Multistable properties of human subthalamic nucleus neurons in Parkinson's disease

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Edited by Peter L. Strick, University of Pittsburgh, Pittsburgh, PA, and approved October 15, 2019 (received for review July 18, 2019)

To understand the function and dysfunction of neural circuits, it is necessary to understand the properties of the neurons participating in the behavior, the connectivity between these neurons, and the neuromodulatory status of the circuits at the time they are producing the behavior. Such knowledge of human neural circuits is difficult, at best, to obtain. Here, we study firing properties of human subthalamic neurons, using microelectrode recordings and microstimulation during awake surgery for Parkinson's disease. We demonstrate that low-amplitude, brief trains of microstimulation can lead to persistent changes in neuronal firing behavior including switching between firing rates, entering silent periods, or firing several bursts then entering a silent period. We suggest that these multistable states reflect properties of finite state machines and could have implications for the function of circuits involving the subthalamic nucleus. Furthermore, understanding these states could lead to therapeutic strategies aimed at regulating the transitions between states.

plateau potentials | multistability | finite state machines | deep brain stimulation | microstimulation

The mammalian basal ganglia play critical roles in motor control and higher functions in normal and pathological conditions (1). The physiology and pathophysiology of basal ganglia circuits, however, are incompletely understood. Many studies over several decades have outlined electrophysiological and connectivity patterns of basal ganglia neurons (1, 2). These studies have led to a number of models to explain, in particular, the pathophysiology of human diseases such as Parkinson's disease (PD; ref. 3).

It is no surprise that understanding circuits has proven to be difficult, with large initiatives launched aiming to understand neural circuits for behavior (e.g., https://braininitiative.nih.gov/, https://www.humanbrainproject.eu/en/). But it has proven difficult to thoroughly understand even the simplest of neural circuits. In the 1980s, Getting outlined a stepwise approach toward understanding motor circuits (4). Two fundamental steps included mapping synaptic connectivity and providing a detailed description of the electrophysiological properties of the neurons involved, as knowledge of wiring alone is insufficient to explain circuit function (5). We would now add to this that knowledge of the neuromodulatory state (e.g., modulation produced by endogenous monoaminergic input) of the neurons and circuits is also required (6). To date, it has not been possible to gain this knowledge to the degree necessary to understand even the simplest of mammalian neural circuits.

Perhaps the most thoroughly studied motor circuit is that of the crustacean stomatogastric ganglion (7). The ∼30 individual neurons of this ganglion and their connectivity have been well characterized, leading to fundamental understanding that circuits are not fixed, but can be reconfigured for different motor behaviors (8). That is, understanding connectivity in the absence of knowledge of neuronal properties and modulatory state is insufficient for the understanding of circuit function. The lessons learned from the study of invertebrate circuits have proven to be invaluable to the study of mammalian, including human, neural circuits underlying behavior. In particular, it is clear that the thorough characterization of complex neuronal properties is critical for understanding the modus operandi of neural circuits.

The connectivity of the excitatory subthalamic nucleus (STN) of the basal ganglia is well understood: it receives inputs from the globus pallidus externa (GPe), motor cortex, and substantia nigra pars compacta, and projects to the GPe, globus pallidus interna, and substantia nigra pars reticulata. Furthermore, the basic electrophysiological properties of these neurons is reasonably well understood, with resurgent and persistent sodium- and calcium-dependent potassium conductances playing key roles for repetitive firing, and low-threshold calcium currents playing a role in postinhibitory rebound (9). Furthermore, in rat STN slice preparations, these neurons have at least 4 different stable states (10). Two of these states are nonspiking (a resting downstate and a silent upstate), and 2 are spiking (rhythmic bursts or tonic firing). The silent upstate, a stable depolarized plateau potential, can be evoked after brief synaptic excitation or after postinhibitory rebound on termination of synaptic inhibition (10). How these multistable states participate in normal physiological or pathophysiological function is not clear, but multistable states have been implicated in neural circuit function, with changes in the perturbation of these states leading to disease phenomena such as epilepsy (11). If human STN neurons are likewise multistable, it is conceivable that dysregulation of this multistability would lead to circuit and behavioral dysfunction.

The STN is a key target for treatment of PD via implanted deep brain stimulating (DBS) devices (12). The mechanisms of action of DBS are not understood, with several potential models proposed (13–15). Understanding the underlying mechanisms by

Significance

Behaviors are realized through concerted activity in neural circuits. This activity results from a combination of neural connectivity and the properties of the involved neurons. By studying the activity of neurons in the human subthalamic nucleus during surgery for Parkinson's disease, we report that these neurons have multiple stable states, and that brief electrical stimuli can lead to transitions between states. We thus suggest that these neurons function as finite state machines. The different states could influence the function of key motor circuits of the basal ganglia, and thus knowledge of these states in disease or in response to treatment could help to define new treatment strategies for people with movement disorders.

Author contributions: H.H. and R.M.B. designed research; R.M.B. performed research; J.W.C. analyzed data; R.M.B. wrote the paper with contributions from all authors; and J.W.C. made figures.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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First published November 11, 2019.

which neuromodulation therapies work is key for the development of new modulation therapies (16). If multistable states are important for normal and pathophysiological function of the STN, then it would be helpful to understand their regulation and the effects that applied DBS current pulses have on these states. Such understanding could lead to more refined treatment strategies, including, for example, medications that specifically alter circuit properties, more specific stimulation strategies, or gene therapies aimed at restoring physiological properties altered in disease (17).

