High-frequency pallidal stimulation eliminates tic-related neuronal activity in a nonhuman primate model of Tourette syndrome

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High-frequency deep brain stimulation targeting the output nucleus of the basal ganglia, the globus pallidus internus, has been suggested as a treatment modality for intractable Tourette syndrome and basal-ganglia-mediated motor tics. Recent studies on the modeling of motor tics induced by focal injections of bicuculline to the striatum, a putative model of Tourette syndrome, have shown that tics induce a widespread modulation within both segments of the globus pallidus. The purpose of this study was to investigate, using the bicuculline-induced Tourette syndrome model, whether and how high-frequency deep brain stimulation targeted to the globus pallidus internus could modulate tic-related activity in the pallidum. The perievent rate changes coinciding with tic expression under the on-stimulation and off-stimulation conditions were examined to determine the effect of high-frequency stimulation on pallidal activity. The results showed that the stimulation blocked tic-related phasic changes in the firing pattern of pallidal cells in parallel with a reduction of the peak amplitude of tic events in the electromyography record. This finding supports the premise that deep brain stimulation targeted to the globus pallidus internus could be a viable treatment option for Tourette syndrome, and the use of pallidal stimulation for motor tics warrants further study. NeuroReport 00:000–000 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.}

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Introduction

Motor tics, as often expressed in Tourette syndrome (TS), are sudden, brief, jerk-like movements that interfere with ongoing activity, and in many cases are associated with abnormalities in the action control pathways of the cortical–basal ganglia–thalamic circuits (CBTC) [1–4]. Recent studies on nonhuman primates (NHPs) have shown that during the expression of tics, widespread, phasic alterations in neuronal activity occur in the globus pallidus (GP), with the external segment (GPe) showing an increased activity and the internal segment (GPi) showing reduced activity before and coincident with the appearance of tics [5,6]. This localization of pathological activity in the output structure of the basal ganglia provides a convenient target for neurosurgical techniques to block the pathology associated with motor tics to prevent it spreading throughout the CBTC.

A recent medical advance for treating CBTC-mediated movement disorders, both hypokinetic and hyperkinetic, for example, Parkinson’s disease and TS, involves continuous high-frequency deep brain stimulation (HF-DBS), at approximately 150 Hz, delivered through a chronically implanted macroelectrode. This electrode is normally targeted to either the GPi, the subthalamic nucleus, thalamus, or pedunculopontine nucleus [7–13]. Although HF-DBS for Parkinson’s disease is an established treatment, its use in TS remains experimental. Although examples of treatment success have been reported in the scientific literature, the underlying therapeutic mechanism has yet to be elucidated, owing largely to ethical and methodological constraints. In this study, we used an established model of TS and motor tics [3,5,6,14–17] and HF-DBS targeting the GPi in NHPs [18] to determine if stimulation could induce a therapeutic response at a neurophysiological level during tic states.

Methods

Animals

One rhesus monkey (Monkey ‘R’; Macaca fascicular, male; weight, 6–7 kg) was used in this study. The animal’s health was monitored by a veterinarian, and his fluid consumption, diet, and weight were monitored daily. All procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and were approved by the RIKEN Animal Experiment Committee.

Surgical procedure

The surgical procedures for cranial implantation were conducted under aseptic conditions using pentobarbital anesthesia (30 mg/kg, intravenously), after induction with...
ketamine HCl (10 mg/kg, intramuscularly). Cranial implantation utilized a rectangular Delrin chamber (27 mm × 27 mm) (Alpha-Omega Engineering, Nazareth, Israel) that was implanted stereotaxically to allow access to the basal ganglia using a coronal approach. The recording chamber was tilted at an angle of 35° in the coronal plane, with the center targeted to the middle of the GP: stereotactic coordinates A17, L8, and H3 [19]. The chambers were fixed into place using bone screws and methylmethacrylate cement. In addition, a head-holder connector and an electroencephalogram screw (Crist Instrument, Hagerstown, Maryland, USA) were attached to the skull. Prophylactic antibiotics and analgesics were administered after the surgery.

