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The Primate Subthalamic Nucleus. III. Changes in Motor Behavior and Neuronal Activity in the Internal Pallidum Induced by Subthalamic Inactivation in the MPTP Model of Parkinsonism

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SUMMARY AND CONCLUSIONS

1. The effects of reversible and irreversible pharmacological manipulations of the neuronal activity in the subthalamic nucleus (STN) on parkinsonian motor signs and neuronal activity in the internal segment of the globus pallidus (GPi) were studied in African green monkeys rendered parkinsonian by treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

2. Muscimol injections (≤ 1 μl, 1 μg/μl) into STN reduced neuronal activity recorded at the injection site within minutes. This was immediately followed by reduced akinesia, tremor, and rigidity, as well as the emergence of dyskinesias in contralateral limbs. The motor effects were accompanied by generalized behavioral activation, lasted between 10 and 60 min, and were strongly dependent on the site of injection, with injections into the lateral "arm area" of STN first affecting contralateral arm movements and injections into the "leg" area affecting leg movements first.

3. Bicuculline injections (≤ 1 μl, 1 μg/μl) into STN marginally increased the neuronal activity and induced neuronal discharge in bursts. Rigidity, akinesia, and tremor in the contralateral limbs were not changed.

4. Injections of ibotenic acid in two animals (2 and 7 μl, 10 μg/μl) resulted in 70 and 51% destruction of STN, respectively. Similarly to the muscimol injections, this resulted in a reduction of the neuronal activity, a reversal of parkinsonian motor signs, and the development of dyskinesias in the contralateral limbs.

5. Although tremor was significantly reduced after STN lesions, periodic oscillatory neuronal activity in GPi persisted. The strength of modulation of the neuronal oscillation was not significantly changed after STN lesion.

6. The percentage of cells in GPi exhibiting increases in discharge in response to torque application was significantly reduced after STN lesion. The magnitude and duration of the responses with increase in firing rate were reduced after STN lesioning.

7. These results support the hypothesis that abnormally increased tonic and phasic activity in STN leads to abnormal GPi activity and is a major factor in the development of parkinsonian motor signs. Furthermore they imply that cells in the basal ganglia have the intrinsic property of discharging in periodic bursts, which is unmasked under parkinsonian conditions.

INTRODUCTION

As outlined in the first paper of this series (Wichmann et al. 1994), the basal ganglia are viewed as components of several larger segregated circuits that also involve the cortex and thalamus (Alexander et al. 1986, 1990; Hoover and Strick 1993). One of these, the "motor" circuit, has particular relevance for the development of movement disorders. This circuit includes precentral motor cortical areas as well as the sensorimotor territory of the striatum, both segments of the globus pallidus, and the subthalamic nucleus (STN). The motor areas in the putamen and in the internal pallidal segment (GPi) are linked via a "direct" monosynaptic inhibitory pathway and a polysynaptic "indirect" pathway, whose first portion consists of an inhibitory connection between the striatum and the external pallidal segment (GPe). GPe efferents then reach GPi via two different routes—a monosynaptic inhibitory pathway (Hazrati et al. 1990; Smith et al. 1992) and a pathway involving an inhibitory connection between GPe and STN, and an excitatory connection between STN and GPi (Kitai and Kita 1987; Smith and Parent 1988).

Metabolic and electrophysiological studies suggest that parkinsonian motor abnormalities are accompanied by disordered activity of the indirect pathway, particularly in the portion involving STN (Bergman et al. 1990, 1994; Filion and Tremblay 1991; Miller and DeLong 1987; Mitchell et al. 1989). For instance, in studies in macaques rendered parkinsonian by treatment with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Filion and Tremblay 1991; Miller and DeLong 1987) the average firing rate of cells decreased in GPe but increased in GPi. As reported in the second paper of this series (Bergman et al. 1994), our studies in African green monkeys demonstrate an increase of the average firing rate in STN and confirm some of the previously described rate changes in GPi. The study also describes changes in the firing pattern of STN and GPi neurons, with the emergence of prominent periodic bursts, predominantly at the parkinsonian tremor frequency of 4–8 Hz. These and other findings (Gerfen et al. 1990; Mitchell et al. 1989) have led to a pathophysiologic scheme for the development of parkinsonism in which loss of the dopaminergic innervation of the striatum leads to an increase of the activity along the inhibitory pathway linking the putamen and GPe, eventually resulting in disinhibition of neurons in STN. This leads to an increased excitatory drive on GPi neurons and consequently increased inhibition of thalamocortical cells. Overactivity in GPi is also caused by disinhibition via the direct pathway and the GPe-GPi connection. The lack of dopamine in the basal ganglia may promote the development of rhythmic burst discharges in neurons of the motor circuit, possibly because of intrinsic membrane properties of basal ganglia neurons (Bergman et al. 1994; Nambu and Llinas 1990). The changes in tonic discharge rates may be associated with the

hypokinetic features of Parkinson's disease, whereas the development of rhythmic discharge patterns may be linked to parkinsonian tremor.