Here, we have asked whether human STN neurons recorded extracellularly during surgery for PD have fingerprints of multistability. We define a stable state as being a state of action potential firing or silence that outlasts the stimulus and continues for at least 1 s before reverting back to the prestimulus state. We used brief microstimulation to locally affect the recorded neurons, and found that doing so can lead to state changes similar to those reported to rat STN neurons in slice preparations (10, 18, 19). That is, we provide evidence that human STN neurons in PD have multiple stable states, and could thus be considered as finite-state machines, units in which a specific transition stimulus leads to a change from one defined state to another. We suggest that understanding any dysfunction of multistable properties can lead to new specific therapeutic strategies.

Results

In total, we analyzed 130 stable recordings (83 in which microstimulation was used) in 32 subthalamic nuclei in 28 patients. Recordings lasted for periods of 28 to 380 s (mean, 106 ± 72 s; means ± SDs used throughout). In each recording, we discriminated 1 to 2 separate neurons and selected for analysis a single neuron that we could clearly discriminate pre- and poststimulation. In the 72 neurons in which we recorded state changes, stable prestimulus recordings lasted 5.2 to 42.9 s (mean, 14.6 ± 9.5 s), during which the firing rates were 4.5 to 71.5 Hz (mean, 26.5 ± 18.4 Hz), as is typical for STN neurons.

We first retrospectively searched for evidence of spontaneous periods of firing cessation, as these might be expected with changes in state (10). We found silent periods lasting greater than 1 s in 14 recordings (11%) from 11 patients; each of these silent periods was followed by resumption of the pre-silent period firing frequencies (Fig. 1*A*). The average duration of spiking cessation was 2.3 ± 1.5 s (range, 1.0 to 6.4 s; Fig. 1*B*). These spontaneous silent periods are compatible with spontaneous state changes in these neurons, either to a downstate or a silent upstate.

We typically use microstimulation through the recording microelectrode as part of our clinical mapping procedure for DBS procedures. On electrode advancement, currents used were typically less than 10 μA at frequencies of 300 Hz lasting 500 ms. None of the microstimuli within the STN was perceived by the patient. Our clinical recording system led to a blanking of the recording during and immediately after microstimulation (lasting ~185 ms after stimulation), so immediate poststimulus firing could not be assessed. Nonetheless, in 72/83 recordings, we saw 4 distinct state changes in response to microstimulation, all followed by recovery to prestimulus states: increased firing, cessation of firing, decreased firing, or transient burst firing followed by a silent period.

In 21 neurons (10 patients), brief microstimulation led to a sustained increase in firing rate (Fig. 2; mean increase, 6.8-fold; pre: 17 ± 11 Hz [range, 7 to 30 Hz]; post: 97 ± 73 Hz [range, 21 to 181 Hz]; $P < 0.0001$). These increases lasted 9.8 ± 9.1 s before recovering to prestimulus firing rates. In some of these cells ($n = 9$), subsequent microstimuli were delivered and similar increases were seen; that is, the responses were repeatable. Although not a change in spiking state per se (the neuron remained tonically active), this result suggests that the brief stimulus can toggle the neuron to at least one other stable firing frequency set point.

In a number of neurons, microstimulation led to a reduction or cessation of firing. In 6 neurons (5 patients), the firing rate decreased (mean decrease, 70%; pre: 48.6 ± 26 Hz; post: 14.6 ± 12.4 Hz; $P = 0.005$) for, on average, 24 ± 35 s (range, 3.1 to 85.0 s) before