**Experimental setup**

The animal was trained to sit in a primate chair and was allowed to complete a simple sensorimotor task for delivery of a liquid reward. Spontaneous behaviors and task execution were observed and recorded during each experimental session using a multichannel video system (GV-800; Geovision, Taipai, Taiwan). The system was designed to visualize behavioral sequences from four different locations: face, upper limbs, lower limbs, and the behavioral task. Digital images from each of the separate cameras were captured at 25 frames per second and stored on a hard disk for offline analysis. In addition, electromyography (EMG) signals were sampled from four muscles: biceps, triceps, zygomaticus major, and ventral orbicularis oris. The EMG wires were constructed from a 100 μm Teflon-coated silver wire (A-M systems, Sequim, Washington, USA). The EMG wires were percutaneous and inserted just before each experimental session using a 27-G hypodermic needle. EMG signals were sampled at 5 kHz and bandpass-filtered (5–450 Hz, 4-pole Butterworth filter).

**Electrophysiological recording**

Following recovery from surgery, the animal underwent microelectrode-guided mapping of the striatum, GPe, and GPi. The striatum and GP were identified by their characteristic neuronal activity and their relation to known anatomical boundaries, as described previously [5]. Mapping was compared with a standard stereotaxic atlas [19]. The preliminary mapping process was followed by experimental sessions. During each experimental session, up to eight glass-coated tungsten microelectrodes (impedance, 250–750 kΩ at 1 kHz) and an injectrode [28-G cannula surrounding a parylene-coated 50 μm microelectrode extending 0.5 mm beyond the tip of the injection cannula (Alpha-Omega)] were introduced using two separate manipulating towers that could move each electrode independently with 2 μm resolution (dual movement tower and electrode positioning system; Alpha-Omega Engineering). The microinjection cannula was connected to a Delrin manifold to a 10 μl syringe (Hamilton Company, Reno, Nevada, USA) filled with bicuculline (Sigma-Aldrich, Tokyo, Japan). The depth of the injection site within the striatum was determined by electrophysiological recording using the injectrode. All electrophysiological data were passed through a low-gain 16-channel headstage (2 Hz–7.5 kHz bandpass) and then digitized at 24 kHz (16-bit resolution, Tucker Davis Technologies, Alachua, Florida, USA). The digitized signals were filtered (300 Hz–6 kHz; first-order Butterworth) and saved to a disk either as continuous data, or as 38-sample-long snippets of the continuous data stream. Once a sufficient number of separable neurons had been identified on the electrodes and were stable for a minimum of 1 min, bicuculline 15 μg/μl (Sigma-Aldrich) dissolved in physiological saline was slowly injected (2.5–8 μl) at a rate of approximately 2 μl/min.

In addition, a dual contact DBS probe (surface areas ~0.5 mm²/contact, impedance < 1 kΩ at 1 kHz) was lowered into the GPi using the same manipulating towers, to allow for the macrostimulation of the target area (Unique Medical, Tokyo, Japan). Stimulation was delivered to the GPi using an isolated stimulator (Model IZ-32; TD, Gainesville, Florida, USA), with symmetric biphasic pulses (cathodal followed by anodal) 60 μs in duration and was delivered at 150 Hz at 1 V for blocks of 30 s. Neuronal data were then collected during an initial 1 min off-stimulation (‘baseline’ or ‘control’) period, followed by 30 s blocks of stimulation delivered once every 60 s. Cells were only considered for offline analysis if at least three 30 s blocks of stimulation were obtained with their corresponding control periods. Stimulation artifacts were removed online using a template subtraction method, with further offline processing using custom MATLAB (V2007B; The Mathworks, Natick, Massachusetts, USA) scripts [18].