To further test this model we explored the effects of pharmacological manipulations of the activity of STN neurons in the same MPTP-treated African green monkeys that had already been studied in the first two parts of this series (Bergman et al. 1994; Wichmann et al. 1994). If overactivity of STN was indeed essential for the development of parkinsonian motor signs, we expected that transient or permanent inactivation of the nucleus should lead to normalization of the discharge rate and pattern in GPi, accompanied by the amelioration of parkinsonian signs. By contrast, pharmacological activation of STN should induce a worsening of the parkinsonian motor signs in parkinsonian animals.

We report here on the behavioral effects of localized injections of the γ-aminobutyric acid-A ($GABA_A$) receptor agonist muscimol and the $GABA_A$ receptor antagonist bicuculline in STN, and on the effects of permanent lesions of STN with the neurotoxin ibotenic acid on the discharge rate and pattern in GPi. Some of the striking behavioral effects of the ibotenic acid injections in these animals have already been reported elsewhere (Bergman et al. 1990). A part of this study was also reported in abstract form (Wichmann et al. 1990).

METHODS

Most of the methods used for this study have been described in detail in the companion papers (Bergman et al. 1994; Wichmann et al. 1994) and will be described here only briefly.

Animals and behavioral conditioning

Three juvenile African green monkeys [*monkeys A* and *B*, used in the previous studies (Bergman et al. 1994; Wichmann et al. 1994) and *monkey D*, *Cercopithecus aethiops aethiops*, weight 3–4 kg] were used for the current study. As described in the companion papers, the monkeys were trained in a torque holding task (Wichmann et al. 1994). Metal chambers permitting recording and injection were positioned over the left cerebral hemisphere in *monkey A* and the right hemispheres in *monkeys B* and *D*. After recording in the normal state (Wichmann et al. 1994) the monkeys were treated systemically with MPTP (Bergman et al. 1994). The present experiments were carried out after completion of neuronal recording after the MPTP treatment.

The experiments were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

Pharmacological treatment

Injections into STN were carried out with a combined recording-injection device (Hamada and DeLong 1992a). This device consist of a 30-gauge injection cannula through which a thin coated tungsten wire (50 μm base diam, 75 μm OD) is threaded. The cut end of the wire protrudes 0.3–0.5 mm from the tip of the cannula, permitting recording neuronal multiunit activity close to the injection site. Pressure injections were performed with the help of a Hamilton syringe that was connected to the injection cannula with a Teflon tube. The injection cannula and the recording wire were protected by a guide tube. The whole assembly was lowered into the brain with a microdrive (MO-95, Narishige, Tokyo) coupled to a linear potentiometer for depth measurements. Once inside the brain the injection cannula was lowered through the guide tube and further advanced (18–25 mm from the tip of the guide tube) toward the injection target. All drugs were applied in 0.1- to 0.2-μl boluses with 60- to 180-s intervals between boluses. Drugs were applied until the appearance of neuronal or behavioral changes, up to a maximal amount of 1 μl per injection. After injection the cannula was left at the injection site for an additional period of 5 min to prevent backflow along the injection site. The following drugs were used: ibotenic acid (Regis, Morton Grove, IL; 10 mg/ml in phosphate-buffered saline), muscimol (Sigma, St. Louis, MO; 1 mg/ml in saline), and (−)bicuculline methiodide (Sigma, St. Louis, MO; 0.25–1 mg/ml). Injections of saline were carried out as controls. Only one injection was carried out per day.

Recording and data acquisition

During the injection sessions the neuronal activity recorded using the tungsten wire was bandpass filtered (200–4,000 Hz), halfwave rectified, and passed through a sample and hold device (PSI-1, Bak Electronics, Germantown, MD). It was sampled with the position signal from a manipulandum in which the monkey's contralateral arm was resting (Wichmann et al. 1994) and stored on video tape with a digital data adaptor (VR-10, Instrutech, Mineola, NY).

The parkinsonian signs (voluntary and involuntary movements, rigidity and, tremor) after the injections were assessed by two examiners. The behavior of the monkeys was videotaped in the primate chair or in the cage after the injection of drugs. A computer-assisted method (Bergman et al. 1990) was later used to quantify movements of the limbs: an observer watched the video and pressed specific keys on a keyboard whenever movements of arm or leg occurred. The computer measured the time and duration a given key was pressed. The data were then divided into 1-min segments and the number of movements analyzed as a function of time.