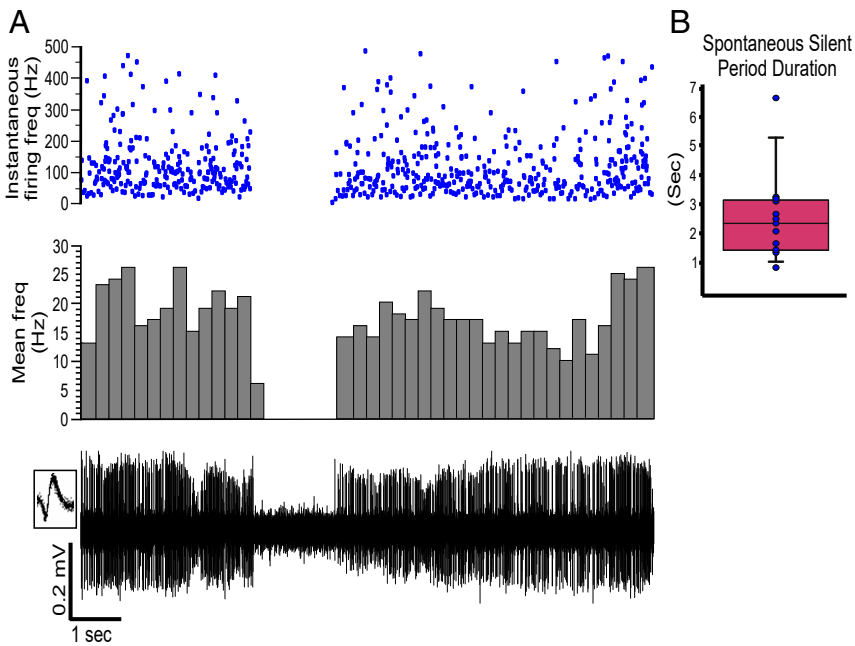

Fig. 1. Spontaneous silent periods in STN neurons. Spontaneous silent periods lasting greater than 1 s were noted in 14 neurons from 11 patients. (*A*, *Bottom*) Trace is a representative recording demonstrating a spontaneous silent period of 1.7 s followed by recovery of the repetitive firing. (*Inset*) Spike waveform. (*Middle*) Mean frequency during the recording (bins of 0.25 s). (*Top*) Instantaneous firing frequencies during the recorded period. (*B*) Median and quartile ranges of the spontaneous silent periods. Each dot represents the silent period duration of an individual neuron.

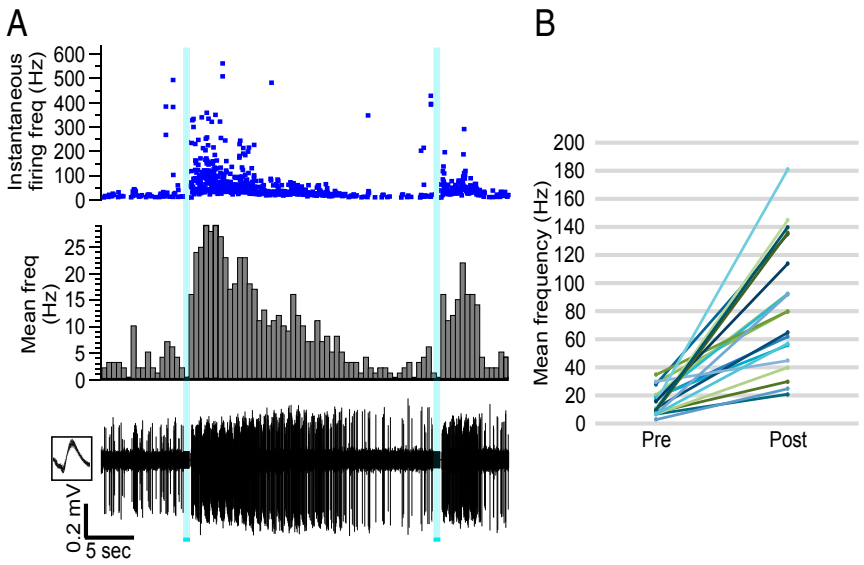

Fig. 2. Increased STN neuron firing in response to microstimulation. Increased firing in response to microstimulation was seen in 21 neurons recorded from 10 patients. (*A*, *Bottom*) Trace is a representative recording in which an identifiable neuron (*Inset*) demonstrates an increased firing rate in response to microstimulation (light blue, 10 μA, pulse duration 500 μs, 300 Hz for 500 ms). (Note amplitude modulations are a result of cardiac rhythm.) (*Middle*) Mean frequency during the recording (bins of 0.25 s). (*Top*) Instantaneous firing frequencies during the recorded period. (*B*) Increase in firing rate after stimulation for each of the neurons. Each line represents an individual neuron that demonstrated an increase in baseline firing (pre) in response to microstimulation (post).

returning to prestimulation firing frequencies. In 6 other neurons (4 patients), stimulation led to a complete cessation of firing followed by recovery to prestimulation firing rates after 5.8 ± 1.7 s (range, 4.1 to 8.3 s; Fig. 3). In an additional 14 neurons, there was a cessation of firing with no or limited recovery during the brief recording period. Interestingly, 5 of these neurons were the only ones microstimulated with amplitudes higher than 10 μA (15, 40, 40, 50, and 60 μA). Thus, it is possible that these 14 neurons were damaged by the stimuli, or that we simply lost the recordings, rather than that these neurons had state changes. Nevertheless, the recovery of about half the neurons (n = 12) suggests that microstimulation could lead to a stable reduction in firing rate, or cessation of firing that could be mediated by either a transient downstate or silent upstate: the state cannot be determined in the absence of membrane potential recording, as can be done in the rodent (10). Note that some of these neurons may have some initial poststimulus firing (vide infra) that was not seen because of amplifier blanking.

Interestingly, in 2 neurons, there appeared to be a single isolated spike after the silent period (Fig. 3, arrow; see also Fig. 4 *A*, *i*, arrow). Such a spike has been seen after silent up-states in rat STN neurons, in which it is thought to be a dendritic spike that leads to termination of the up-state (19). In our extracellular recordings,

