

# Synchronous Unit Activity and Local Field Potentials Evoked in the Subthalamic Nucleus by Cortical Stimulation

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**Magill, Peter J., Andrew Sharott, Mark D. Bevan, Peter Brown, and J. Paul Bolam.** Synchronous unit activity and local field potentials evoked in the subthalamic nucleus by cortical stimulation. *J Neurophysiol* 92: 700–714, 2004. First published March 24, 2004; 10.1152/jn.00134.2004. The responses of single subthalamic nucleus (STN) neurons to cortical activation are complex and depend on the relative activation of several neuronal circuits, making theoretical extrapolation of single neuron responses to the population level difficult. To understand better the degree of synchrony imposed on STN neurons and associated neuronal networks by cortical activation, we recorded the responses of single units, pairs of neighboring neurons, and local field potentials (LFPs) in STN to discrete electrical stimulation of the cortex in anesthetized rats. Stimulation of ipsilateral frontal cortex, but not temporal cortex, generated synchronized “multiphasic” responses in neighboring units in rostral STN, usually consisting of a brief, short-latency excitation, a brief inhibition, a second excitation, and a long-duration inhibition. Evoked LFPs in STN consistently mirrored unit responses; brief, negative deflections in the LFP coincided with excitations and brief, positive deflections with inhibitions. This characteristic LFP was dissimilar to potentials evoked in cortex and structures surrounding STN and was resistant to fluctuations in forebrain activity. The short-latency excitation and associated LFP deflection exhibited the highest fidelity to low-intensity cortical stimuli. Unit response failures, which mostly occurred in caudal STN, were not associated with LFPs typical of rostral STN. These data suggest that local populations of STN neurons can be synchronized by both direct and indirect cortical inputs. Synchronized ensemble activity is dependent on topography and input intensity. Finally, the stereotypical, multiphasic profile of the evoked LFP indicates that it might be useful for locating the STN in clinical as well as nonclinical settings.

## INTRODUCTION

The basal ganglia (BG) are a group of subcortical brain nuclei intimately involved in movement and cognition (DeLong 1990; Gerfen and Wilson 1996; Graybiel 1995). The cerebral cortex, the principal afferent of the BG, directly transfers information to the BG via projections to the neostriatum (NS) and subthalamic nucleus (STN). Although not accounted for in classic models of BG function (DeLong 1990; Wichmann and DeLong 1996), recent studies contend that the input operations of STN are critical for the processing of cortical information in the BG, not least because the cortico-subthalamic pathway represents the swiftest route by which the cortex can influence the activity of STN and its targets, the

globus pallidus (GP) and the output nuclei of the BG (for reviews, see Mink 1996; Nambu et al. 2002; Smith et al. 1998).

The responses of STN neurons to cortical input can be “multiphasic” and vary according to the relative activation of distinct BG circuits, including the NS and the reciprocally connected STN-GP network (Fujimoto and Kita 1993; Kolo-miets et al. 2001; Maurice et al. 1998; Nambu et al. 2000). The corticostriatal projection can be quantitatively appreciated in terms of the degrees of convergence/divergence and the spatial arrangements of connections between the neurons that are engaged by cortical activation (Kincaid et al. 1998; Zheng and Wilson 2002). However, such an appreciation is not yet possible for corticosubthalamic or striatopallidal projections or the reciprocal connections within the STN-GP network (for reviews, see Bevan et al. 2002b; Smith et al. 1998). It is therefore difficult to predict how neighboring STN neurons will respond to cortical activation. Furthermore, although electrophysiological studies at the single-cell level *in vitro* suggest that GABAergic inputs from the GP could act as a powerful synchronizing force in the STN (Bevan et al. 2002a,b), little information is available concerning the response of STN neurons to cortical inputs and the interaction of cortical inputs with the mechanisms underlying the autonomous activity and unusual input-output properties of STN neurons (Beurrier et al. 2000; Bevan and Wilson 1999; Hallworth et al. 2003; Otsuka et al. 2001). Indeed, cortical excitatory postsynaptic potentials (EPSPs) can be inefficiently coupled to the generation of action potentials in another class of autonomously active neuron in the BG, the cholinergic interneuron of the NS (Bennett and Wilson 1998).

The complex single-cell and network interactions within cortico-basal ganglia circuits, together with the paucity of detailed anatomical data, make the theoretical extrapolation of single neuron responses to the population level a difficult task. Yet the manner in which local ensembles of STN neurons might be recruited and coordinated by cortical input is of fundamental importance for our understanding of the roles of the STN and indeed, the BG, in behavior. Recent evidence suggests that the activity of STN neurons may be highly synchronized under some circumstances (Brown et al. 2002; Magill et al. 2000, 2001). The population response in STN, and particularly the degree of synchronization associated with it, is all the more significant because cortical afferent activity itself may be highly synchronized during behavior (for reviews, see

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Engel and Singer 2001; Engel et al. 2001; MacKay 1997). Furthermore, inappropriate synchronization of neuronal ensembles may underlie, or at least contribute to, dysfunction in BG diseases (for reviews, see Bergman et al. 1998; Bevan et al. 2002b; Boraud et al. 2002; Brown 2003).

To gain a more complete understanding of the degree of synchrony that can be imposed on STN neurons and associated neuronal networks by the cortex, we recorded the responses of single units and pairs of neighboring neurons in STN to focal electrical stimulation of the ipsilateral frontal cortex in anesthetized rats. When the subthreshold and suprathreshold activities of neural ensembles are sufficiently synchronized, it is often evident at the level of the local field potential (LFP) (for reviews, see Hubbard et al. 1969; Mitzdorf 1985). Thus to test the hypothesis that activity in STN can be synchronized to a high degree by cortical input, we simultaneously recorded the LFPs evoked together with the unit responses after cortical stimulation.

## METHODS

### *Electrophysiological recordings and labeling of recording sites*

Experimental procedures were carried out on adult male Sprague-Dawley rats (Charles River, Margate, UK) and were conducted in accordance with the Animals (Scientific Procedures) Act 1986 (United Kingdom) and the American Physiological Society's *Guiding Principles in the Care and Use of Animals*.

Electrophysiological recordings were made in 13 rats (230–350 g). Anesthesia was induced with isoflurane (Isoflo, Schering-Plough, Welwyn Garden City, UK) and maintained with urethan (1.3 g/kg ip; ethyl carbamate, Sigma, Poole, UK), and supplemental doses of ketamine (30 mg/kg ip; Ketaset, Willows Francis, Crawley, UK) and xylazine (3 mg/kg ip; Rompun, Bayer, Germany) as described previously (Magill et al. 2001). All wound margins were infiltrated with the local anesthetic, bupivacaine (0.75% wt/vol; Astra, Kings Langley, UK), and corneal dehydration was prevented with application of Hypromellose eye drops (Norton Pharmaceuticals, Harlow, UK). Animals were then placed in a stereotaxic frame. Body temperature was maintained at  $37 \pm 0.5^\circ\text{C}$  (mean  $\pm$  SD) with the use of a homeothermic heating device (Harvard Apparatus, Edenbridge, UK). Anesthesia levels were assessed by examination of the cortical electroencephalogram (EEG; see following text) and by testing reflexes to a cutaneous pinch or gentle corneal stimulation. Electrocardiographic (ECG) activity and respiration rate were also monitored constantly to ensure the animals' well being (see following text). Mineral oil or saline solution (0.9% wt/vol NaCl) was applied to all areas of exposed cortex to prevent dehydration.

Parallel bipolar stimulating electrodes (constructed from nylon-coated stainless steel wires; California Fine Wire, Grover City, CA), with tip diameters of  $\sim 100 \mu\text{m}$ , a tip separation of  $\sim 150 \mu\text{m}$ , and an impedance of  $\sim 10 \text{ k}\Omega$ , were implanted into the right frontal and right temporal cortex and then affixed to the skull with dental acrylic cement (Associated Dental Products, Swindon, UK). The coordinates of the frontal stimulation site [AP:  $-4.2 \text{ mm}$ , ML:  $-3.5 \text{ mm}$  (bregma reference), and a depth of  $2.3 \text{ mm}$  below the dura] (Paxinos and Watson 1986) correspond to the border region between the lateral and medial agranular fields of the somatic sensorimotor cortex (Donoghue and Parham 1983; Donoghue and Wise 1982). The coordinates of the temporal stimulation site (AP:  $+5.2 \text{ mm}$ , ML:  $-6.9 \text{ mm}$ , and a depth of  $2.3 \text{ mm}$  below the dura) approximately correspond to the primary auditory cortex (see Kolomiets et al. 2001).

The EEG was recorded via a 1 mm diam steel screw juxtaposed to the dura mater above the right frontal cortex (AP:  $-4.5 \text{ mm}$ , ML:

$-2.0 \text{ mm}$ , which corresponds to the medial agranular field of the somatic sensorimotor cortex) (Donoghue and Wise 1982) and referenced against an indifferent electrode placed adjacent to the temporal musculature. Raw EEG was band-pass filtered (0.1–2,000 Hz,  $-3 \text{ dB}$  limits) and amplified (2,000 times, NL104 preamplifier; Digitimer, Welwyn Garden City, UK) before acquisition. The ECG was differentially recorded via two silver wires that were inserted subcutaneously into the ipsilateral forelimb and hindlimb. Raw ECG was band-pass filtered (10–100 Hz) and amplified (5,000 times, NL104; Digitimer) before acquisition. The chest movements accompanying respiration were recorded using a miniature accelerometer (AP19, Bay Systems, Somerset, UK) and charge amplifier (Type 5007; Kistler Instrumente AG, Winterthur, Switzerland). The signal from the accelerometer allowed the depth and rate of respiration to be accurately assessed on- and off-line.