Whenever possible the monkeys were left in the recording chair after the injection. In the primate chair one arm was placed at 75° abduction of the shoulder joint in a low-friction manipulandum. The elbow joint was located over the axis of rotation of the manipulandum, thus allowing the application of flexion and extension torques (0.1 Nm, 60 ms) to the elbow. The displacement of the elbow by torque pulses, reflecting the compliance of the elbow joint, was further studied. For this we applied series of 15–20 flexion and extension torque pulses with a manipulandum at regular intervals (for details see Wichmann et al. 1994). The maximal displacement obtained after the torque pulse was averaged over the series of torques given at a particular time point and taken as a measure for muscular tone (Lang and Fhan 1989; Teravainen et al. 1989). Statistical comparison of the averaged displacement before and after injection was performed with two-tailed *t* tests. If the monkey was too agitated, all restraints except for the neck plate of the primate chair were removed. Behavioral changes were observed for ≤5 h after the injections and were recorded on video tape.

After completion of STN lesion, the activity of GPi neurons was studied with standard platinum-iridium microelectrode recordings. The dyskinesias, and the markedly increased arousal level of the animals after the lesion prevented a meaningful interpretation of firing rates. Only the results of the analysis of firing pattern and response to torque will be further discussed. Full description of the recording techniques is given in the companion papers (Bergman et al. 1994; Wichmann et al. 1994).

Data analysis

Time interval histograms and autocorrelation functions were calculated for all neurons with acceptable recording quality, as

explained in the companion paper (Bergman et al. 1994). A feature-extracting program was used to detect and quantify grouping of discharge that could not be explained as a random process (Karmon and Bergman 1993). The average waveforms of action potentials of neurons in GPi were analyzed as described in the second paper of this series (Bergman et al. 1994).

Computerized statistical methods (Crutcher and Alexander 1990; Wichmann et al. 1994) were used to analyze the neuronal responses to the application of torque pulses. The polarity (increases or decreases), average magnitude, duration, and latency of these responses were computed. Comparisons between the activity of GPi neurons in the normal state, after treatment with MPTP, and after STN lesion were carried out with two-tailed t tests and χ^2 tests.

Histological analysis

The histological procedures are described in the previous papers of this series (Bergman et al. 1994; Wichmann et al. 1994). In addition to cresyl violet stains, tyrosine hydroxylase stains were also evaluated.

Images of the cresyl violet sections of the lesioned STN areas were digitally sampled and analyzed to estimate the extent and localization of the lesions (Nikon, Microphot-FXA, total magnification ×62.5; Image 1.40, NIH software package).

RESULTS

Data base

The data base includes the behavioral results of 10 muscimol, 8 bicuculline, and 2 saline injections into STN in *monkeys B* and *D* as well as behavioral and neuronal recording results before and after ibotenic acid lesions of STN of *monkeys A* and *B*.

As in the companion papers, the data base of neuronal activity contained different sets of neurons for different parts of the study. Data from earlier phases of the experiment from *monkeys A* and *B*, discussed in detail in the companion papers (Bergman et al. 1994; Wichmann et al. 1994), were used as controls.

After lesioning of STN with ibotenic acid, 112 GPi neurons could be recorded with adequate quality to allow the analysis of their discharge pattern. Data from 90 spike trains could be used for the study of neuronal responses to application of torque pulses to the elbow.

Behavioral effects of muscimol injections to STN

Muscimol injections in STN always reduced the neuronal activity close to the injection site. This usually occurred within a few minutes after the injections. In some cases there was a sharp decrease of the multicellular activity recorded with the combined recording-injection system (Fig. 1*A*), but in other cases the decrease followed a more prolonged time course over several minutes.

The decrease in neuronal activity in STN was followed by the emergence of dyskinesias. At the same time parkinsonian motor signs were greatly ameliorated. The animals began again to move their extremities in a purposeful manner. The emergence of increased movements (voluntary and involuntary) is demonstrated in Figs. 1*B* and 2. Although we did not formally measure the amount of tremor after the injections of muscimol, it seemed to be greatly reduced in the contralateral limbs after these injections.