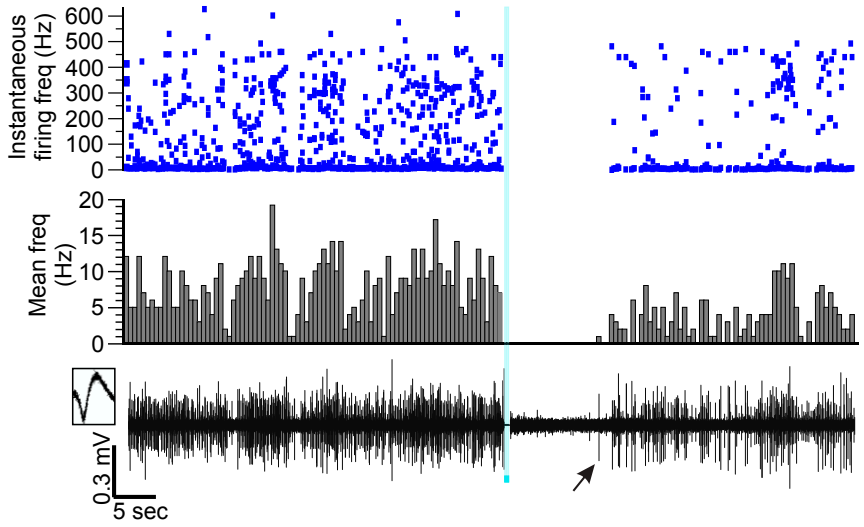

Fig. 3. Cessation of STN neuron firing after microstimulation. Microstimulation led to a silent period followed by recovery to prestimulation firing rate in 6 neurons from 4 patients. (*Bottom*) Trace is a representative recording in which a discriminated neuron (*Inset*) demonstrates cessation of firing in response to microstimulation (light blue, 10 μA; pulse duration, 500 μs at 300 Hz for 500 ms). Arrow indicates a possible terminating spike. Note that although this recording includes more than 1 neuron, the analysis is on a single neuron based on spike discrimination and PCA as described. Note that the peak-to-peak amplitudes of the analyzed neuron ranged from 120 to 420 μV, and were thus at least twice the amplitude of the background activity (60 μV). (*Middle*) Mean frequency during the recording (bins of 0.25 s). (*Top*) Instantaneous firing frequencies during the recorded period.

however, we could not discriminate any distinguishing characteristics in the spike shape of this terminating spike compared with the shape of either earlier or subsequent spikes, so we cannot speculate as to whether or not it was dendritic in origin.

Microstimulation often led to a more complex response ($n = 14$ in 6 patients) consisting of a short period of irregular spiking ($n = 3$), a single burst ($n = 2$), or multiple ($n = 9$) repetitive bursts, with each of these response types preceding a silent period (Fig. 4 *A* and *B*). In the 11 bursting cells, after the ∼185-ms amplifier blanking period, there were 1 to 8 bursts (mean, 3.6 ± 2.2 bursts) comprised of 3 to 11 spikes per burst (mean, 5.3 ± 2.1 spikes), with each burst lasting 5.7 to 26.1 ms (mean, 13.5 ± 6.9 ms; Fig. 4*C*). Within the bursts, there was a progressive slowing of instantaneous firing rate (Fig. 4 *A, iii* and Fig. 4*B*), consistent with what is seen during spike frequency adaptation (20). The bursts occurred at frequencies between 3.5 and 35.0 Hz (mean, 10.5 ± 7.9 Hz; Fig. 4*D*). In 4 neurons, repeated stimuli led to a qualitatively similar pattern, but the burst parameters were not stereotypical. After these bursts, there was a silent period that lasted 3.1 to 5.8 s (mean, 4.4 ± 0.8 s; Fig. 4*E*) before recovering to prestimulus firing rates. There was no correlation between the duration of the bursting episodes (0.2 to 1.1 s post blanking period) and the duration of the silent period (Fig. 4*F*).

Finally, in some neurons ($n = 11$ in 7 patients), repeated stimuli led to different state changes. For example, neurons that were silenced or had a reduced firing frequency in response to