Extracellular recordings of LFPs and action potentials in the ipsilateral STN were simultaneously made with glass electrodes (6–12 M $\Omega$  measured at 10 Hz in situ, tip diameters of 2.0–3.0  $\mu\text{m}$ ) that were filled with a 0.5 M NaCl solution containing 1.5% wt/vol Neurobiotin (Vector Labs, Peterborough, UK). Electrodes were lowered into the brain using a computer-controlled stepper motor (Burleigh IW-711; Scientifica, Harpenden, UK), which allowed the electrode depth to be determined with a resolution of 0.5  $\mu\text{m}$ . Extracellular signals from the electrode were amplified (10 times) through the active bridge circuit of an Axoprobe-1A amplifier (Axon Instruments, Foster City, CA), bifurcated, and then differentially filtered to extract LFPs and unit activity. The LFPs were recorded after further amplification (100 times; NL-106 AC-DC Amp, Digitimer) and low-pass filtering (between DC and 2 kHz; NL125 filters, Digitimer). Single units were recorded following AC-coupling, further amplification (100 times; NL-106, Digitimer), and band-pass filtering (between 0.4 and 4 kHz; NL125, Digitimer). A HumBug unit (Quest Scientific, Vancouver, Canada) was used in place of a traditional "notch" filter to eliminate mains noise at 50 Hz (Brown et al. 2002). Action potentials were typically between 0.4 and 1.2 mV in amplitude and always exhibited an initial positive deflection.

The responses of the STN to cortical stimulation were determined by focal electrical stimulation of the cortex (Kolomiets et al. 2001; Maurice et al. 1998). Electrical stimuli, which consisted of single square-wave current pulses of 0.3 ms duration and variable amplitude (75–600  $\mu\text{A}$ ), were delivered to the ipsilateral frontal or temporal cortices at a frequency of 0.67 Hz using a constant current isolator (A360D; World Precision Instruments, Stevenage, UK) that was gated by a programmable pulse generator (Master-8; AMPI, Jerusalem, Israel).

The final recording location in each experiment was marked by a discrete, extracellular deposit of Neurobiotin [100 nA anodal current; 1-s (50%) duty cycle for 60 min] (Magill et al. 2001). After a period of 1–2 h for the uptake and transport of the Neurobiotin by neurons and glia at the recording sites, animals were given a lethal dose of ketamine anesthetic and perfused via the ascending aorta with 100 ml of 0.01 M phosphate-buffered saline (PBS) at pH 7.4, followed by 300 ml of 4% wt/vol paraformaldehyde and 0.1% wt/vol glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, and then by 150 ml of the same solution without glutaraldehyde. Brains were then postfixed in the latter solution at  $4^\circ\text{C}$  for  $\geq 12 \text{ h}$  before sectioning.

### *Histochemistry*

Standard techniques were used to visualize the Neurobiotin deposits (Kita and Armstrong 1991; Magill et al. 2001). Briefly, the fixed brain was cut into 60  $\mu\text{m}$  thick sections in the coronal plane on a vibrating blade microtome (VT1000S; Leica Microsystems, Milton Keynes, UK). Sections were washed in PBS and incubated overnight in avidin-biotin peroxidase complex (1:100; Vector) in PBS containing 0.2% wt/vol Triton X-100 and 1% wt/vol bovine serum albumin (Sigma). After washing, the sections were incubated in hydrogen

peroxide (0.002% wt/vol; Sigma) and diaminobenzidine tetrahydrochloride (0.025% wt/vol; Sigma) in the presence of nickel ammonium sulfate (0.5% wt/vol; Sigma) dissolved in Tris buffer (0.05 M, pH 8.0) for 15–30 min. Neurobiotin-filled cells were intensely labeled with an insoluble, black/blue precipitate. Finally, sections were dehydrated, cleared and mounted for light microscopy using standard techniques (Bolam 1992). All final recording sites and the locations of the stimulation electrodes were histologically verified.

### Data acquisition and analysis

Evoked LFPs and unit activity were sampled at 5 and 10 kHz, respectively. The EEG signal was sampled at 5 kHz. The ECG and respiration signals were each sampled at 400 Hz. All biopotentials were digitized on-line with a PC running Spike2 acquisition and analysis software (version 4; Cambridge Electronic Design, Cambridge, UK). Evoked LFPs and EEG were high-pass filtered at 0.25 Hz off-line to remove slow “DC drift” (Spike2). Data from the recording session were first scrutinized for ECG-related artifacts; LFP data contaminated with such artifacts were rejected. For recordings of pairs of neurons, spikes were sorted off-line and under supervision using the automatic waveform discrimination function of Spike2 (see Levy et al. 2002b). All paired recording data were visually inspected to verify the high quality of spike sorting. Because some data sets did not follow a normal distribution (1-sample Kolmogorov-Smirnov test for “normality”), statistical comparisons of unpaired data were performed using the Mann-Whitney *U* test. The criterion for significance was the 95% level (unless stated otherwise). Data are expressed as means  $\pm$  SD.

Peristimulus time histograms (PSTHs) were constructed from 200 consecutive stimulation trials with a bin size of 1 ms. The cumulative sum (CUSUM) technique, a sensitive method for quantitatively assessing trends in PSTH profiles with respect to a prestimulus control, was used to statistically define the responses of units to cortical stimulation (Ellaway 1978). The criteria used to establish significant excitatory or inhibitory responses were changes in CUSUM above or below, respectively, thresholds set at the mean CUSUM score during the 95 ms immediately preceding stimulation  $\pm 2$  SDs of this mean. Response latencies were calculated according to the first bin in which a response reached significance. The “peak” of an excitation response was defined as the bin with the highest spike count. The peak of an

inhibition response was defined as the bin with the lowest spike count or, in the case of a cessation in firing, the bin in the middle of the statistically significant inhibition. Peristimulus averages of the evoked LFPs were generated from the same 200 stimulation trials used for the PSTHs. Positive or negative deflections in the average evoked LFP were considered significant if the peaks and troughs of such deflections exceeded threshold voltages defined as  $\pm 2$  SD of the prestimulus mean. Normalized frequency histograms of the unit responses and evoked LFPs were calculated by assigning a score of 1 when the parameter in a given bin (1 ms duration) exceeded the upper significance level (set at mean  $+2$  SD) and a score of  $-1$  when the parameter fell below the lower significance level (set at mean  $-2$  SD). Bins in which the responses were not significantly different from baseline were assigned a score of 0. LFPs were down-sampled to 1,000 Hz (Spike2) for direct comparison of evoked LFPs with unit responses. Significant relationships were tested by standard linear regression (Microsoft Excel).

## RESULTS

### Unit activity evoked by cortical stimulation

The responses of a total of 69 neurons to stimulation of the ipsilateral frontal cortex were recorded throughout STN. The spontaneous activities of these STN neurons were similar to those previously described (Magill et al. 2000, 2001), i.e., mean firing rates of  $\sim 10$  Hz, with tonic, irregular firing or periodic, bursting patterns. The majority of the STN neurons (61%) responded to frontal cortical stimulation at an intensity of  $300 \mu\text{A}$  (or  $600 \mu\text{A}$ ; see following text) in a typical, “multiphasic” fashion (Figs. 1–4). These neurons responded with, in turn, a short-latency brief excitation, a short-latency brief inhibition (27 of 42 responsive neurons), or a marked reduction in firing (15 neurons), a long-latency excitation, and finally a long-latency, long-duration inhibition (Fig. 1, *A* and *B*). The mean latencies of these ordered responses were  $4.5 \pm 1.6$ ,  $10.4 \pm 3.0$ ,  $16.6 \pm 4.7$ , and  $30.6 \pm 5.5$  ms, respectively. All neurons that responded in this characteristic manner were located in the rostral half of STN (Fig. 5). The responses of a

