels for sedation based on STANPUMP models<sup>27,28</sup> and the protocol we used would be predicted to produce a peak effect site concentration of 1.34  $\mu\text{g/ml}$  at 1 min, 40 s postinjection (approximately 0.4  $\mu\text{M}$ ), using the Schnider model and a  $k(e0)$  with a fixed time-to-peak of 1.6 min.<sup>28</sup> It should be noted that we did not administer the sedatives as an infusion, only as a bolus. This is comparable to a steady-state infusion dose of 50  $\mu\text{g/kg/min}$  – a typical sedative dose in these patients. Of course, individual patients will exhibit varying degrees of sedation in response to this infusion dose, and this should only serve as an approximation for initially targeting appropriate doses for the clinical management of each individual, in any subsequent DBS surgeries. Data from the STANPUMP model closely paralleled our drug kinetic results (fig. 4B), with effect site concentrations peaking within 2 min and falling below effective levels within 10 min. Patients reported feeling sleepy, calm, and/or relaxed after the bolus injection, but none lost consciousness. Additional studies will be needed to determine how microelectrode mapping in this nucleus will be altered while using these sedatives, with voluntary or imposed joint movements driving evoked discharge; however, it is clear that spontaneous background discharge activities are not markedly disrupted. Sedation would be desirable in these patients because growing anxiety and discomfort are leading causes of concern during these surgeries and often contribute to increases in blood pressure that can increase intracranial bleeding.

### STN Neuron Discharge Patterns are Unique and Remain Stable Over Time

STN neuron discharge patterns were quite variable, although mean discharge frequencies were very consistent. The variability can be seen in joint-interval graphs (figs. 3



and 4) comparing different neuron firing patterns, where unimodal, fanlike patterns, bimodal low- and high-frequency clusters, and multimodal discharge patterns can be seen. It was interesting that each neuron's discharge pattern remained mostly stable for the duration of each recording (10–15 min; fig. 4) even in the presence of propofol or remifentanyl. It is not known whether these discharge patterns reflect the individual electrical properties of each cell, or its position within a neural circuit, or to what extent the Parkinson disease pathology contributes to these firing patterns. It was clear, however, that feedback GABA-mediated inhibition largely determines the limit for short-interval discharges, because this was consistently increased in each neuron after propofol administration (figs. 3 and 4). To our knowledge, this is the first report to demonstrate both the uniqueness and stability of STN neuron firing patterns. It will be useful to explore joint-interval analyses for comparing STN discharge patterns in animal models of Parkinson disease, as well as in control recordings of STN neurons, because this may reveal interesting discharge properties of the disease state.

### Propofol Prolongs GABA-mediated Inhibition of STN Neurons

Propofol produced an increase in refractory periods for STN neuron discharge, and this effect was completely consistent with a prolongation in the time course of feedback GABA-mediated inhibition from globus pallidus neurons. Drugs such as propofol have been shown to prolong GABA-mediated inhibition in a number of brain regions. In fact, this is the most consistently reported effect produced by propofol from a number of independent laboratories.<sup>19,29</sup> We confirmed that STN neurons receive GABA-mediated synaptic inhibition, using voltage clamp recordings from STN neurons in rat brain slices. We also showed that propofol prolongs the decay time constant of IPSCs recorded from these cells. Furthermore, the degree of IPSC prolongation was entirely consistent with the increase in postspike inhibition seen in human recordings (fig. 5E).

The concentration of propofol we applied to brain slices was chosen to approximate effect site concentrations thought to pertain *in vivo* during sedation. We arrived at this finding by first measuring the brain concentrations achieved in rats after an anesthetic dose of propofol.<sup>24</sup> We then used both chemical measures and diffusion modeling to determine what the applied propofol concentration should be, to achieve this effect site concentration in our brain slices.<sup>24</sup> We then followed this up with physiologic measures of the ability of this applied concentration to achieve steady-state effects in our brain slices. We found that it required more than 5 h for propofol to achieve steady-state effects, because of the slow diffusion into brain slices. We found an excellent agreement between our chemical measures, model predictions, and actual physiologic measures.<sup>24</sup> That said, it should be noted as a major caveat that actual propofol effect site concentrations

are not known—and have never been directly measured. Neither is it known whether an effect site concentration in rats is the same as in humans, but it is likely that the higher doses needed to sedate rats, compared with humans, are the result of both pharmacokinetic and pharmacodynamic differences between species. Our observation of the similar increase in time course of inhibition seen in both human recordings and rat brain slice responses lends support to the idea that our model of propofol kinetics appears to closely approximate real conditions.<sup>24</sup>

Surprisingly, STN neurons do not appear to express tonic GABA receptors, because propofol did not depress current-evoked discharge in current clamp recordings from rat neurons (fig. 5A), nor were holding currents changed in voltage clamp (fig. 5D), nor was there any indication that propofol produced a general depression of human STN neuronal activity (figs. 1, 3, and 4). Propofol has been shown to potently activate tonic GABA channels in neurons from hippocampus,<sup>19,30</sup> thalamic,<sup>31</sup> and neocortical regions.<sup>32</sup> Even GABAergic inhibitory interneurons have been shown to express propofol-sensitive tonic GABA currents.<sup>30</sup> CA1 pyramidal neuron,<sup>30</sup> spinal neuron,<sup>33</sup> and neocortical neuron<sup>34</sup> spontaneous discharge activity is markedly depressed by propofol acting through enhanced tonic GABA currents. To the best of our knowledge, STN neurons are the only cells that do not have these tonic currents. Thus, some of the results from our study may not generalize to other brain regions where neurons do express tonic currents. However, the propofol-induced prolongation of GABA-mediated IPSCs would be expected to occur throughout the brain.<sup>29</sup>

This finding raises an interesting observation—propofol's actions on STN neurons can be explained by local actions on these neurons, even though it is obvious that this sedative would affect neurons providing input to the STN, especially layer V cortical pyramidal neurons<sup>2</sup> as well as neurons downstream from the STN that project back to STN (*e.g.*, globus pallidus GABAergic neurons). It appears that effects on other brain regions do not markedly affect STN neurons, or that whatever effects do contribute to altered STN discharge are largely masked by the local effects produced directly on the STN cells. Another interpretation could be that compensatory responses to propofol can balance effects throughout the brain; for example, perhaps the tonic inhibition of pyramidal neurons is balanced by tonic inhibition of inhibitory interneurons such that the net excitatory drive from neocortex to STN neurons remains constant, especially for the relatively low doses used in our study.

### Different Sedatives Operate by Different Mechanisms

Different chemical/pharmacologic classes of drugs produced different effects on STN action potential discharge activity. Propofol depressed short-interval discharge activity, whereas remifentanyl did not. These observations are consistent with a growing body of literature indicating that depressants ap-

pear to operate through quite different mechanisms to achieve a similar degree of sedation.<sup>19,26</sup> There appear to be different pharmacologic routes to the endpoint of sedation. Additional studies will need to focus on the differences between propofol's and remifentanyl's actions on STN neurons, especially regarding the burst discharge clusters we observed after administration of the opiate. Opiates have been shown to produce disinhibition by depressing GABA-mediated synaptic inputs to pyramidal neurons,<sup>35</sup> so it is possible that a similar effect is occurring on STN neurons or on neurons upstream from these cells.

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