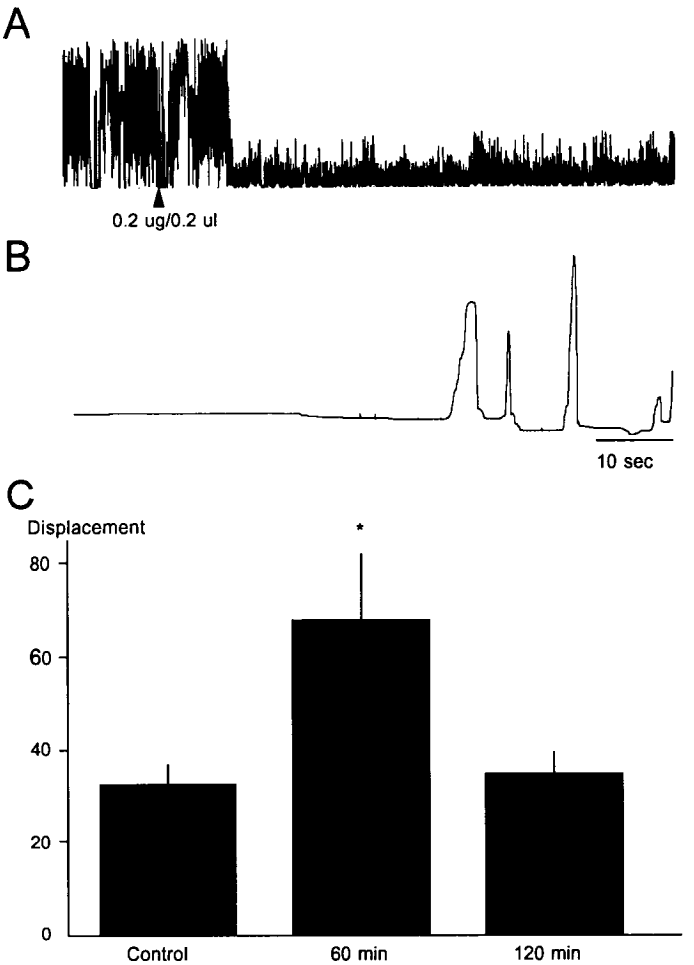

FIG. 1. Effects of transient inactivation of subthalamic nucleus (STN) by injection of muscimol (0.2 μg) on the neuronal activity in STN, on elbow movement, and on parkinsonian rigidity. *A*: neuronal activity. Multiunit activity was half-wave rectified and integrated with a sample and hold integrator. *B*: elbow position. Elbow movements simultaneously recorded with the neuronal activity in *A*. Movement data were recorded from the output of a potentiometer that was coupled to a manipulandum in which the monkey's arm was placed during the injection. *C*: torque displacement data. Torque pulses, here used to obtain a measure of limb compliance, were applied to the elbow by a torque motor that was attached to a manipulandum in which the monkey's arm rested. The elbow displacement is expressed in arbitrary units as average of 15–20 measurements at each time point. Asterisk: significant ($P < 0.05$, 2-tailed t test) deviation from preinjection controls.

Muscular tone (assessed by displacement of the elbow joint induced by a standardized torque pulses) also decreased significantly (Fig. 1*C*). The response to injections occurred usually in <1 min; the longest response latency was 5 min; the response lasted for a variable amount of time (between 10 and 60 min), after which the parkinsonian signs returned.

Injections were carried out only in the dorsal half of STN to selectively affect the sensorimotor territories of STN and to prevent as much as possible the spread of drugs into the substantia nigra. The effects of injections in different areas of STN differed significantly. Injections into the central STN first induced leg movements (Fig. 2*A*), which were followed 2–10 min later by arm movements. Injections in the more lateral and anterior areas (1.9 mm more lateral

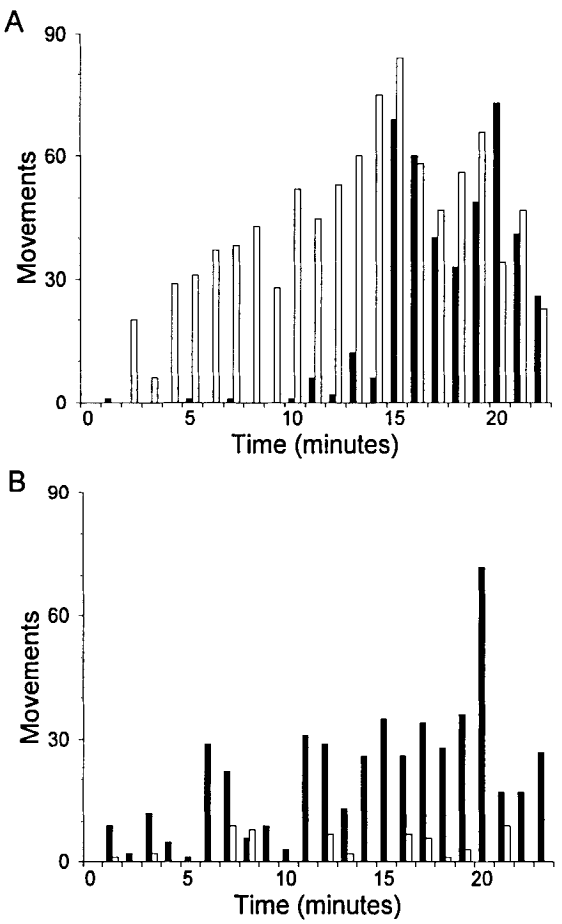

FIG. 2. Time course of the development of voluntary and involuntary movements in the arm (filled bars) and leg (open bars) after muscimol or ibotenic acid injections in STN. *A*: effects of muscimol injection (1 μg/μl) into the medial STN; the time axis starts at the end of injection. *B*: effects of muscimol injection (1 μg/μl) into the lateral STN; the time axis starts at the end of injection. A computer-assisted method was used to measure the occurrence and duration of limb movements (see details in METHODS). The data were divided into 1-min segments and the number of movements displayed as a function of time.

and 1.3 mm more anterior than the previous injection) resulted almost immediately in arm movements (Fig. 2*B*). Injections between the "arm" and "leg" areas resulted in nearly synchronous appearance of leg and arm dyskinesias.