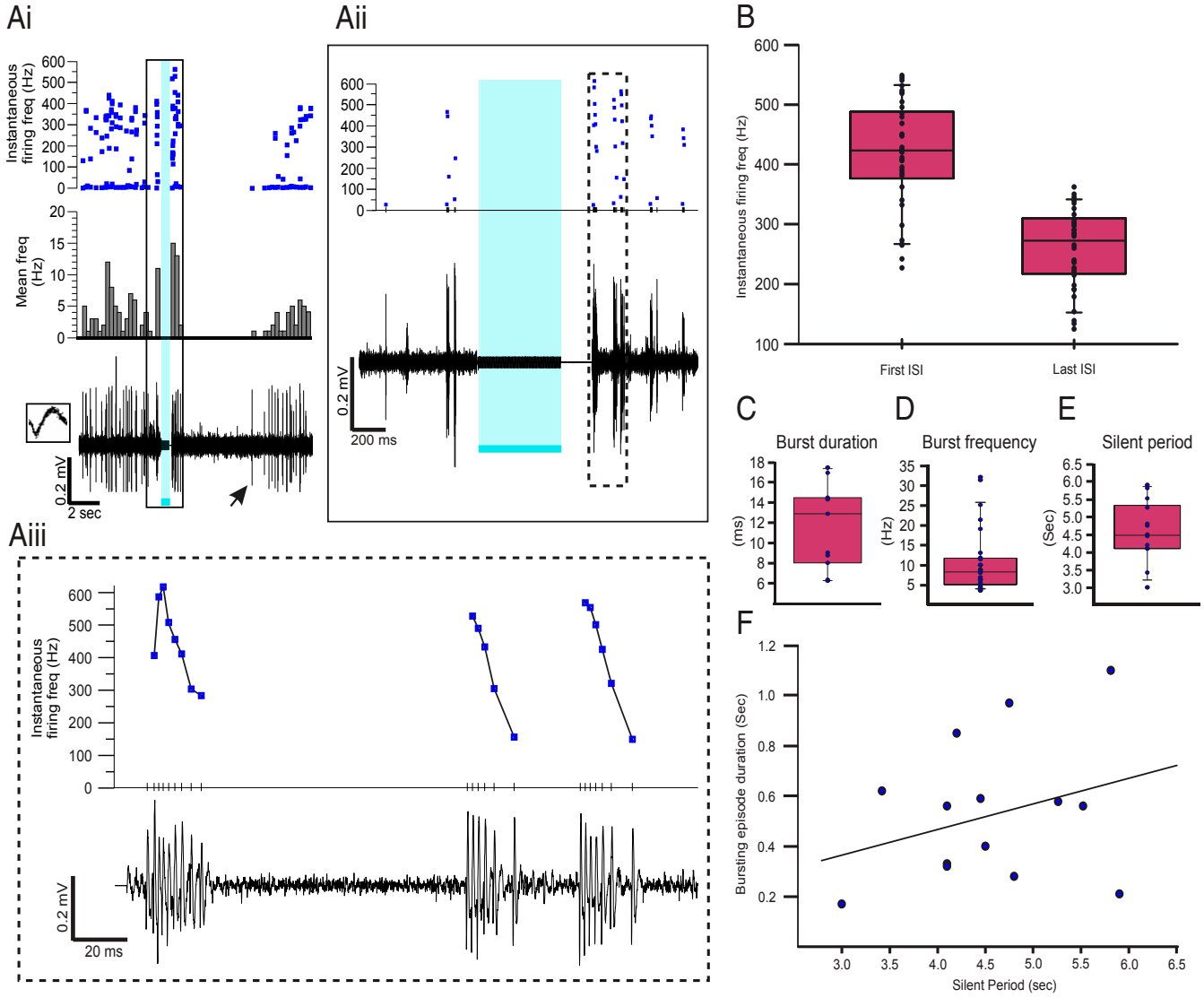

Fig. 4. Bursting followed by silent periods in response to microstimulation. After microstimulation a brief burst of firing followed by a silent period was seen in 14 neurons from 9 patients. (*A*, *i*, *Bottom*) Trace is a representative recording in which an identifiable neuron (*Inset*) has this type of response after microstimulation (light blue, 5 μA, pulse durations 500 μs at 300 Hz for 500 ms). Arrow indicates a possible terminating spike. (*Middle*) Mean frequency during the recording (bins of 0.25 s). (*Top*) Instantaneous firing frequencies during the recorded period. (*A*, *ii*) Expanded view of square box in *A*, *i*, depicting bursting activity after microstimulation. (*A*, *iii*) Expanded view of dotted box in *A*, *ii*, depicting 3 individual poststimulus bursts. *Top* is instantaneous firing frequency for each burst. Note within-burst spike frequency adaptation. (*B*) Median and quartile ranges of the first and last interspike interval for each burst in all cells, showing spike frequency adaptation. Each dot represents an individual burst. (*C*) Median and quartile ranges for burst duration. Each dot represents the average burst duration for an individual neuron. (*D*) Median and quartile ranges of the interburst interval. Each dot represents an individual interburst interval. (*E*) Median and quartile ranges for the silent period duration that followed the bursting activity postmicrostimulation. Each dot represents an individual silent period. (*F*) There was no relationship between bursting episode duration and the subsequent silent period ($r^2 = 0.08$; $P = 0.827$).

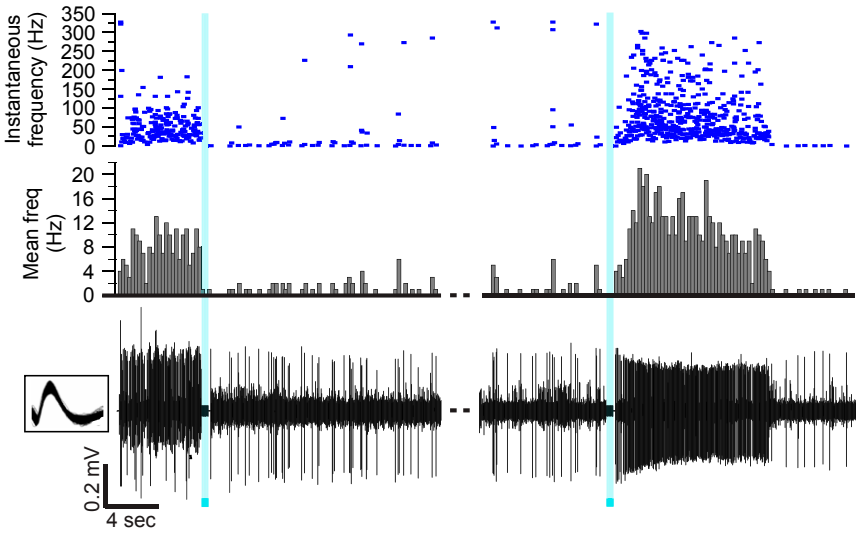

Fig. 5. State changes to repeated microstimulation in STN neurons. Repeated microstimulation led to different state changes in firing rates of 11 neurons from 7 patients. In this example, initial microstimulation led to a decrease in firing rate. The next, identical, stimulus led to an increase in firing rate (above prestimulation firing rate). (*Bottom*) Raw recording of an identifiable neuron (*Inset*) showing responses to microstimulation (light blue, 5 μA; pulse duration of 200 μs at 300 Hz for 500 ms). (*Middle*) Mean frequency during the recording (bins of 0.25 s). (*Top*) Instantaneous firing frequency during the recorded period. Dotted line indicates a period of recording (17 s) removed for clarity.

the first stimulus could switch to tonic or increased firing rates in response to a subsequent stimulus (Fig. 5). This suggests that individual neurons can assume multiple states, and that neuronal responses to the stimuli are state-dependent.