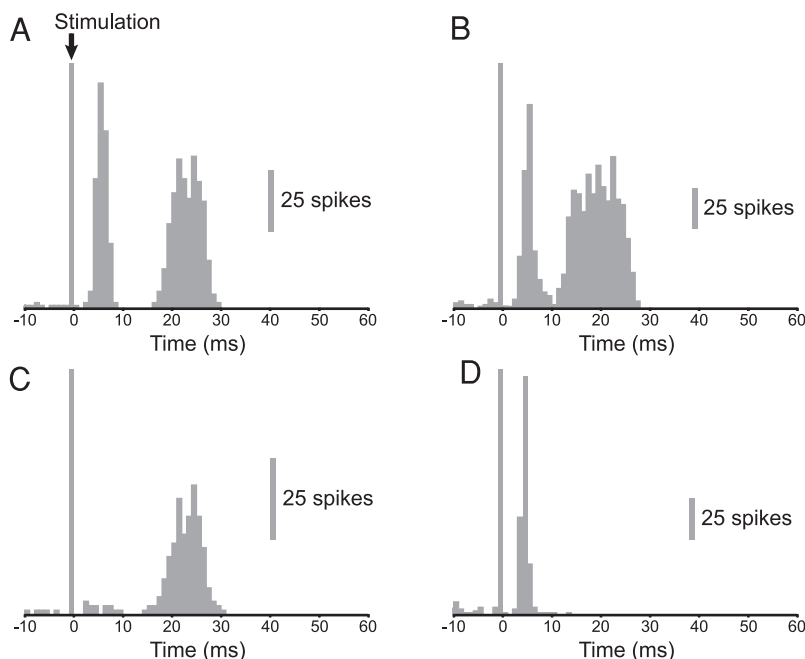


FIG. 1. The responses of neurons in the rostral half of subthalamic nucleus to stimulation of the ipsilateral frontal cortex are often multiphasic. Peristimulus time histograms (spike count vs. time) of the responses of single subthalamic nucleus (STN) neurons to frontal cortical stimulation ( $300 \mu\text{A}$ ). In this and subsequent figures, stimulation occurred at time 0. *A*: typical “multiphasic” unit response that consisted of a short-latency brief excitation, a short-latency brief inhibition, a long-latency excitation, and, finally, a long-latency, long-duration inhibition. *B*: the two excitation peaks displayed by some neurons were separated by a marked reduction in firing, rather than a significant period of inhibition, during which firing ceased. *C* and *D*: the responses of a small number of neurons included only select phases of the stereotypical response, e.g., no short-latency excitation (*C*) or a short-latency excitation followed by a long-lasting inhibition (*D*). Stimulation artifacts in this figure and subsequent figures are truncated for clarity.



small minority of STN neurons ( $n = 8$ ) were more variable; only some of the phases of the typical, multiphasic unit response were expressed, e.g., no short-latency excitation (Fig. 1C), a short-latency excitation only (Fig. 1D), or a long-latency excitation and/or long-latency inhibition only (Figs. 6 and 10). Most of the STN neurons that did not respond to cortical stimulation (300 or 600  $\mu\text{A}$ ; 15 of 19 neurons) were located in the caudal half of the nucleus (Fig. 6). These data suggest that, in agreement with previous studies (e.g., Kolomiets et al. 2001), the responses of single STN neurons are complex and topographically organized.

The stereotyped response of single neurons in the rostral half of STN to each cortical stimulus (exemplified by the structured form of individual PSTHs), together with the fact that the responses of neurons at different recording sites within rostral STN were alike (exemplified by the similarities between the PSTHs of different neurons), raises the possibility that unitary responses may be synchronized within a small time window by each cortical stimulus. To characterize better the degree of synchrony imposed on STN neurons by cortical input, 10 pairs of neurons were recorded with single electrodes during stimulation. In all but one case, the responses of both neurons in the pair were qualitatively and quantitatively similar. Seven of the 10 pairs responded to cortical stimulation in the typical multiphasic manner (Fig. 2A). The short-latency brief excitations and inhibitions of the seven responsive pairs were synchronized to within a few milliseconds (mean timing differences of  $1.0 \pm 0.1$  and  $2.7 \pm 0.5$  ms, respectively, across 7 pairs). The long-latency excitations and the starts of the long-latency, long-duration inhibitions were also tightly synchronized ( $3.2 \pm 1.0$  and  $2.0 \pm 0.9$  ms, respectively). The responses of one exceptional pair of neurons were not similar or synchronized (Fig. 2B). The remaining two pairs of neurons were located in caudal STN and did not respond to cortical stimulation (data not shown).

The fact that the majority of responses of neighboring neurons were similar suggests that descending cortical input can have a synchronizing influence on the STN and the circuits involved in STN responses. To test the hypothesis that larger, more spatially distributed populations of STN neurons are synchronized in a similar manner by cortical stimulation, we recorded LFPs evoked simultaneously with the responses of units. Because LFPs are the result of the synchronized, sub-threshold, and suprathreshold activities of local neural populations, they may be faithful indicators of synchrony in the STN (for reviews, see Hubbard et al. 1969; Mitzdorf 1985).

#### *Correlations between evoked unit responses and LFPs*

Multiphasic unit responses in rostral STN were evoked together with distinct LFPs in a robust and consistent manner (Fig. 3). On stimulation, action potentials rode on negative and positive fluctuations in the LFP in a predictable manner; these LFPs were seen on a trial-by-trial basis (Fig. 3A). Peristimulus averaging confirmed that LFPs evoked in rostral STN by frontal cortical stimulation consistently mirrored the simultaneously recorded single-unit responses, such that the two types of responses were again synchronized to within a few milliseconds (Figs. 3B and C, 4A and 5A). A brief negative deflection in the LFP ("N1"; mean latency to the peak of the deflection of  $5.4 \pm 1.3$  ms;  $n = 42$ ) occurred in time with the short-latency excitation seen in the unit response. A brief

positive deflection in the LFP ("P1"; mean latency to the peak of the deflection of  $11.9 \pm 2.8$  ms) occurred at the time of the short-latency inhibition or the reduction in firing. A second negative deflection ("N2"; mean latency to peak of  $21.6 \pm 3.9$  ms) was closely associated with the long-latency excitation response. The mean latencies of the peaks of the N1, P1, and N2 deflections were not statistically different from the mean latencies of the "peak" of each of the corresponding phases of the unit response (the mean peaks of the short-latency excitation, short-latency inhibition, and long-latency excitation responses occurred at  $6.0 \pm 2.0$ ,  $12.8 \pm 3.5$ , and  $22.7 \pm 3.9$  ms, respectively). The final LFP deflection, a second positive deflection ("P2"; mean peak latency of  $30.1 \pm 3.1$  ms), coincided with the start of the long-latency, long-duration inhibition ( $30.7 \pm 5.3$  ms; see Figs. 3C, 4A, and 5A). The close relationship between unit responses and LFPs was also evident in spike-triggered averages of the LFP (Fig. 3D) and in the frequency histograms of the unit and LFP responses for all 42 responsive neurons recorded in rostral STN (Fig. 3E). Increases in unit activity coincided with negative shifts in LFPs, and decreases in unit activity coincided with positive shifts in LFPs. Linear regression analysis of the frequency histograms of unit activity and LFPs (0–30 ms) demonstrated a significant negative correlation ( $r = -0.74$ ,  $P = < 0.00001$ ).

#### *Dependence of evoked responses on cortical connectivity*

The LFPs evoked in STN were not closely related to field potentials that were simultaneously evoked in the frontal cortex itself, as measured in the EEG, suggesting that volume conduction of cortical activity was not responsible for the subthalamic nucleus LFP (Figs. 4A and 8). Electrical stimulation of temporal cortex, which, unlike the frontal cortex, does not project directly to the STN (Canteras et al. 1990; Kolomiets et al. 2001), failed to elicit stereotypical, multiphasic unit and LFP responses in any of the STN regions examined (14 neurons tested; Fig. 4B). Recordings of frontal cortical EEG during stimulation of temporal cortex suggested that stimulation of temporal areas did not lead to an excitation of frontal cortex (Fig. 4B). If widespread excitation of cortical neuronal structures by excessive current flow was occurring, then one would expect similar responses in STN when stimulating either of these distant and functionally-distinct cortical regions. Thus the relatively low-intensity electrical stimuli used in this study probably activated only a small, circumscribed area of cortex, further implying that unit and LFP responses were dependent on cortical connectivity and not volume conduction effects of cortical stimulation per se.

Although these stereotypical, multiphasic LFPs were tightly correlated to unit responses, they were unlikely to be the result of suprathreshold activity in the soma and dendrites of just one or two neurons in close proximity to the tip of the recording electrode because the LFPs could be recorded easily when unit activity could not (Fig. 5A). Moreover, the evoked LFPs were robust and repeatable, such that N1, P1, N2, and P2 deflections could be clearly discerned without averaging i.e., "on-line" in response to a single stimulus (Fig. 5B).

