Neurotransmitter release from high-frequency stimulation of the subthalamic nucleus

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Object. High-frequency stimulation (HFS) delivered through implanted electrodes in the subthalamic nucleus (STN) has become an established treatment for Parkinson disease (PD). The precise mechanism of action of deep brain stimulation (DBS) in the STN is unknown, however. In the present study, the authors tested the hypothesis that HFS within the STN changes neuronal action potential firing rates during the stimulation period by modifying neurotransmitter release.

Methods. Intracellular electrophysiological recordings were obtained using sharp electrodes in rat STN neurons in an in vitro slice preparation. A concentric bipolar stimulating electrode was placed in the STN slice, and electrical stimulation (pulse width 50–100 μsec, duration 100–2000 msec, amplitude 10–500 μA, and frequency 10–200 Hz) was delivered while simultaneously obtaining intracellular recordings from an STN neuron.

High-frequency stimulation of the STN either generated excitatory postsynaptic potentials (EPSPs) and increased the action potential frequency or it generated inhibitory postsynaptic potentials and decreased the action potential frequency of neurons within the STN. These effects were blocked after antagonists to glutamate and γ-aminobutyric acid were applied to the tissue slice, indicating that HFS resulted in the release of neurotransmitters. Intracellular recordings from substantia nigra pars compacta (SNc) dopaminergic neurons during HFS of the STN revealed increased generation of EPSPs and increased frequency of action potentials in SNc neurons.

Conclusions. During HFS of STN neurons the mechanism of DBS may involve the release of neurotransmitters rather than the primary electrogenic inhibition of neurons.

KEY WORDS • deep brain stimulation • dopamine • electrophysiological monitoring • Parkinson disease • subthalamic nucleus

High-frequency stimulation of the STN through implanted electrodes has become an established treatment for PD. Nonetheless, the precise mechanism of DBS in the STN is unknown. Because the effects of HFS are usually similar to those of a lesion, it has been hypothesized that this type of DBS acts by silencing neurons within the stimulated structure. Beurrier, et al., used the blind patch-clamp technique in a rat brain slice preparation to demonstrate that HFS blocked action potential generation in STN neurons during the poststimulation period. These investigators argued that the same blocking effect occurred during stimulation as well, but they were unable to verify this assertion in their preparation because of stimulus artifacts. Recently, Magarinos-Ascone, et al., demonstrated in a similar rat brain slice preparation that sustained high-frequency (> 100 Hz) electrical stimulation could result in steady membrane depolarization, an increased firing rate, followed by bursts, and finally total silence of tonic cells. Both groups of researchers posited that the mechanism of DBS within the STN in PD was a consequence of the HFS-mediated inhibition of neuronal firing within the STN.

This hypothesis is challenged by recent findings, however. For example, HFS of the STN in MPTP-treated monkeys caused a short-latency excitation of both the globus pallidus internus and externus, indicating that activation of efferent fibers in the STN resulted from HFS. Furthermore, analysis of in vivo observations has revealed that HFS may produce its beneficial effect by activating axons of STN cells, STN afferents, or fibers passing close to the stimulation site. Thus, there are conflicting reports of the exact effect of HFS of STN neurons. In the present study we report that HFS of the STN generated both EPSPs and IPSPs in STN neurons and modified the firing rate of action potentials resulting from neurotransmitter release.

Materials and Methods

Tissue Slice Preparation

For the preparation of tissue slices, 4- to 6-week-old male or female Sprague–Dawley rats were deeply anesthetized using sodium pentobarbital (30–40 mg/kg) and then were killed by decapitation. The forebrain was rapidly removed and the hemispheres were separated with a midline incision. Tissue slices (400 μm thick) were cut in the coronal plane by using a vibratome (Ted Pella, Inc., Redding, CA). During preparation, the tissue slices were placed in a chilled...
FIG. 1. Tracings obtained during an extracellular multiunit recording from the STN, revealing poststimulus inhibition of action potential generation. A single 10-second duration electrical stimulus at 100 Hz delivered within approximately 200 μm from the STN recording electrode produced a stimulation artifact, followed by the inhibition of neuronal firing lasting approximately 30 seconds and the gradual return of spontaneous activity. Due to the stimulation artifact, it was impossible to see what happened during stimulation.