Injections of muscimol sometimes induced nystagmus, gaze deviation to the injection side, and counterclockwise circling 1–2 h after the injection, probably resulting from diffusion to structures adjacent to STN, e.g., the substantia nigra pars reticulata.

Behavioral effects of bicuculline injection to STN

In contrast to the significant reduction of the neuronal activity after injections of muscimol, bicuculline injections marginally increased neuronal activity (Fig. 3*A*). Frequently the neuronal activity assumed a bursting pattern after the injections. Movements of the contralateral limbs were somewhat decreased and muscular rigidity tended to increase (Fig. 3*B*). However, these changes were not statistically significant. The late effects of bicuculline injections were similar to those of muscimol.

Effects of saline injections to STN

Saline (1 μl) was injected into two areas that previously had shown pronounced effects with injection of muscimol. The neuronal activity in STN was slightly reduced (80% of control level) after these injections. No overt behavioral effects were seen after saline and the tone of muscles around the elbow joint, as assessed with the application of torque pulses, was not significantly changed.

Behavioral effects of ibotenic acid injections into STN

Monkey A received four injections of ibotenic acid (total amount of 7 μl) along the anterior-posterior axis of STN. In *monkey B* a single injection of 2 μl ibotenic acid was sufficient to eliminate any detectable electrical activity in STN.

The acute effects of ibotenic acid were similar to those described above for muscimol. After the injection there was a significant reduction of the background neuronal activity,

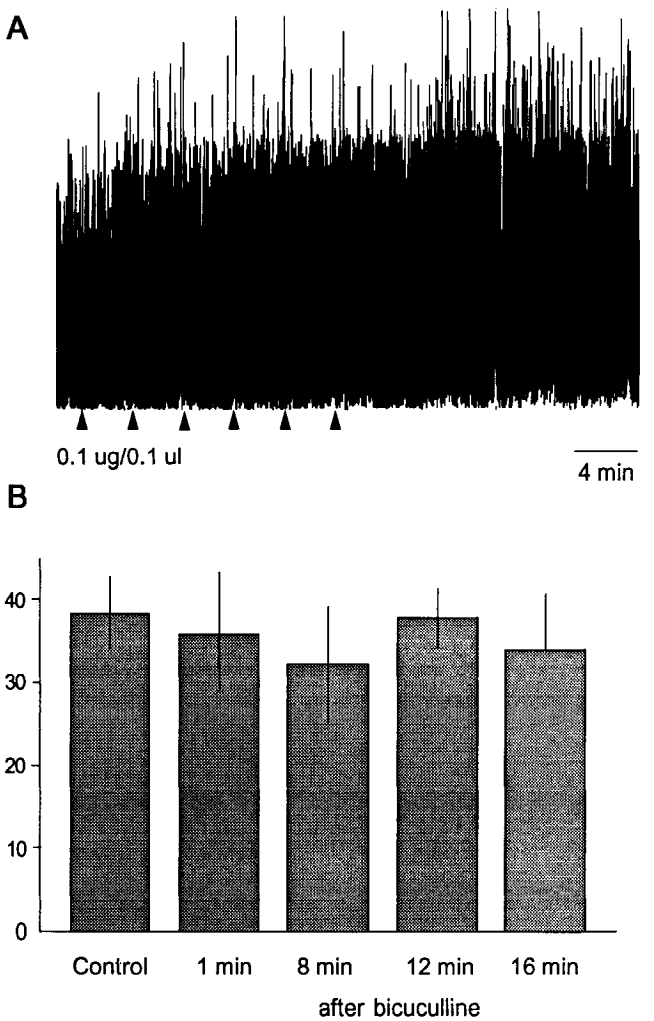

FIG. 3. Effects of activation of STN by injection of bicuculline (1 μg/μl) on the neuronal activity in STN and on rigidity. *A*: neuronal activity. Multiunit activity was half-wave rectified and integrated with a sample and hold integrator. *B*: torque displacement data. Torque pulses, here used to obtain a measure of limb compliance, were applied to the elbow by a torque motor that was attached to a manipulandum in which the monkey's arm rested. Displacement is expressed in arbitrary units and averaged for 15–20 measurements. The bicuculline injection had no significant effect on rigidity (2-tailed *t* test).

FIG. 4. Reconstruction of the extent of STN lesions in *monkeys A* and *B*. The outline of STN is plotted with the damaged areas shown filled. The parasagittal sections are arranged from medial (*top left*) to lateral (*bottom right*). The distance between individual sections is 0.35 mm (*monkey A*) and 0.3 mm (*monkey B*). Numbers above sections: estimated distance from the midline.

followed almost immediately by dyskinetic movements of the contralateral limbs.