**Offline analysis of neuronal activity**

The action potentials of individual neurons were sorted offline (OfflineSorter V2.8.7; Plexon, Plano, Texas, USA) using the high passed signal (200 Hz, 4-pole, high-pass Butterworth filter). Offline sorting enabled high fidelity of neuronal identification even for neurons that changed gradually their firing properties and spike shapes over time. Neurons were accepted for further analysis if they met the following criteria: (a) the recording was from a location within the GP. (b) The acquired action potentials of the neuron were of a consistent distinct shape that could be fully separated with a high degree of certainty from the spike waveforms of other neurons and background noise. (c) The interspike intervals of the neuron were confirmed to have a minimum refractory period of 1.5 ms.

**Data analysis**

Following offline sorting of the neuronal data, the spike trains were correlated with behavioral events using a MATLAB (V2007B; The Mathworks) and Neuroexplorer.
Electromyography (EMG) and neuronal data during tics with globus pallidus internal segment high-frequency deep brain stimulation (GPi-HF-DBS). (a) Examples of raw data from EMG and neuronal activity in the pallidum after administration of bicuculline and during HF-DBS. The top trace shows EMG recorded from the orofacial region, in which the large voltage transients are tic events. The bottom two traces are simultaneous recordings from both segments of the pallidum (GPe and GPi) during expression of the above tics. (b) Quantification of the reduction in tic-related EMG peak voltage amplitude. The histogram shows the EMG signals aligned to tic onset. The inset shows a graphical representation of the t-test across bins between the two experimental conditions and the reduction in peak amplitude of the tic-related EMG ($P < 0.001$). (c) Modulation of the activity in a GPe neuron by tics (black trace) and HF-DBS (red trace). Neuronal activity is aligned to tic onset. Note that the large increase in activity under the off-stimulation condition was abolished by HF-DBS. The dashed lines represent confidence intervals of 99% and are color coded to each condition. (d) Modulation of the activity in a GPi neuron by tics (black trace) and HF-DBS (red trace). The activity is aligned to tic onset. Note that the decrease in activity under the off-stimulation condition was abolished during HF-DBS. GPe, globus pallidus external segment; GPi, globus pallidus internal segment.

(V4; Nex Technologies, Littleton, Massachusetts, USA) environment. The EMG signals were processed offline and the envelope was estimated by rectifying the signal, followed by low-pass filtering (10 Hz, 8-pole Butterworth filter). Detection of tics utilized the rectified and filtered EMG signal by a threshold crossing method (3 SD over the steady state mean). Behavioral events were identified using frame-by-frame video analysis and aligned to EMG activity to exclude nonrelevant muscular activity, as described previously [5]. Significant differences between the two states, on-stimulation and off-stimulation, in the peak amplitude of the EMG were tested for by performing a $t$-test across the rectified, concatenated prestimulation and poststimulation matrices of EMG traces, which gave a $P$-value for each analyzed bin (10 ms) with a significance threshold at $P = 0.001$ (Fig. 1b inset). The time stamps from the EMG that were a consequence of myoclonic tics were then used to construct perievent
histograms (using 10 ms bins) for neuronal data. The significance of neuronal activity in peri-event histograms was analyzed by constructing 99% confidence limits on the basis of the mean and SD of neuronal activity within the tail of the histogram (activity at 2-0.2 s before the tic). A neuron was considered to have a significant peri-event response when its activity crossed the assigned confidence level for three successive bins, and the z-score was greater than 3.

Results

A total of eight bicuculline injections were administered into the sensorimotor putamen. Analysis of EMG activity and video data revealed that the animal developed abnormal tic movements in the orofacial and forelimb regions in five sessions (Table 1).

Although the study was principally designed to test for short-latency neuronal effects of HF-DBS rather than long-term behavioral responses, the 30 s trains of stimulation were capable of reducing tic-related muscular activity. An example of a raw EMG trace is shown in Fig. 1a, in which periodic voltage deflections correspond to individual tic movements. By plotting the rectified EMG activity aligned to tic onset (Fig. 1b), we confirmed that GPI-HF-DBS significantly reduced the amplitude of tic-related EMG activity (see Fig. 1b inset for statistical justification). Although it would appear in Fig. 1a, that there was also a reduction in the frequency of tic movements with stimulation, the analysis of the whole data set showed that the tic frequency was unaffected (Supplementary Fig. 1, http://links.lww.com/WNR/A172).