FIG. 2. a: Tracing of an intracellular recording from an STN neuron revealing that electrical stimulation could generate action potentials at 53 to 78 Hz during the stimulation period. At the end of stimulation, there was an inhibition of neuronal firing lasting 1.8 seconds. b: Tracing demonstrating that the membrane potential of the STN neuron was held at −80 mV with the intracellular injection of hyperpolarizing direct current. A single electrical stimulus applied at 10 Hz resulted in the generation of subthreshold EPSPs. c: Magnification of segment featured in b. d: Tracing obtained by increasing the stimulation frequency to 20 Hz, revealing the frequency-dependent summation of EPSPs such that, even at −80 mV membrane-holding potential, stimulation was able to generate action potentials. e: Magnification of segment featured in d. f: Tracing obtained in another STN neuron, revealing that 20-Hz stimulation resulted in IPSPs and blockage of action potential generation. g: Magnification of segment featured in f.
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Fig. 3. Tracings obtained during electrical stimulation (100 Hz frequency) in the continuous presence of glutamate and GABA receptor antagonists, showing complete blockage of the postsynaptic potentials.

Results

Extracellular STN Recordings

Using an extracellular microelectrode, we recorded from spontaneously firing STN neurons (five tissue slices). A single stimulus of 100 Hz delivered within approximately 200 μm of the stimulating electrode produced a stimulation artifact, followed by the inhibition of neuronal firing lasting for approximately 30 seconds and the gradual return of spontaneous activity (Fig. 1). Because of the stimulation artifact, we could not see what happened to neuronal activity during the stimulation period.

Intracellular STN Recordings

To study the cellular effects of HFS, we performed intracellular recordings from STN neurons by using sharp electrodes. These recordings were obtained from STN neurons within approximately 200 μm of the stimulating electrode during a single brief (~200 msec) electrical stimulus delivered at 100 Hz. The frequency of action potentials increased in the majority of recorded cells (11 of 16 cells; Fig. 2a). At the end of stimulation, there was a profound inhibition of spontaneous activity, as had occurred with the extracellular recordings. To study the subthreshold responses to electrical stimulation of the STN, we hyperpolarized the recorded neurons with an intracellular injection of direct current to hold the membrane potential at −80 mV. At this membrane potential, electrical stimulation at 10 Hz revealed EPSPs (Fig. 2b and c). The onset latency of these postsynaptic events was as brief as the delay imposed by the synapse (~1 msec), indicating that the pathway between stimulated neurons and recorded neurons was monosynaptic. To study the effect of the frequency of stimulation on the pattern of neuronal activity, the stimulation frequency was increased to 20 Hz. A frequency-dependent summation of the EPSPs was apparent, and even at a holding potential of −80 mV, the HFS was able to generate action potentials (Fig. 2d and e). In a subset of neurons, electrical stimulation blocked the generation of action potentials while the stimulus was applied (five of 16 cells; Fig. 2f). The current-clamp traces revealed that IPSPs followed each stimulation pulse (Fig. 2g).

Analysis of these results indicates that HFS of the STN may result in both EPSPs and IPSPs, probably by stimulating synaptic release of neurotransmitters.

Neurotransmitter Blockage

To determine whether the effects of HFS were mediated by synaptic transmission, we bath-applied 6-cyano-7-nitroquinoxaline-2,3-d 20 μM (an AMPA/kainate subtype glutamate antagonist), D-(-)-2-amino-5-phosphopentanoic acid 40 μM (an N-methyl-D-aspartate subtype glutamate antagonist), and bicuculline 10 μM (a GABAA receptor antagonist). These antagonists blocked all cellular effects of HFS. Thus, HFS had no effect on action potential generation in cells held at −55 mV, and only the stimulation artifact occurred at holding potentials of −65 mV during the stimulus period (five cells; Fig. 3). Analysis of these results indicates that the effects of HFS are mediated by neurotransmitter release and that HFS elicited both EPSPs and IPSPs by releasing endogenous neurotransmitters such as glutamate.
Effects of HFS on the Activity of STN Neurons