In summary, in response to microstimulation, 72/83 (87%) of neurons showed evidence of state changes (Fig. 6). Of these, 58 recovered to their prestimulus firing rates, and of these 58 neurons, 21 had increases in firing rate, 6 had decreases in firing rate, 14 had spiking or bursting followed by a silent period, and 6 responded with a silent period. An additional 11 changed to different states with repeated stimuli. In many cases, the state-change responses to microstimulation were similar to those reported in rat STN slices in response to stimulation (10).

Discussion

In this study, we provide evidence that human STN neurons, recorded during surgery for Parkinson's disease, can undergo state changes in response to microstimulation. These state changes outlast the stimulus by at least 1s, a feature of stable membrane states (21). Although these states can last many seconds, it is not surprising that in the awake human, the transitions can be spontaneous as well: Not only are there spontaneous silent periods, but these periods end spontaneously. In addition, when firing rates are increased, there may be frequency adaptation of these rates before the cell reverts to its prior state. Because of the similarity of the results seen in the reduced rodent preparation (10, 19), we suggest that human STN neurons in PD have multiple stable states, including, at least, a tonic firing state (or multiple tonic firing states), a silent state, and a transient bursting state followed by a silent period. These states were revealed using low-amplitude, brief stimulus trains through the recording microelectrode. Thus, we provide evidence that human STN neurons are multistable and that their states can be switched by focal, low-amplitude microstimulation.

Silent periods have previously been observed after microstimulation of the STN (22). These authors reported that the silent periods were dependent on the frequency of stimulation, having investigated from 1 to 100 Hz at higher amplitudes (100 μA) than those that we used. Further, they suggested that there may be a relation between the evoked silent period duration and patient outcome. This latter finding could explain their previous failure to demonstrate evoked STN silent periods in which they studied only 4 patients; perhaps the lack of response predicted a poorer outcome for these patients (23).

Cellular Electrophysiological Properties and Circuit Function. Although the use of extracellular recording precludes study of the

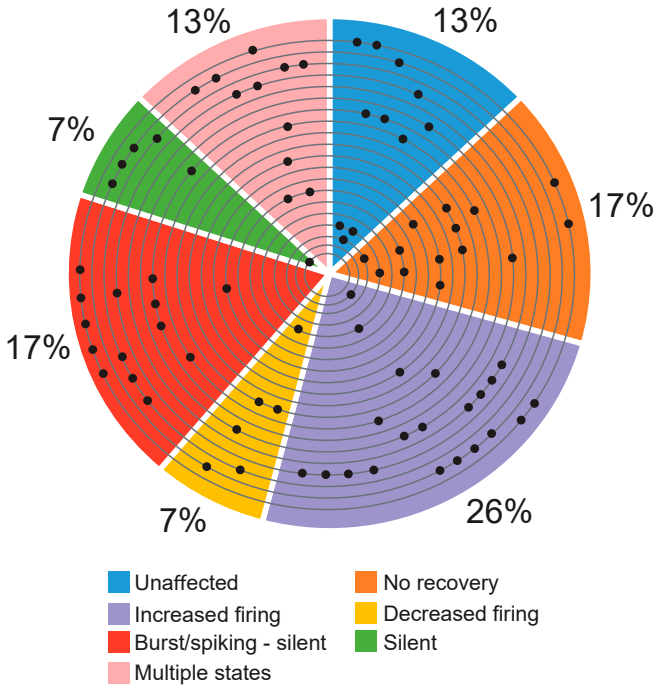

Fig. 6. Summary of state changes identified in response to microstimulation. Pie chart showing responses of 83 neurons recorded during microstimulation, with overall percentages indicated for each response type. Each concentric circle represents a patient, and each point on these circles indicates the response of an individual neuron recorded from that patient.

cellular membrane processes responsible for these multistable states, comparisons to analogous findings from studies using whole-cell patch clamp techniques in reduced preparations from rodents can yield insight into these processes (10, 14). Note that we were also limited by the inability to record the first 185 ms after stimulus termination, making comparison of early poststimulus events impossible. As it is difficult to differentiate cellular from circuit mechanisms in the human, comparison with animal preparations is essential.

In rat STN neurons recorded in slice preparations, neuronal state changes could be induced with brief stimuli, delivered either intracellularly or synaptically (10). Neurons could be switched from a resting downstate to a silent upstate after either excitatory or inhibitory (leading to postinhibitory rebound) stimulation. An intermediate, tonic firing state was also demonstrated, as was a rhythmic bursting state.