#### *Topographical organization of evoked responses within the subthalamic nucleus*

Unit response failures to frontal cortical stimulation, which most commonly occurred in the caudal half of STN, were not

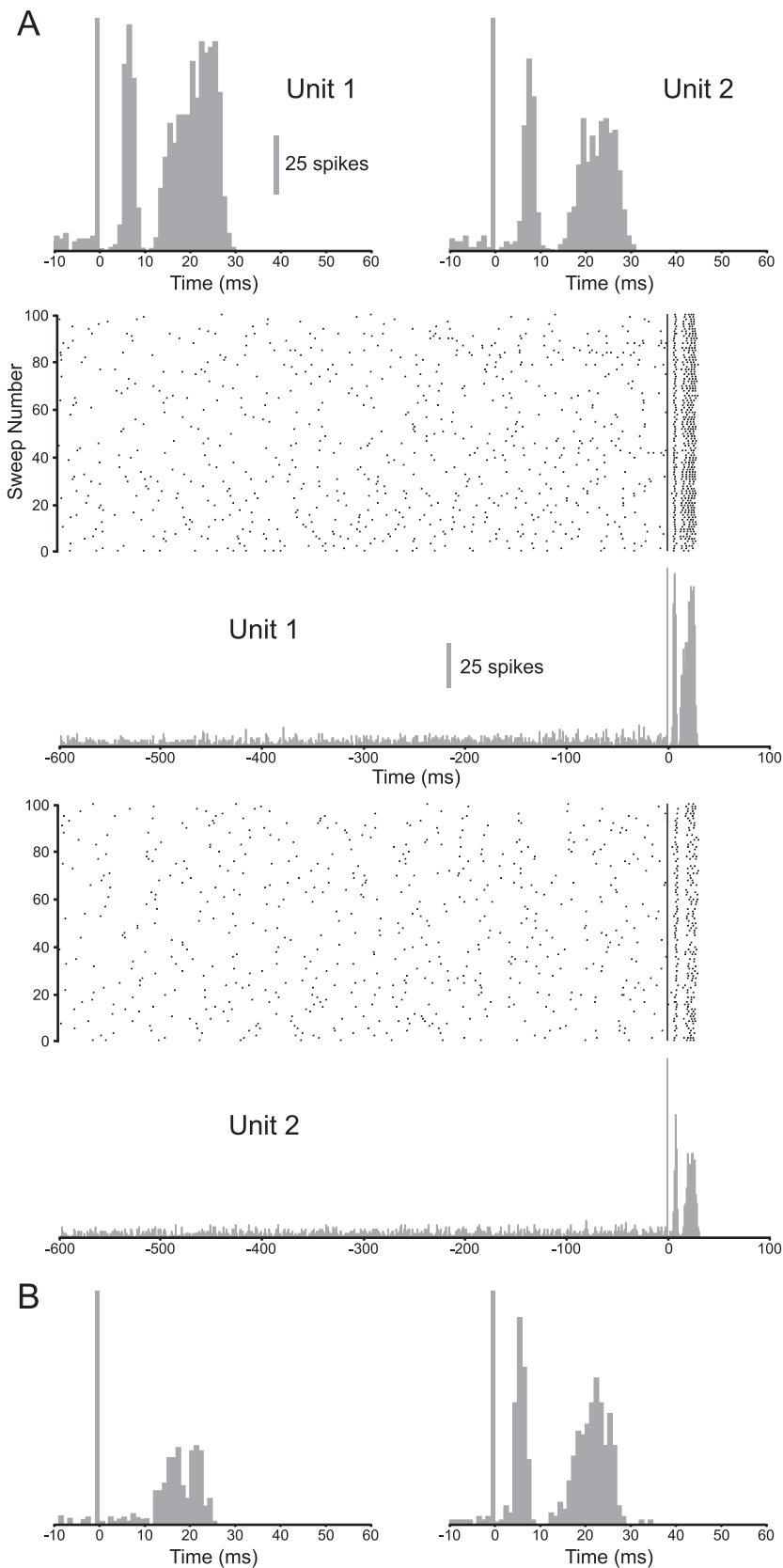


FIG. 2. The responses of neighboring subthalamic nucleus neurons are often synchronized by frontal cortical stimulation. *A, top*: responses of 2 neighboring neurons in rostral STN that were simultaneously recorded using the same electrode. Note that the response profiles were qualitatively similar and that the peak excitations and inhibitions of both neurons were synchronized to within a few milliseconds. *Bottom*: rasterplots and longer peristimulus time histograms did not show significant increases or decreases in firing before cortical stimulation, suggesting that the discharges of the 2 neurons were not synchronized before stimulation. *B*: the responses of the exceptional pair of rostral STN neurons were not similar or synchronized. Calibration bars for unit 1 also apply to unit 2. Calibration bars in *A, top*, also apply to *B*.

accompanied by the stereotypical evoked LFP ( $n = 16$ ; Fig. 6), further indicating that these LFPs were topographically organized and that the stimulation current was not excessive.

Although neurons in the caudal half of STN did not demonstrate the multiphasic response typical of cells in rostral STN, two neurons did respond with a long-latency excitation and a

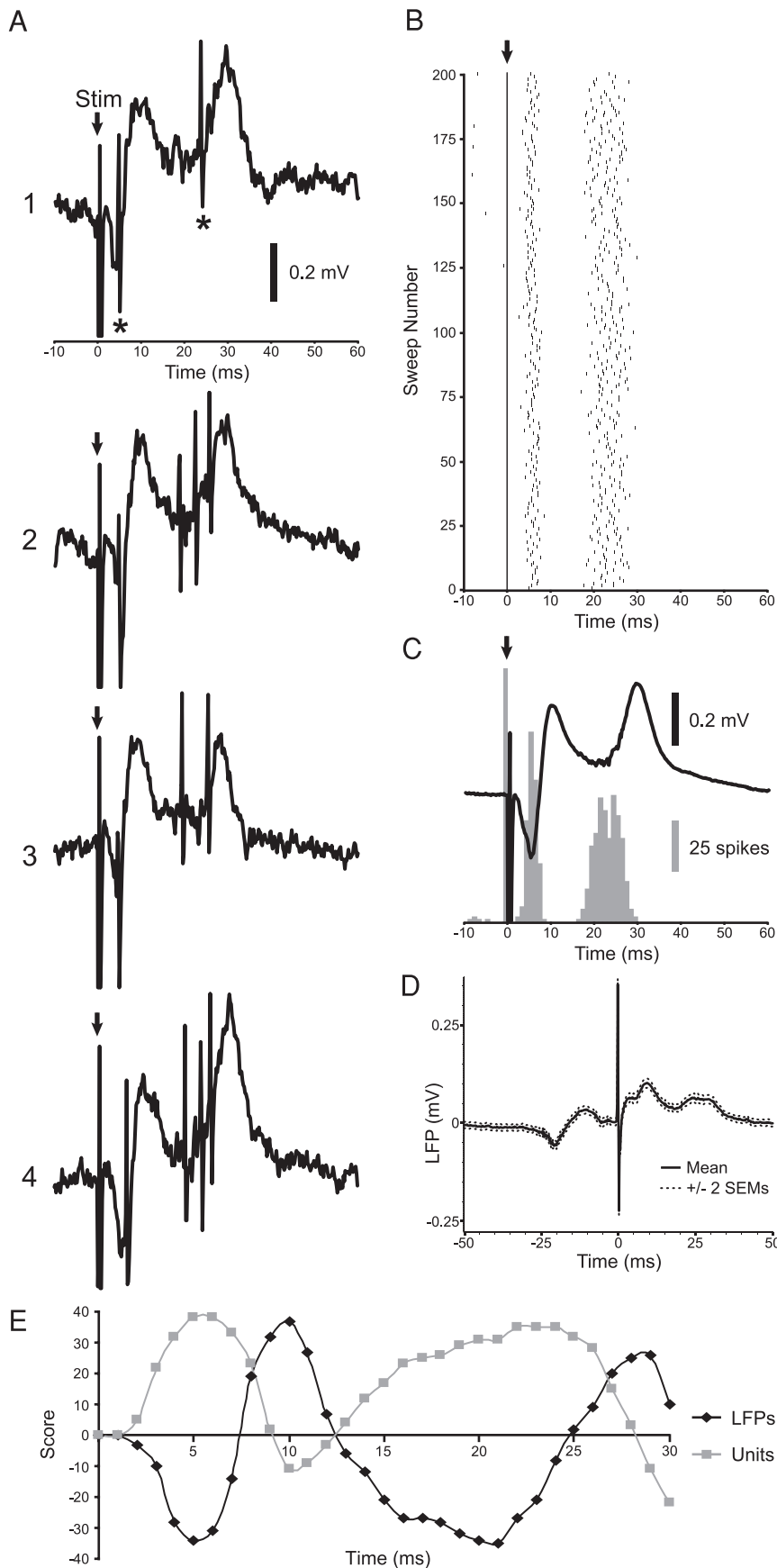


FIG. 3. The unitary responses of subthalamic nucleus neurons are consistently evoked together with distinct local field potentials. *A*: typical unit responses and local field potentials (LFPs, 1–4) evoked in rostral STN by 4 sequential stimuli delivered to the frontal cortex ( $300 \mu\text{A}$  pulses at 0.67 Hz). Units (sharp spikes; see \* in 1) and LFPs were recorded from the same electrode (0 to 2 kHz band-pass filtered). *B*: raster plot showing the typical “multiphasic” unit responses evoked by 200 sequential stimuli. *C*: peristimulus time histogram of unit responses and peristimulus average of LFPs evoked in the 200 stimulation trials shown in *B*. *D*: spike-triggered average of LFPs evoked in the 200 stimulation trials shown in *B*. Periods of increased likelihood of firing were coincident with significant negative deflections in the LFP. The large deflection at *time 0* corresponds to the spike in the wide-band filtered LFP. *E*: frequency histograms of the unit responses and evoked LFPs for all 42 responsive neurons recorded in rostral STN demonstrated a stereotyped, inverse relationship between unit activity and LFPs. In this and the following figures, positivity is signified as upward deflections of LFPs. Same STN neuron and recording site in *A–D*. Calibration bar in 1 of *A* also applies to 2–4 in *A*.