The experimental setup allowed simultaneous recording of the single-neuron activity in both segments of the GP during the expression of tics during the on-stimulation and off-stimulation periods (GPe = 31, GPI = 26). Of the recorded cells, 84% exhibited tic-related activity changes (GPe = 25, GPI = 21). Figure 1a shows the raw neuronal data obtained during the animal’s tic state. A close inspection of the trace revealed that during the off-stimulation period, the neurons in both segments of the GP showed typical tic-related discharge. Specifically in the example shown, the GPe cell exhibited a phasic increase of activity that was coincident with tics (Fig. 1c, black), whereas the GPI cell exhibited a phasic reduction of activity (Fig. 1d, black). These responses were consistent across all the neurons from each segment of the pallidum.

We found that the application of HF-DBS in the GPI abolished the abnormal discharges in both the GPe (Fig. 1c, red) and the GPI (Fig. 1d, red). A population-scale analysis showed that GPI-HF-DBS induced the abolition of tic-related neural responses in 71% of the recorded neurons (GPi = 22, GPI = 18). The reduction in the number of neurons showing tic-related activity was significant ($P < 0.001$ for both GPe and GPI, $\chi^2$-test). During stimulation the recorded neurons would typically still remain active with firing rates often mirroring the stimulation frequency (Fig. 1c, red), although several examples of transient and complete inhibition were observed.

Discussion

GPI-HF-DBS has been suggested as a treatment modality for intractable TS and motor tics [11,20–22]. Although theoretical models and clinical reports suggest that this treatment modality is and should be effective, and a previous study in NHPs has shown at a behavioral level that subthalamic nucleus stimulation reduces bicuculline-induced stereotypy [23], to date there are no studies that have directly recorded tic-related neuronal activity in the pallidum during GPI-HF-DBS. This study has attempted to address this issue by recording single unit activity within the GP during tic states while applying HF-DBS to the GPI.

The results of this study show that consistent with previous reports, focal disinhibition of the striatum with bicuculline induces myoclonic like motor tics, and that the expression of tics is associated with widespread phasic changes in pallidal activity aligned to tic onset [3,5,6,14–17]. These reported neurophysiological changes from the NHP model closely match those recorded from the TS patients [24].

A recent theoretical model has suggested that the phasic alteration in pallidal activity associated with tics is related to a ‘loss of physiological specificity’ within the pallidum. Specifically, this hypothesis suggests that during tic states there is a breakdown of normal physiology, such that cells that would not typically be associated with the anatomical locality of the tic become responsive to and implicated in the abnormal movements [25]. The results of this experiment show that HF-DBS can block the phasic changes in neuronal activity associated with tics that may indicate a loss of physiological specificity within the pallidum. The maintenance of cell firing during the stimulation period resembles neuronal responses to HF-DBS in the parkinsonian condition [18], and suggests that a frequency following response underlies the observed changes. In addition to modulated pathological

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*AC, anterior commissure.

The anatomical plane at which the injections were placed relative to the AC. All injections were administered caudally to the AC.
neuronal activity, it was observed that HF-DBS could significantly attenuate the peak amplitude of tic events. Therefore, the results of this study would appear to support clinical observations that GPi-HF-DBS can ameliorate the motor symptoms associated with TS, but with the caveat that other critical nodes in the CBTC may also prove to be therapeutic.

Conclusion

The study shows that GPi-HF-DBS abolishes phasic responses in the pallidum that are associated with tic expression. It is possible that this elimination is the therapeutic mechanism of GPi-HF-DBS in TS and basal-ganglia-mediated tic disorders.

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Conflicts of interest

There are no conflicts of interest.

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