The chronic behavioral effects of ibotenic acid injection have already been described in detail (Bergman et al. 1990). Briefly, all three major parkinsonian signs (akinesia, rigidity, and tremor) were reduced on the contralateral side. The previously severely akinetic monkeys were again able to feed and groom themselves with their contralateral limbs and showed increased generalized activity and locomotion. Strikingly, the monkeys appeared generally much more alert, maintained a more upright position, and were more responsive to stimuli in their environments. During the first 1–3 weeks (*monkeys A* and *B*, respectively), dyskinesias of the contralateral limbs gradually disappeared. The motor disability on the ipsilateral side, however, remained constant until the time of killing 64 and 53 days after the MPTP treatment (*monkeys A* and *B*, respectively).

Histological analysis of STN lesion

In both monkeys the lesioned STN showed marked gliosis and loss of neuronal elements in cresyl violet stains. In *monkey A* the lesion was patchy (Fig. 4*A*) and covered 51% of the nucleus, whereas *monkey B* had a more continuous lesion that amounted to 70% of the total volume of the nucleus (Fig. 4*B*).

The lesions were confined to STN, with no evidence of damage to nearby structures such as the substantia nigra, globus pallidus, thalamus, zona incerta, or reticular nucleus of the thalamus.

Spike waveforms and spontaneous activity in GPi

The waveforms of extracellularly recorded action potentials (spikes) in GPi did not change after lesions of STN. The spike duration was 800 ± 120 (SD) μs (n = 75) before and 810 ± 140 μs (n = 93) after lesioning. Other parameters, e.g., the amplitude and duration of positive and negative portions of the waveform, also remained unchanged.

Although tremor was significantly reduced after STN lesions, the periodic oscillatory neuronal activity in GPi persisted (Fig. 5). The percentage of cells oscillating at 8–20 Hz decreased, whereas the population of cells with periodic oscillations at 4–8 Hz was not significantly changed and the proportion of cells with oscillations at >20 Hz increased. The oscillation frequency of most (16 of 17) of the higher-

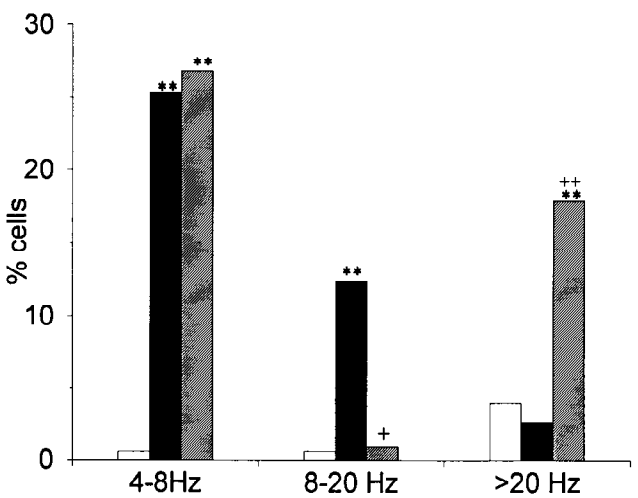

FIG. 5. Frequency of neurons in internal segment of globus pallidus (GPi) that discharged with periodic bursts in untreated monkeys, and in the same animals after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment before and after the lesion of STN. Only neurons with oscillation grades >5 (see METHODS) were included. The oscillation frequency was determined by the feature-extracting algorithm. Open bars: normal state. Filled bars: MPTP-treated state. Striped bars: postlesion state. Double asterisk: significant difference ($P < 0.01$, χ^2 test) from normal state. Plus sign: significant difference ($P < 0.05$, χ^2 test) from MPTP-treated state. Double plus sign: significant difference ($P < 0.01$, χ^2 test) from MPTP-treated state.

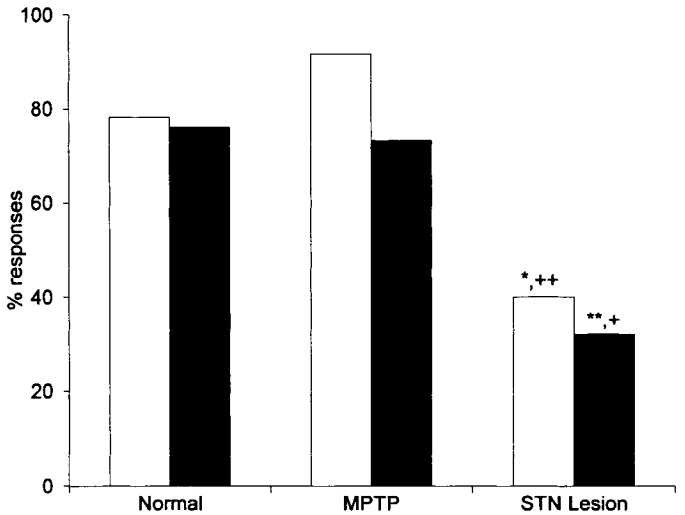

FIG. 6. Percentages of increase in discharge after application of elbow torque pulses in GPi in normal monkeys and in the same animals after MPTP treatment, before and after the lesion of STN. Open bars: responses to extension torque. Filled bars: responses to extension torque. Asterisk: significant difference ($P < 0.05$, 2-tailed t test) from normal values. Double asterisk: significant difference ($P < 0.01$, 2-tailed t test) from normal values. Plus sign: significant difference ($P < 0.05$, 2-tailed t test) from the MPTP-treated state. Double plus sign: significant difference ($P < 0.01$, 2-tailed t test) from the MPTP-treated state.

frequency oscillators was >40 Hz (Average oscillation frequency 63.66 ± 15.74 Hz).