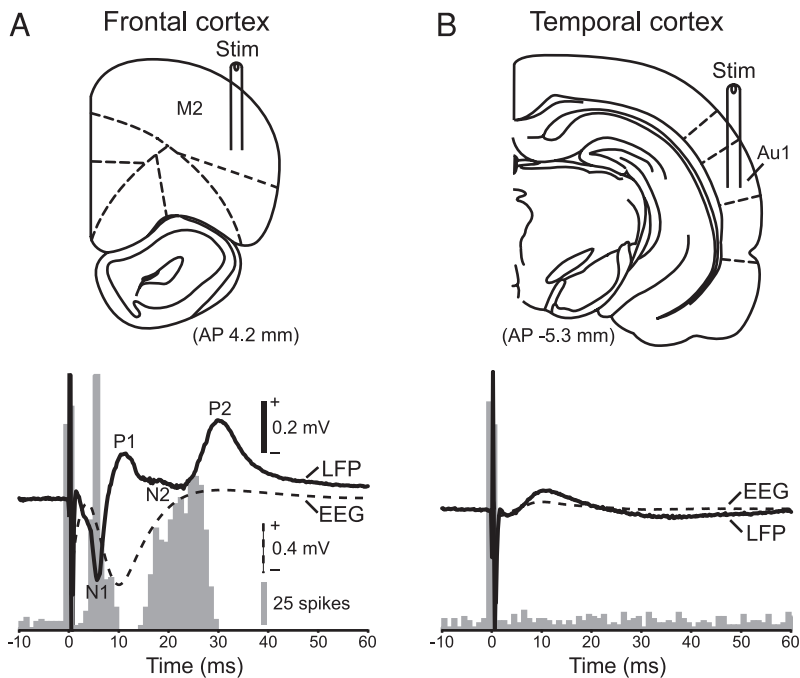


FIG. 4. Evoked unit activity in the subthalamic nucleus is tightly correlated with LFPs and is organized according to cortical region. *Top*: schematic representations of the positions of the stimulating electrodes (Stim) in the frontal (*A*) and temporal (*B*) cortices. *A*: stimulation of the frontal cortex at 300  $\mu\text{A}$  evoked a characteristic unit response that was consistently mirrored by contemporaneous deflections in the LFP (—, peristimulus average of LFP) recorded from the same electrode. Excitations at the single-unit level were accompanied by negative deflections in the LFP (N1 and N2), while inhibitions were associated with positive deflections in the LFP (P1 and P2). The LFP in STN was dissimilar to the field potential evoked in the frontal cortex by the same stimuli [---; electroencephalogram (EEG)]. *B*: stimulation of the temporal cortex (600  $\mu\text{A}$ ) did not evoke a response from the same neuron. Accordingly, field potentials in the STN and frontal cortex exhibited a different, smoother profile. Calibration bars in *A* also apply to *B*. AP (anterior-posterior) numbers denote positions with respect to bregma. M2, secondary motor cortex, Au1, primary auditory cortex.

weak, long-latency long-duration inhibition (Fig. 6). The absence of short-latency responses was correlated at the level of the LFP by a lack of distinct N1 and P1 deflections. Moreover, the long-latency unit responses were associated with small N2 and P2 deflections (also see Fig. 10).

#### *Spatial specificity of evoked responses*

To test whether the multiphasic evoked LFP was confined to STN, unit and LFP responses were also recorded from neighboring brain regions. The LFP typically evoked in rostral STN was not observed in surrounding structures, such as the zona incerta, the cerebral peduncle and the internal capsule (Figs. 5*A* and 7), supporting the idea that the LFP is due to the synchronous activity of neighboring STN neurons and is not strongly influenced by activity in surrounding (proximal) structures. Most neurons in the ventral division of the zona incerta (ZIV) did not respond to cortical stimulation (13 of 20 neurons tested; Fig. 7*A*). Evoked LFPs recorded with nonresponsive ZIV neurons were of small amplitude and were unpredictable (Figs. 5*A* and 7*A*). The discharges of the responsive ZIV neurons were highly variable and were not clearly correlated with the wide variety of LFPs that were evoked in this region (Fig. 7, *B–E*). Neither the unit activity nor LFP bore a strong relationship to the responses in STN. Neurons in the cerebral peduncle (CP) were only rarely observed ( $n = 3$ ). None of the CP neurons responded to cortical stimulation and evoked LFPs were relatively smooth and featureless (Figs. 5*A* and 7*F*).

#### *Relationship of evoked responses to stimulus intensity*

To ensure that stimulation at a current intensity of 300  $\mu\text{A}$  was effectively maximal and to test the possibility that different phases of the characteristic unit and LFP responses were differentially sensitive to input intensity, current-response relationships were studied. The profile of the LFP and the unit responses evoked in STN by frontal cortical stimulation were

both dependent on the intensity of the current that was delivered (Fig. 8). There were no qualitative differences in the response profiles evoked by stimulation at 600 and 300  $\mu\text{A}$  (Fig. 8, *A* and *B*), and although the absolute magnitudes of the phasic responses varied slightly, no significant differences in latencies were observed ( $n = 9$  neurons). Thus the responses to stimulation at 300  $\mu\text{A}$  were maximal with respect to the pattern of the response. However, reducing the current intensity to 150 or 75  $\mu\text{A}$  resulted in distinct and corresponding changes in the unit and LFP responses (Fig. 8, *C* and *D*). The short-latency responses were attenuated, as were the associated LFP deflections (N1 and P1), and the longer-latency unit responses tended to fail together with the related LFP deflections (N2 and P2). The short-latency excitation and N1 responses were the last to fail at the lowest current intensity (Fig. 8*D*).

#### *Relationship of evoked responses to brain state*

The urethan-anesthetized rat is a good model for determining the impact of extremes of forebrain activity on the BG (Magill et al. 2000, 2001). Activity in the cortex spontaneously shifts from “slow-wave activity” (Fig. 9*A*), which is similar to activity observed during natural sleep, to a state of “global activation” (Fig. 9*B*), which contains patterns of activity that are more analogous to those observed during the awake state and vice versa (Steriade 2000). To test whether alterations in ongoing forebrain activity could affect the profiles of the evoked responses in STN, we recorded and compared responses evoked during both slow-wave activity and global activation. Spontaneous shifts in the global brain state of the animal, as assessed from the cortical EEG, did not greatly affect the multiphasic responses to cortical stimulation (6 neurons; Fig. 9, *A* and *B*). There were no qualitative differences in the patterns of the unit responses or LFPs evoked by stimulation during the two brain states, and although the absolute amplitudes of the multiphasic responses varied slightly, no significant differences in latencies were observed.





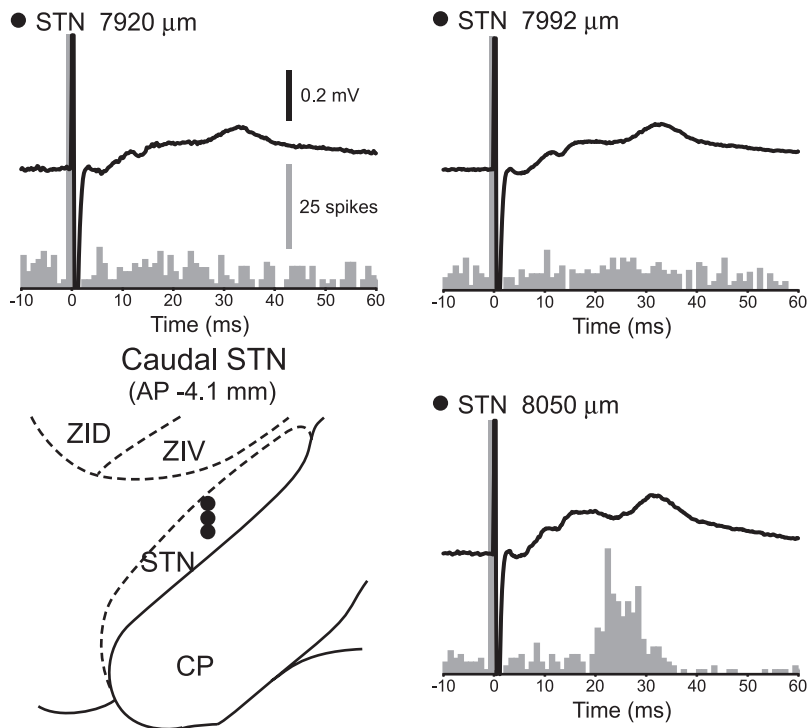


FIG. 6. Unit responses and LFPs evoked in the subthalamic nucleus by frontal cortical stimulation are topographically organized. *Bottom left*: schematic representation of recording sites in 1 vertical pass of the electrode through the caudal half of the STN. Figures denote depths of recording sites from the cortical surface. These neurons were recorded from the same animal as shown in Fig. 5. *Top left* and *right*: the multiphasic unit and LFP responses that were observed in rostral STN were not observed in caudal STN. Caudal STN neurons did not usually respond to frontal cortical stimulation (STN neurons at depths of 7,920 and 7,992  $\mu\text{m}$ ) and robust LFP responses of the kind recorded in rostral STN were not observed. Neurons located within a few hundred micrometers of each other generally shared similar "response" profiles (neurons at 7,920 and 7,992  $\mu\text{m}$ ). However, 2 cells did respond with a long-latency excitation and a weak, long-latency, long-duration inhibition, which were reflected in the LFP (e.g., the neuron at 8,050  $\mu\text{m}$ ). Note the absence of short-latency responses was correlated at the level of the LFP by a lack of distinct N1 and P1 deflections. Calibration bars for units and LFP in STN (7,920  $\mu\text{m}$ ) apply throughout. AP (anterior-posterior) number denotes position with respect to bregma. CP; cerebral peduncle, ZID, dorsal division of the zona incerta; ZIV, ventral division of the zona incerta.