The effects of high-frequency DBS are usually similar to those of a lesion, indicating that this type of stimulus acts by silencing the neurons of the stimulated structure. In agreement with this hypothesis, Beurrier, et al., used the patch-clamp technique in a rat slice preparation to demonstrate that high-frequency electrical stimulation blocked action potential generation in STN neurons in the poststimulation period. The inhibitory effect of HFS was thought to be due to a blockage of voltage-sensitive Na⁺ channels. Similarly, Magarinos-Ascone, et al., who used the in vitro rat brain slice technique, demonstrated that sustained high-frequency (> 100 Hz) electrical stimulation could depolarize membrane potential and trigger action potentials. They also observed IPSPs in a subset of cells tested. Garcia, et al., showed that HFS of the STN suppressed both spontaneous activity and the generation of spikes, neither of which had been prevented by antagonists of the metabotropic and ionotropic glutamate and GABA receptors. Nonetheless, tetrodotoxin, the Na⁺ channel blocker, suppressed all HFS-evoked spikes. Therefore, these authors concluded that HFS drives STN neuronal activity by directly activating the neuronal membrane.

The excitatory inputs into the STN are thought to originate in the cerebral cortex, whereas the inhibitory inputs are from the globus pallidus. In the rat STN, both prefrontal and motor cortices stimulation induces an excitatory response as a result of activation of the direct corticostriatopallidal pathway. Analysis of these results indicates that the EPSPs and IPSPs in the current study may originate from the stimulation of the descending cortical input to the STN (which relies on glutamate as the neurotransmitter) and globus pallidal input to the STN (which relies on GABA as the neurotransmitter), respectively. The finding in the current study that glutamate and GABA antagonists were able to block the postsynaptic potentials lends support to the hypothesis that the effects of HFS are mediated by releasing endogenous neurotransmitters.

Given that the STN projections are also thought to be glutamatergic, one might predict that the activation of STN neurons by HFS would lead to elevated glutamate release in STN target structures. Indeed, previous data have shown that electrical stimulation of the STN causes a significant increase in the extracellular glutamate concentration in the ipsilateral globus pallidus and substantia nigra, both of which have axonal projections arising from the STN. Furthermore, the activity of SNr cells is increased with STN stimulation; this increase is probably a consequence of activating glutamatergic subthalamiconigral projections, particularly considering that the latency of the evoked excitation was consistent with the conduction time of the subthalamiconigral neurons. In addition, data from a study of the effects of HFS of the STN on neuronal activity of the globus pallidus in two rhesus monkeys rendered parkinsonian by the administration of MPTP revealed both activation of the STN efferent fibers and resultant changes in the temporal firing pattern of neurons in the globus pallidus externus and rostrum.
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Internus. Analysis of the results in the current study indicates that HFS of the STN activates the STN neurons. Note, however, that STN hyperactivity is thought to be the crucial pathological problem in PD as the dopaminergic connections degenerate. How further activation of the nucleus may lead to a therapeutic benefit is currently unknown.

Activation of SNc Neurons by STN HFS

One important target structure of the STN, as demonstrated in an anatomical study, is the dopaminergic SNc, in which synaptic terminals arising from the STN were identified and formed synapses on dendrites within the substantia nigra. This anatomical relationship indicates that HFS-mediated activation of the STN may increase activity of the dopaminergic SNc neurons. In the present study, we found that HFS of the STN generated EPSPs and action potentials in SNc neurons. From these results one can infer that HFS increased the firing of SNc neurons recorded either extracellularly or intracellularly. The EPSPs in SNc neurons are thought to arise from a direct monosynaptic excitatory glutamatergic input from the STN.

Furthermore, analysis of data from neurochemical studies demonstrates that HFS in the rat STN increases brain activity.
dopamine\textsuperscript{11,13,15} or dopamine metabolites.\textsuperscript{26} Clinically, bilateral stimulation of the STN improves most parkinsonian symptoms, decreases\textsuperscript{13} or eliminates\textsuperscript{15} the need for levodopa, and ameliorates motor fluctuations and dyskinesias in a way that is quantitatively comparable to results obtained with levodopa. Importantly, the beneficial effects of STN stimulation occur in the dopamine off period, but not during the dopamine on period. High-frequency stimulation of the STN may even result in dyskinesias that resemble those that occur when excess levodopa is given.