The modulation depth (the ratio of the oscillation amplitude to the average firing frequency) was not significantly changed by STN lesion. The modulation depth of the 4- to 8-Hz oscillators was 0.53 ± 0.28 before and 0.57 ± 0.39 after STN lesion.

Neuronal responses to elbow torque pulses in GPi

The percentage of cells with increases in discharge after application of torque pulses to the elbow was significantly reduced after STN lesion (Fig. 6). Increases accounted for 93% (40 of 43 responses) and 81% (22 of 27 responses) of the total number of torque responses in the normal and the post-MPTP state, respectively (see also Bergman et al. 1994; Wichmann et al. 1994). However, they accounted only for 35% (13 of 37) of the responses after STN lesion. In all behavioral states there were no significant differences between the responses to flexion and extension torques (Fig. 6).

The average duration and magnitude and total number of discharges per response during increases in neuronal discharge after torque increased after MPTP treatment and were reduced to more normal levels after STN lesioning (Fig. 7). The average magnitude of decreases in discharge rate to torque was decreased in the parkinsonian state and again increased after STN lesion, whereas the reverse was true for their duration.

DISCUSSION

The results of this study support the view that abnormal activity in STN is important in the development of the major parkinsonian motor signs, because transient or permanent inactivation of STN in MPTP-treated monkeys led to an amelioration of parkinsonian motor signs paralleled by a decrease of neuronal responses with increases in discharge rate to somatosensory input (torque application) in GPi. The persistence of periodic oscillatory activity in GPi after destruction of STN was an unexpected finding that provided further insights into the pathophysiology of parkinsonian tremor.

Behavioral effects of manipulation of STN activity in parkinsonian primates

The striking reversal of all parkinsonian motor signs after inactivation of STN strongly supports the current model of parkinsonian pathophysiology, in which changes of activity along the indirect pathway assume a major role in the development of all major motor signs of parkinsonism. Changes in the activity STN neurons may be central in this regard, because this nucleus with its projections to both pallidal segments and the substantia nigra pars reticulata is in a unique position to influence the activity of large portions of the basal ganglia output. It is particularly interesting that akinesia and tremor were reduced after STN inactivation. On the basis of experience with earlier neurosurgical lesions in the thalamus (Hassler et al. 1960; Narabayashi 1990), akinesia had been considered to be a "negative" parkinsonian sign, i.e., a sign resulting from loss of function unrelated to activity changes in remaining structures and therefore not reversible by subsequent lesions. The fact that inactivation of the sensorimotor territory of STN reversed akinesia strongly suggests that akinesia results from increased activity in the basal ganglia. Although the motor circuit is most strongly implicated by our experiments with injections of muscimol into discrete areas of the sensorimotor territory of STN, a contributing role of associative or limbic circuits in some aspects of akinesia cannot be ruled out with certainty, because injections may have spread to nearly nonmotor portions of the nucleus that were not explored systematically in our experiments. In previous clinical reports, tremor seemed to respond better to lesions in the thalamus than to lesions in the pallidum (Narabayashi 1954, 1990), and was therefore thought to arise from oscillatory activity in thalamocortical circuits, with little involvement of the basal ganglia. The present study, as well as other recent reports (Aziz et al. 1991; Benazzouz et al. 1993; Guridi et al. 1993; Sellal et al. 1992; Signore et al. 1993; Yamada et al. 1992), by showing that parkinsonian motor signs are reduced after STN inactivation, suggests strongly that tremor also develops as a consequence of abnormal activity in the motor portions of the indirect pathway, likely a combination of excessive and abnormally patterned neuronal discharge. In line with the notion that all parkinsonian motor abnormalities arise from abnormal discharge in the basal ganglia are reports that interruption of parts of the motor circuit in parkinsonian patients by stereotaxic lesions of the ventrolateral posterior GPi has striking effects on akinesia, rigidity, and tremor (Baron et al. 1993; Laitinen et al. 1992; Svennilson et al. 1960).