primary motor, cingulate and, to a lesser extent, somatosensory cortex (see review by Smith et al. 1998). The first response of most neurons in the rostral half of STN to stimulation of the

ipsilateral frontal cortex was a brief, but powerful, excitation with a mean latency of 4.5 ms. This short-latency excitation has been described by others and appears to be driven by the

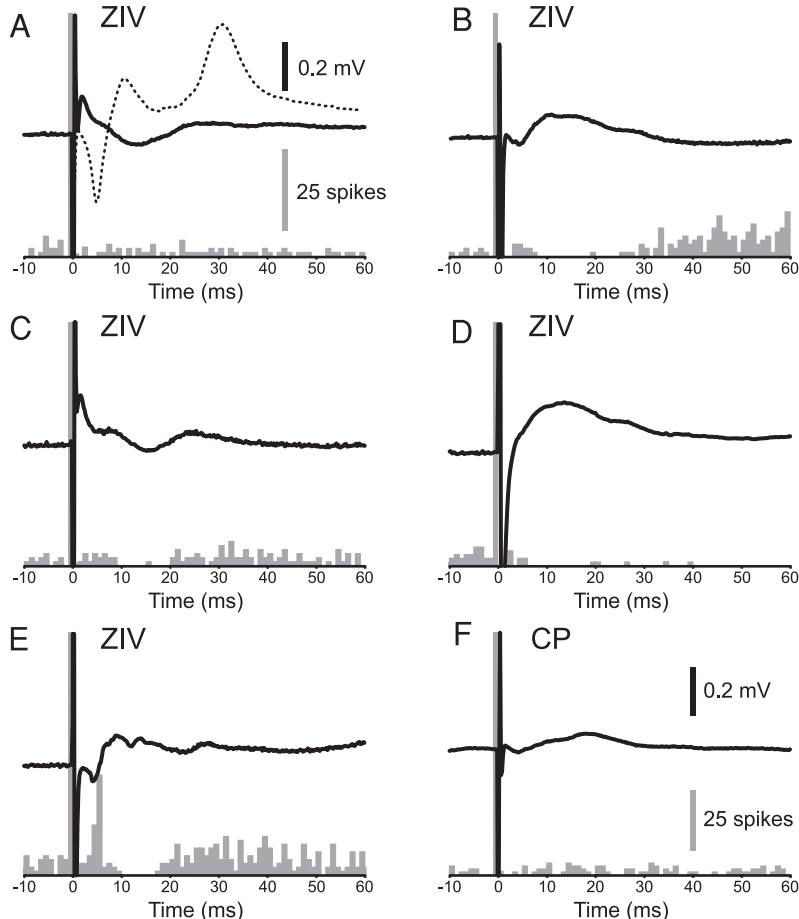


FIG. 7. Unit responses and LFPs evoked in the zona incerta and cerebral peduncle are variable and are not strongly correlated. *A*: most neurons in the ventral division of the zona incerta (ZIV) did not respond to cortical stimulation (300–600  $\mu\text{A}$ ). Evoked LFPs recorded with nonresponsive ZIV neurons were of small amplitude and were unpredictable. *...*, multiphasic LFP evoked in rostral STN for comparison. *B–E*: in contrast to the subthalamic nucleus, the discharges of the responsive ZIV neurons were variable and were usually not clearly correlated with the evoked LFPs. *F*: the small number of neurons recorded in the cerebral peduncle (CP) did not respond to cortical stimulation, and LFPs evoked in the peduncle were relatively smooth and featureless. Calibration bars in *A* apply to *B–E*.

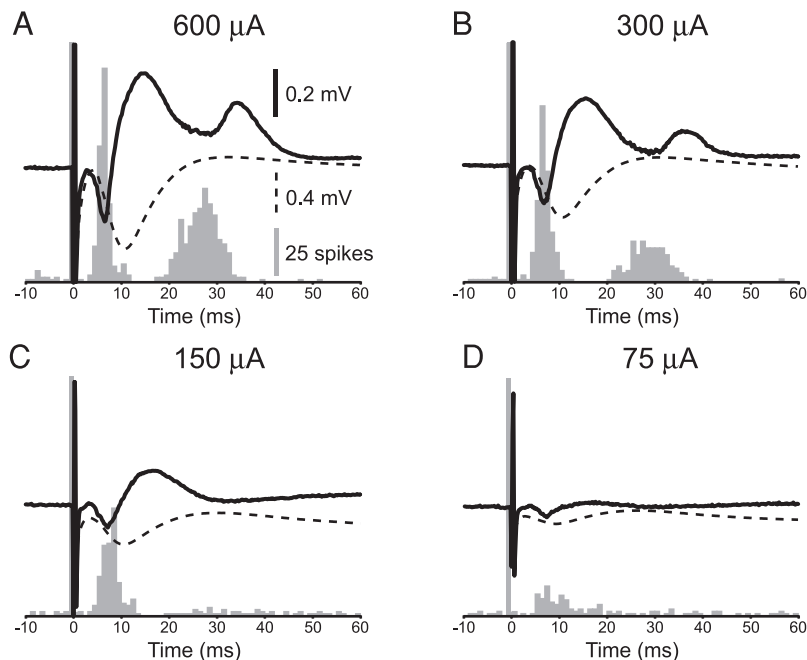


FIG. 8. Unit responses and LFPs evoked in the subthalamic nucleus covary with stimulus intensity. *A*: typical responses of a rostral unit, the LFP, and the cortical EEG (---) to frontal cortical stimulation at an intensity of 600  $\mu$ A. *B*: reducing the stimulus intensity to 300  $\mu$ A did not alter the qualitative nature of the responses although there were small, but commensurate, changes in the absolute magnitudes of the responses. *C*: a further reduction in current intensity to 150  $\mu$ A resulted in an attenuation of the short-latency excitation and inhibition responses and a failure of the long-latency responses. Changes in the evoked LFP were commensurate with these alterations in the unit response; the amplitudes of the N1 and P1 deflections were reduced, whereas the N2 and P2 responses virtually disappeared. *D*: only the weak, short-latency excitation, with a corresponding small, negative deflection in the LFP, remained at a stimulus intensity of 75  $\mu$ A. Same neuron recorded in *A–D*. Calibration bars in *A* apply to all panels.

direct, excitatory corticosubthalamic projection (Bevan et al. 1995; Fujimoto and Kita 1993; Kitai and Deniau 1981; Kolomiets et al. 2001; Maurice et al. 1998; Nambu et al. 2000; Rouzair-Dubois and Scarnati 1987; Ryan and Clark 1991, 1992). The multiphasic nature of the typical unit response develops from disynaptic and polysynaptic interactions, which are slower to manifest. The second phase of the unit response, a brief, short-latency (mean: 10.4 ms) inhibition, probably arises from feed-forward excitation of GP neurons by inputs from activated STN neurons and then feed-back inhibition of STN neurons by reciprocally connected neurons of the GP (Bevan et al. 2002a,b; Fujimoto and Kita 1993; Kita and Kitai 1991; Maurice et al. 1998; Nambu et al. 2000; Ryan and Clark 1991, 1992; Smith et al. 1998). Disinhibition of STN neurons, mediated by feed-forward connections through the NS and GP and then on to STN, has been proposed to account for the third phase of the response, a long-latency (mean: 16.6 ms) excita-

tion (Maurice et al. 1998; Nambu et al. 2000). Alternatively, this late excitation may represent the latter phase of a prolonged barrage of cortical EPSPs and/or a long-lasting response of STN neurons to short-lasting cortical input (Fujimoto and Kita 1993; Otsuka et al. 2001; Ryan and Clarke 1992).