Alternatively, HFS of the STN may result in stimulation of axons passing near or through the STN. The SNc fibers themselves may be susceptible to this type of activation with HFS of the STN. If a relative improvement in motor symptoms is correlated with the electrical energy required for chronic stimulation, the best coefficient was observed for active electrode poles projecting onto white matter dorsal to the STN.\textsuperscript{41,49} Anatomical correlates may be the pallidal thalamic bundle (including Field H of Forel and the thalamic fascicle), the pallidolentamic tract, and/or the zona incerta. Another anatomical correlate may be the axons of the SNc neurons themselves that are apposed to the dorsal surface of the STN.\textsuperscript{17} Thus, an increase in dopamine levels in the brain may well be responsible for the efficacy of HFS of the STN in parkinsonian patients.

Two crucial data are required to demonstrate that an increase in brain dopamine levels is responsible for the efficacy of HFS of the STN. First, HFS-induced dopamine release must be directly measured. A technique that may be used to measure directly the change in stimulation-induced dopamine levels is the electrochemical technique of amperometry.\textsuperscript{3,10,16} Other investigators using amperometry have shown that the release of dopamine in the striatum of anesthetized rats is evoked by electrical stimulation of the mesolimbic dopaminergic pathway.\textsuperscript{12,13,16,40,41} Interestingly, electrical stimulations mimicking the bursting pattern of discharge by SNc neurons were twice as potent as regularly spaced stimulations in enhancing the dopamine extracellular concentration,\textsuperscript{12} thus indicating that dopamine release might be facilitated by increasing the impulse frequency. The high extracellular overflow evoked by a burst might also be due to an accumulation of the released dopamine, whereas at lower frequencies dopamine might be readily eliminated between every action potential.\textsuperscript{12}

Second, to show that neurotransmitter release is the reason for the beneficial effect, one must demonstrate that the beneficial effect can be blocked by specific dopamine antagonist drugs during HFS of the STN. If dopamine release is indeed the reason for the beneficial effect of HFS of the STN, then a dopamine antagonist will likely block the clinical benefit of stimulation.

Note, however, that results of \textsuperscript{\textsuperscript{11C}}raclopride positron emission tomography scanning demonstrated no significant difference in \textsuperscript{\textsuperscript{11C}}raclopride binding, despite significant improvements in Unified PD Rating Scale motor scores following unilateral stimulation of the STN.\textsuperscript{1} From this finding one can infer that STN stimulation does not mediate its anti-PD effects through the release of dopamine. The question of whether HFS of the STN improves symptoms of PD via the release of dopamine is unresolved but deserves further investigation.


Relationship Between Stimulation Frequency and Clinical Effectiveness

An investigation into the relationship between stimulation frequency and clinical effectiveness in patients with PD who had been treated using DBS of the STN demonstrated that stimulation rates of 10 and 50 Hz were ineffective but that stimulation at 90, 130, and 170 Hz was clinically effective.\textsuperscript{28} Interestingly, a graph of the stimulation frequency plotted against the frequency of action potential generation in the present study revealed that maximal firing was induced at approximately 100 Hz. Given that the pattern of discharge of STN neurons during HFS may play a critical role in the beneficial effect of DBS, it is tempting to correlate the present results of HFS in vitro to the beneficial effects in MPTP-treated monkeys\textsuperscript{3} and patients with PD.\textsuperscript{3,26}

Conclusions

Analysis of the data from this study indicates that the mechanism of HFS in the STN is more complex than previously thought. Consider, for example, the exciting possibility that DBS may be able to activate neurotransmitter systems, thus producing EPSPs and IPSPs in the postsynaptic cell. One of these neurotransmitter systems that may be activated by polysynaptic release of glutamate from the STN onto SNc neurons is the dopaminergic system.

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References

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