During physiological or pathophysiological operation of circuits involving the STN, it is not clear which inputs lead to state changes. Microstimulation in the human is nonspecific: the structures stimulated will depend on the location of the electrode relative to afferent axons (excitatory, inhibitory, and modulatory), neuronal somata, and initial segments, and will also depend on the rheobases and chronaxies of these different elements. The STN receives excitatory inputs directly from the cortex (the hyperdirect pathway) and parafascicular nucleus of the thalamus (in rats, ref. 24), as well as inhibitory inputs from the GPe (via the indirect pathway). In humans with an electrode in the STN, we cannot stimulate excitatory versus inhibitory inputs selectively, but the different state changes observed in different neurons could result from preferential stimulation of 1 or the other of these inputs (e.g., due to location of axons from different sources relative to the electrode). For example, based on data from the rat, it could be that stimulation of excitatory input increases the firing rate to a new stable state (Fig. 2), and stimulation of inhibitory input, depending on the current state, could lead to either a stable reduction of firing rate (Fig. 3) or transient postinhibitory bursting followed by a silent upstate (Fig. 4). The effects of stimulation would also depend on the current state of the neurons recorded (see *Multistable STN Neurons as Finite State Machines*). Thus, it is not surprising that different effects are seen in different neurons or in the same neuron at different times.

The most intriguing pattern of firing produced by microstimulation was perhaps the transient bursting followed by a silent period. Although this could be produced by the activation of an oscillatory circuit between the STN and GPe (25, 26) or within the STN itself (27), several factors point to at least a significant contribution of intrinsic properties of STN neurons. First, STN neurons have intrinsic bursting properties (9, 18), and these properties can be accentuated in certain conditions, including in response to hyperpolarization (28), to metabotropic glutamate receptor activation (29), and to conditions in which small conductance calcium activated potassium conductances are reduced (as may occur in PD; refs. 25 and 26). Second, in some instances, microstimulation can lead to inhibition of STN neurons, which respond with rebound depolarization. The hyperpolarization can lead to deinactivation of sodium and calcium conductances, and the rebound excitation can be initiated by low-threshold transient calcium conductances (24, 30–33). These could then lead to the activation of other conductances, including persistent and resurgent (activated during repolarization) sodium conductances (28) and L-type calcium conductances, which are likely involved in prolonging the depolarization (18). In rat STN neurons, such rebound excitation can lead to a prolonged silent upstate (10); the underlying conductances of these silent upstates are not clear (vide infra). Third, the time course of spike frequency adaptation in the poststimulus bursts is consistent with intrinsic adaptation properties seen in other neurons (34), as well as STN neurons (30). Taken together, it is clear that STN neurons have intrinsic properties that can lead to multiple states, making it likely these intrinsic processes significantly contribute to these complex patterns of firing.

It is interesting to consider whether the silent periods could represent a silent upstate, as characterized in rats (10), and how this state could change neuronal integration, and thus circuit function. In the rat, silent upstates are high-conductance states (5- to 6-fold increase in conductance) with membrane potentials clamped around −40 mV (10). This high-conductance state would result in synaptic inputs near the soma being shunted until the upstate is terminated. In other words, the depolarization of the silent upstate could result in the dendrites being temporarily sensitive to small amplitude inputs, whereas the somatic high conductance reduces proximal synaptic currents. Thus, there would be changes to neuronal integration, and consequently to circuit function: since cortical inputs are dendritic, and thalamic afferents are located proximally (35), the sensitivity of STN neurons to the hyperdirect corticosubthalamic pathway could be increased for the duration of the silent upstate. The mechanisms underlying termination of the silent upstate are not clear, but the high-conductance state decreases slowly (19), perhaps as a result of slow inactivation of sodium conductances (see ref. 28), and it may ultimately be terminated by the afterhypolarization following a dendritic calcium spike (19). In other words, activity in the hyperdirect pathway may lead to its termination.

Multistable STN Neurons as Finite State Machines. The demonstration that transient perturbations can cause state changes is not a novel finding. For example, mammalian spinal motoneurons have at least 2 stable states (36), and rat STN neurons have at least 4 stable states (10). It may therefore be useful to consider STN neurons as finite state machines, units in which a defined transition stimulus leads to a change from one defined state to another (37). In terms of such a model, it becomes clear that to understand circuits, it is necessary to understand the different possible states of each neuronal type in the circuit, as well as the conditions (transition stimuli) leading to changes from one state to another.

State changes in response to transition stimuli may also depend on the current behavioral status of the organism. For example, neurons may undergo a different state change in response to a given transition stimulus, depending on the level of arousal or neuromodulation (e.g., dopaminergic or other monoaminergic tone; ref. 38) or on the behavior at the time of the stimulus (e.g., locomotion vs. at rest; ref. 39).

Multistable states (finite states) have been implicated in disease models (11). Although these properties would be necessary for normal circuit function, it is possible that diseases may alter the states and/or transition properties, leading to symptoms and signs. For example, in the epileptic brain, an otherwise normal transition stimulus may lead to seizures (11). The consideration and use of such models may help to understand the physiology and pathophysiology of the STN and basal ganglia.