The short-latency excitation responses of most pairs of neighboring STN neurons were similar such that they were synchronized within a few milliseconds, and, when present, covaried; this demonstrates that the connectivity of the activated corticosubthalamic projection could support the synchronous recruitment of neighboring target neurons. Because short-latency inhibitions were also synchronized, it is also likely that the activity of neurons in GP, and subsequently in STN, was synchronized by the correlated discharges of STN neurons that were driven by corticosubthalamic input. Synchronous, long-latency excitations suggest that precisely timed, disinhibitory processes were also widespread. These findings argue that,

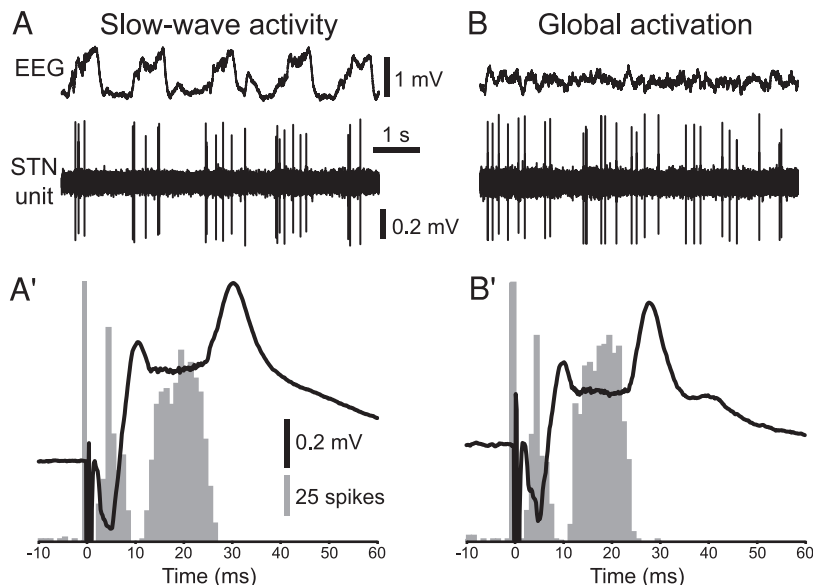


FIG. 9. Unit responses and LFPs evoked in the subthalamic nucleus by cortical stimulation are not strongly dependent on brain state. *A*: during slow-wave activity, cortical activity (EEG) was dominated by a large-amplitude, slow oscillation. Unit activity in STN was closely related to the slow-wave activity present in the cortex; STN neurons commonly exhibited low-frequency oscillations in firing. *A'*: unit and LFP responses evoked during slow-wave activity (a few seconds after recording shown in *A*) were robust and were of stereotypical, multiphasic natures. *B*: global activation was characterized by a prolonged loss of the large-amplitude, slow oscillation in the cortex and was associated with a change in the activity of the STN neuron to irregular, tonic firing at a higher rate. *B'*: the multiphasic responses of the same STN neuron and associated LFP as evoked during global activation (a few seconds after recording in *B*) did not substantially differ from responses evoked during slow-wave activity. Calibration bars in *A* apply to *B*, bars in *A'* apply to *B'*.

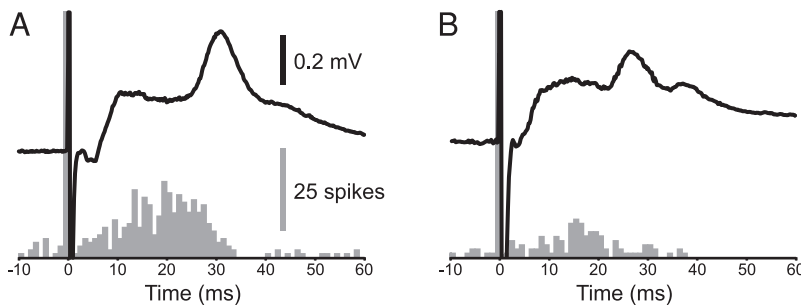


FIG. 10. Atypical unit responses and LFPs evoked in the subthalamic nucleus by cortical stimulation. *A* and *B*: a small number of rostral neurons did not exhibit short-latency excitation or short-latency inhibition responses to stimulation (at 300–600  $\mu$ A) but did exhibit long-latency excitations and inhibitions. The LFPs evoked in these cases were also unusual but still reflected unit activity; short-latency N1 and P1 deflections were small or absent from the corresponding LFPs, but the N2 and P2 deflections were still present. Calibration bars in *A* apply to *B*.

despite potentially complex circuit interactions, responses within small, local populations of neurons tend to be similar because the neurons are recruited by related afferent circuitry.

Unit responses were topographically organized within STN. A small minority of STN neurons, most of which were located in the caudal half of STN, did not respond to cortical stimulation. These findings are in good agreement with previous anatomical (Afsharpour 1985b; Canteras et al. 1990; Kolomiets et al. 2001) and physiological (Kolomiets et al. 2001) studies, which have shown that the frontal cortical areas stimulated in the present study project throughout most of the dorsoventral axis of the rostral two-thirds of STN only. The caudal one-third of the STN receives input from the caudal aspects of the medial frontal cortex (Afsharpour 1985b), which was presumably not activated by the electrical stimulus used in this study. The present data are also in keeping with studies demonstrating that the (auditory) temporal cortex does not project directly to STN (Canteras et al. 1990; Kolomiets et al. 2001). Response differences were unlikely to be due to insufficient current flow at the site of stimulation because responses were maximal with respect to pattern at the stimulus intensities used. Similarly, the unresponsive nature of these neurons was probably not a function of anesthetic depth because the pattern of responses did not dramatically alter across brain states. The fact that unit and LFP responses to corticosubthalamic input were topographically organized and thus, did not conflict with the known anatomy, adds further functional significance to these stimulation data and implies that the currents used stimulated relatively restricted functional areas of cortex.

#### Neural basis of LFPs evoked in the subthalamic nucleus

The recording of LFPs evoked together with unit responses allowed us to test the hypothesis that the responses of larger, more spatially distributed populations of STN neurons were also synchronized to the high degree exhibited by pairs of neighboring cells. A complex and nonlayered organization of neurons and fibers, as occurs in STN (Afsharpour 1985a; Kita et al. 1983; Smith et al. 1998; Yelnik and Percheron 1979), can make the interpretation of current flows, and hence extracellular potentials, challenging (Hubbard et al. 1969). Despite this, several temporal and spatial correlations between evoked LFPs and unit activity were reliably observed.

The first response of most STN neurons to cortical stimulation, i.e., the short-latency excitation, coincided with a prominent negative deflection (N1) in the LFP (Fig. 11A). Theoretical and experimental studies suggest that LFPs are a consequence of current flow related to synchronized, postsynaptic potentials rather than current flow across presynaptic and axonal membranes (Hubbard et al. 1969; Mitzdorf 1985). Thus

we propose that the N1 deflection in the LFP was the result of concerted subthreshold and suprathreshold population activity in STN that was driven by monosynaptic cortical input (Fig. 11, *A* and *B*). In agreement with this, the activity of neighboring STN neurons was synchronously increased by the stimulus (also see Ryan et al. 1992) and, when single units did not respond with a short-latency excitation, the N1 deflection was much smaller or absent. The second phase of the unit response to cortical stimulation, a brief reduction in activity, which was likely caused by feed-back inhibition from GP neurons (see preceding text) (Bevan et al. 2002b), was significantly associated with a brief, positive deflection (P1) in the LFP (Fig. 11A). This positive deflection likely reflected the synchronous hyperpolarization of STN neurons by pallidal inputs (Fig. 11, *A* and *C*). Support for this comes from intracellular recordings (Fujimoto and Kita 1993; Kitai and Deniau 1981) and the fact that when single units did not respond with a short-latency inhibition, the P1 deflection was not distinct. The subsequent long-latency excitation, presumably due to the disinhibition of STN neurons and/or the continued excitation of STN neurons by cortical input (see preceding text), was accompanied by a second negative deflection (N2) in the LFP (Fig. 11, *A* and *D*). The cellular and synaptic mechanisms underlying the final phase of the unit response, a long-duration inhibition, are unknown, although “cortical disfacilitation” has been hypothesized to be the cause (Fujimoto and Kita 1993). The start of this phase again was associated with a positive deflection (P2) in the LFP (Fig. 11A), a finding corroborated by previous intracellular data indicating that the long-duration inhibition is due to membrane hyperpolarization (Kitai and Deniau 1981). These significant temporal correlations between unit and population responses suggest that synchronous frontal cortical inputs can impose widespread synchronization within the rostral half of the STN and associated feed-back and feed-forward neuronal circuits.

In agreement with the topographic organization of unit responses, spatial correlations between units and LFPs were also consistently observed in addition to temporal correlations. Neurons in the caudal half of STN did not respond in the multiphasic way that was typical of neurons in rostral STN. In these cases, the characteristic evoked LFP was either very small, or, more commonly, not seen at all. These observations substantiate the idea that the LFPs were the result of the synchronous activity of ensembles of functionally related STN neurons and argue against a significant contribution to LFPs from volume-conducted activity.

The stereotypical unit responses and LFPs observed in STN were not found in the zona incerta (ZI) or CP. Furthermore, in ZI, unit responses were not clearly related to LFPs, despite the

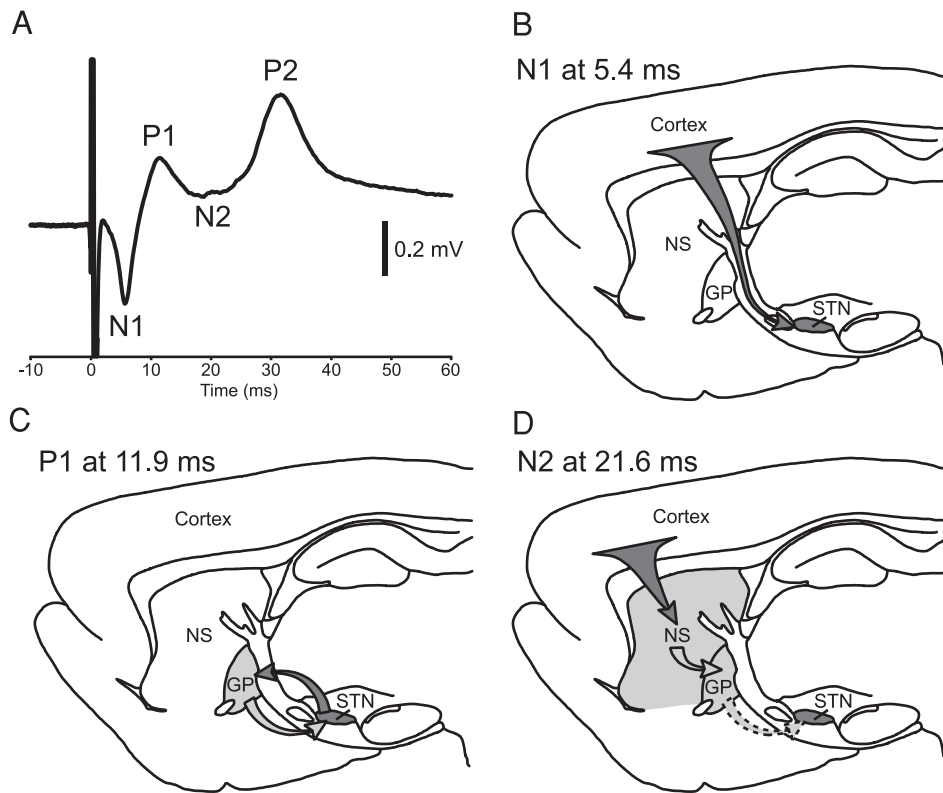


FIG. 11. Hypothetical scheme of the neuronal circuits that likely underlie the cortical stimulation-evoked LFP in subthalamic nucleus. *A*: the stereotypical, multiphasic profile of the LFP evoked in STN. The LFP was likely a consequence of activity in monosynaptic and polysynaptic circuits. *B–D*: times are mean latencies to peak deflections. Shaded basal ganglia nuclei contribute to that particular phase of the response in STN; white nuclei do not contribute. Dark gray arrows, excitatory pathways; light gray arrows, inhibitory pathways. *B*: the short-latency, negative deflection, N1, was probably due to activation of the direct corticosubthalamic pathway. *C*: the short-latency, positive deflection, P1, probably arose as a consequence of feed-forward excitation of globus pallidus (GP) by STN and then feed-back inhibition of STN by GP. *D*: the long-latency, negative deflection, N2, was most likely due to disinhibition of STN through inhibition of GP by neostriatum (NS) in a feed-forward manner. Arrow with dashed line represents disinhibition. The circuits that may underlie the long-latency (mean: 30.1 ms), positive deflection, P2, are unknown.

fact that this area also receives monosynaptic inputs from frontal cortical areas (Mitrofanis and Mikuletic 1999). This implies that the synchronization of unitary responses in STN is not simply the result of the synchronized corticofugal volleys induced by stimulation but must also entail some fundamental differences in the organization of direct cortical inputs to neighboring neurons in STN as compared with neurons in ZIV and/or the intrinsic composition of these nuclei. The unit and population responses recorded in ZI and the CP thus act as useful controls for the data from STN recordings that support the idea that the multiphasic LFP in STN is the result of the synchronized activity of a local population of neurons and, as such, is characteristic of STN.

The high predictability and specificity of the evoked LFPs, together with their topographic nature, have two important implications. First, these data suggest that the geometry of the relevant local dipoles dictating current flow in STN i.e., the somata and dendrites of neurons, may be more ordered than previously thought. Second, the correlations we have observed between units and evoked LFPs may strengthen the interpretation of LFPs commonly observed in STN in other paradigms (see review by Brown 2003), particularly high-frequency LFP oscillations because rapid sequences of excitations and inhibitions at the unit level are reflected in the LFP with good time resolution.

#### Functional implications

Although the electrical stimulation used in this study may in itself ensure a synchronous cortical output, it does not follow that the subthreshold and suprathreshold responses of neighboring STN neurons will also be synchronous. Glutamatergic EPSPs and GABAergic inhibitory postsynaptic poten-

tials (IPSPs), whether they are compound or unitary, are often inefficient at phase-locking the responses of neurons (e.g., Bennett and Wilson 1998; Carter and Regehr 2002; Fricker and Miles 2000). However, we found that activation of direct and indirect inputs to STN neurons could often synchronize the discharges of specific neuronal populations in the STN. Our finding strongly suggests that the neuronal networks engaged by cortical activation are designed to promote precise coupling between synaptic potentials and action potentials in STN neurons, which, in turn, generate correlated activity in neighboring STN neurons during synchronized cortical activity. The mechanisms underlying this precise coupling are unknown but could be extrinsic, e.g., each STN neuron in a given functional domain is innervated to a similar degree by the associated regions of cortex and GP. Alternatively, or additionally, the mechanism may be intrinsic in nature, e.g., STN neurons exhibit an unusual enhanced sensitivity to excitatory input and little spike-frequency adaptation during high-frequency firing (Beurrier et al. 2000; Bevan and Wilson 1999; Hallworth et al. 2003; Wilson et al. 2004). Whatever the mechanism(s) responsible for precise coupling in STN, it is important to note that the same stimuli did not have the same effect on the ZI despite the fact that it also receives inputs from frontal and/or prefrontal cortices (Mitrofanis and Mikuletic 1999).

The significance of our findings is supported by studies that suggest that the STN, under normal and pathological conditions, is subject to synchronous cortical inputs *in vivo*. Neurons in cortical areas projecting to the STN exhibit a wide range of synchronous network activity during natural behaviors (Aoki et al. 1999; Donoghue et al. 1998; Kristeva-Feige et al. 1993; Murthy and Fetz 1992, 1996). In particular, synchronized oscillations, which are caused by periodic, phase-locked discharges of cortical cell ensembles, are frequently observed in



cortex during sensory-motor integration and other complex behaviors that are likely to involve the basal ganglia (Engel and Singer 2001; Engel et al. 2001; MacKay 1997; Roelfsema et al. 1997). Corticosubthalamic input might therefore be highly synchronized during these behaviors, and, in turn, generate synchronous activity in the STN. Consistent with this hypothesis, emergent cortical oscillations synchronize (oscillatory) unit activity in STN (Allers et al. 2002; Magill et al. 2000, 2001; Wichmann et al. 2001). Furthermore, the STN may express synchronous oscillations, as evinced in unit or LFP recordings, under both normal and pathological conditions (Boraud et al. 2002; Brown et al. 2002; Brown 2003; Levy et al. 2002a). Some oscillations in STN are related to movement and are dependent on dopamine, suggesting they are of functional significance (Brown et al. 2001, 2002; Levy et al. 2002a; Williams et al. 2002, 2003). Moreover, simultaneous recordings have shown that oscillatory population activity in STN may be significantly coherent with that in cortex and that the time lags between oscillations are consistent with the cortex playing a role in synchronizing activity in the basal ganglia (Cassidy et al. 2002; Marsden et al. 2001; Williams et al. 2002).

The present data highlight two additional considerations of importance. The response evoked in the cortex itself, as measured in the EEG, was entirely different to the evoked STN response, as recorded in the LFP, suggesting that BG circuits are able to generate a different form of activity than that expressed by the cortex. Second, the fact that the short-latency excitation response of STN neurons and associated LFP deflection (N1) were last to fail on reduction of stimulus intensity suggests that STN neurons receiving monosynaptic cortical input are likely to respond most vigorously and reliably to that input when activated, compared with subsequent inputs derived from polysynaptic circuit interactions. Taken together, the current data add weight to the proposed importance of the corticosubthalamic projection (also see Magill et al. 2001; Mink 1996; Nambu et al. 2002) and synchronous ensemble activity in information processing in cortico-basal ganglia circuits (Engel et al. 2001).

#### *Clinical implications*

The STN is an important target for the surgical treatment of Parkinson's disease and perhaps, in the future, intractable epilepsy, partly because of the proven clinical benefits of "deep brain stimulation," in which neuronal activity is modified by electrical stimulation through electrodes implanted in STN (Chabardès et al. 2002; Kumar et al. 1998; Limousin et al. 1998; Loddenkemper et al. 2001). However, surgical implantation of electrodes is challenging because of the difficulties of unequivocally locating the STN and key neighboring structures (Starr 2002; Voges et al. 2002). The finding that LFPs evoked in STN by stimulation of the cerebral cortex are distinct from those evoked in surrounding structures indicates that evoked LFPs could act as electrophysiological "fingerprints" that might greatly aid the identification of the STN and, perhaps more importantly, specific regions within or above STN (Saint-Cyr et al. 2002; Voges et al. 2002). Electrophysiological recordings of STN activity are now routinely used in implantation surgery to refine the positioning of the stimulation electrode (Priori et al. 2003; Starr 2002) and because the

evoked LFPs are relatively resistant to changes in brain state, patient anesthesia may not be an issue. Furthermore, LFPs can be recorded in STN from microelectrodes, or the macroelectrodes that are used for deep brain stimulation (Brown et al. 2001; Dinner et al. 2002; Levy et al. 2002a; Liu et al. 2002), and importantly, evoked LFPs could be evaluated after only a few cortical stimuli (i.e., within a few seconds intraoperatively). There is also some evidence to suggest that our approach could potentially be extrapolated for use with a noninvasive stimulation technique, such as transcranial magnetic stimulation, thereby increasing its potential clinical value (Kumar et al. 1999). Finally, our definitive demonstration of the neural basis of LFPs in the STN confirms that LFP studies in humans with implanted electrodes provide valuable insight into the pathophysiological mechanisms underlying idiopathic Parkinson's disease (see Brown 2003).

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