Multistability and Parkinson's Disease. It is clear that the activity patterns of STN neurons change in PD. For example, STN neurons in the MPTP-treated nonhuman primate model of PD had higher rates of firing than controls, an increase in 4- to 8-Hz oscillations, and an increase (40) and prolongation (41) of beta range activity. STN beta burst activity, which may normally serve to adapt motor performance (42), increases in people with PD. Dopaminergic medications reduce beta bursts, with the reduction related to improvements in symptoms and signs (43). It has recently been shown that there is little intrinsic connectivity within the STN, with inputs arising from afferents with low divergence, leading to the suggestion that synchronous firing in the STN relies on its afferent inputs (44). We would add that, conceivably, some neuronal states (silent states) facilitate beta bursts in response to

these inputs; for example, through reduced membrane time constants that would promote synchronous responses. Higher probabilities of such states could then result in the motor symptoms of PD. Transitions from these states, for example, through dendritic activation by the hyperdirect pathway, could lead to a reduction of beta bursting and amelioration of motor symptoms. Consistent with this concept, recent evidence points to improved clinical outcome in patients with PD during therapeutic stimulation of regions of the STN with strong cortico-subthalamic inputs (45).

In summary, we have shown here that human STN neurons in patients with Parkinson's disease have multiple electrophysiological states, and can be triggered to switch states by brief extracellular microstimulation. The relationship of these states to physiology or pathophysiology of the STN or its associated microcircuits is not yet clear. Nor is it clear how these states are affected by medications or DBS. To fully understand the states and their transition requirements would require experiments in animals, comparing transitions in normal vs. PD models, and their responsiveness to dopamine. It is conceivable that this understanding would enable tailored therapies to restore more normal transition properties and lessen the symptoms and signs of PD. Perhaps this is one of the hidden roles of therapies for PD, including dopaminergic medications and DBS.

Experimental Methods

Patients undergoing STN deep brain stimulation surgery for Parkinson's disease at Capital Health in Halifax, Nova Scotia (associated with Dalhousie University), between 2000 and 2015 routinely had awake (sometimes with occasional low-dose sedation) stereotactic (frame-based) MRI, and microelectrode guided procedures for localization of the subthalamic nucleus. Preoperative UPDRS motor scores ranged from 25 to 67 (median, 42; mean, 44 $\pm$ 13). All targeting was based on T1-weighted sequences, although as imaging advanced over the years and the STN could be better visualized, additional sequences were added (e.g., T2-FLAIR-CUBE sequences) and fused with the T1-weighted sequences to improve accuracy. All patients stopped their Parkinson's medications the evening before surgery. Data from 17 male and 11 female patients (aged 42 to 73 y; mean, 59 y; median, 58 y) were examined, and stable recordings selected for further study. All patients had bilateral procedures on either 1 or 2 (staged) days.

Our routine was to use microelectrode recording for identification of physiological characteristics of STN neurons, and microstimulation for identification of any adverse effects that may indicate off-target effects. All patients consented to the procedure, as well as to the collection and use of their data for research purposes. The protocol was approved by the Capital Health Research Ethics Board.

The recording system used (Axon Guidance 3000, purchased at that time from Molecular Devices, Sunnyvale, CA) allowed for both recording and stimulation through a single microelectrode. Electrode (initially made by W. Hutchison, Toronto, ON, Canada; later purchased from FHC, Bowdoin, ME) impedances were 500 to 1,000 Ω. Recording gains were adjusted to maximize use of the analog-digital converter range (although were sometimes clipped), with signals typically band-pass filtered 300 to 5,000 Hz. Stable recordings, indicated by the continued presence of the same unit as identified by spike morphology throughout the period of interest, were selected for analysis.

Stimulation parameters during initial recordings (during step-wise advancement of the electrodes through the STN) were typically $<$10 μA with pulse durations of 200 to 500 μs in trains of 300 Hz for 0.5 s. During step-wise electrode withdrawal, higher-amplitude stimuli were used for verification, but we did not record neuronal responses during withdrawal.

All patients had early postoperative MRIs to verify DBS electrode placement.

Extracellular microelectrode recordings were imported into Spike 2 (CED, Cambridge, UK) for offline analysis. Spikes were sorted using the wave event template function to discriminate spikes based on a principal component analysis of spike properties including amplitude, width, and shape. For each recording, a single neuron that we could clearly discriminate pre- and post-stimulation was selected for analysis. Mean firing frequencies were calculated and binned as the average firing rates in 250-ms windows during the entire recording period. To quantify increases or decreases in firing rates post-microstimulation, mean firing rates were calculated for 1-s periods before and 1-s periods after microstimulation. In neurons that demonstrated rebound burst firing, the mean firing rate was calculated as the average firing rate for the duration of the burst postmicrostimulation. The premicrostimulation mean firing rate was calculated as the average firing rate for a 1-s period immediately preceding microstimulation. Student's *t* tests were used to compare firing rate pre- and postmicrostimulation with a statistical significance level set at $P < 0.05$. Results are reported as means $\pm$ SDs.

As approved by the research ethics board, all nonidentifiable data (secondary use data; in this case, electrophysiological data) will be readily available on request to the corresponding author.

ACKNOWLEDGMENTS. We thank the undergraduate students who assisted with various parts of the project over the years, including Aafiah Hamza and Marshall Dunn. We also thank Isabelle Mintz and Jason Kass for valuable discussions, Asher Wright for discussions about finite state machines, Ron Hill for technical assistance during the operations, and Paula Chiasson for her hard work that was solely aimed at improving the lives of our patients. R.M.B.'s position is supported by Brain Research UK.

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