The fact that transient inactivation of STN by microinjections of muscimol reversed parkinsonian motor signs and produced dyskinesias in a somatotopically organized

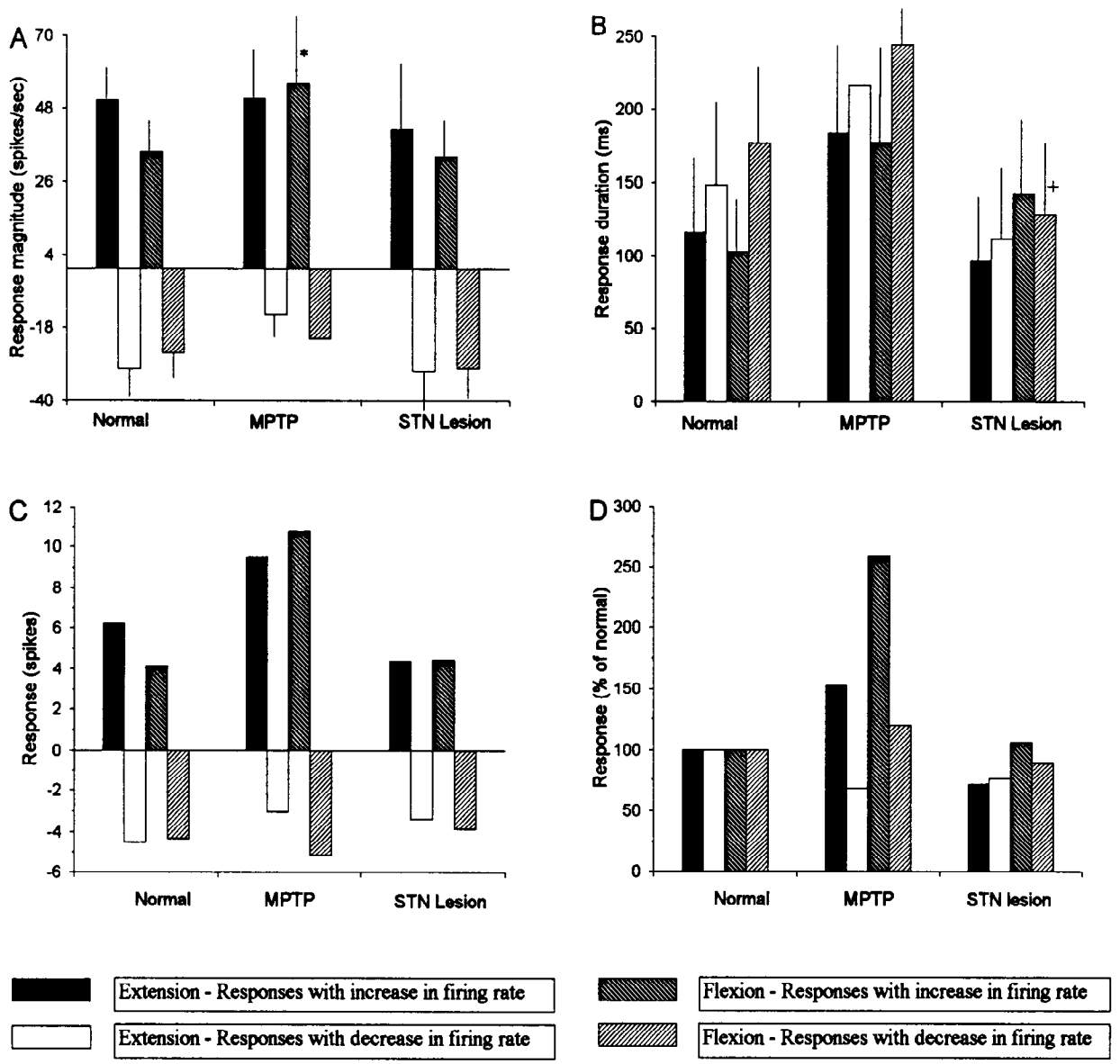

FIG. 7. Neuronal responses of GPi cells to elbow torque pulses in GPi in the normal state and after MPTP treatment before and after lesions of STN. The neuronal responses to randomly applied elbow torque pulses were analyzed with a computerized method (see METHODS). *A*: response magnitude, calculated as the difference between the average firing rate during the response and the mean discharge rate during the pretrial control period. *B*: duration of neuronal responses. *C*: total number of discharges (magnitude × duration). *D*: relative neuronal response. To generate *D*, data in *C* were normalized with the respective response in the normal state representing 100%. Bars: means ± SD. Asterisk: significant difference ($P < 0.05$, 2-tailed t test) from normal values. Plus sign: significant difference ($P < 0.05$, 2-tailed t test) from the MPTP-treated state. Filled bars: increases in discharge in response to extension torque pulses ($n = 18$ before and $n = 11$ after MPTP treatment, $n = 6$ after STN lesion). Open bars: decreases in discharge in response to extension torque pulses ($n = 5$ before and $n = 1$ after MPTP treatment, $n = 9$ after STN lesion). Bars with leftward striping: increases in discharge in response to flexion torque pulses ($n = 16$ before and $n = 11$ after MPTP treatment, $n = 7$ after STN lesion). Bars with rightward striping: decreases in discharge in response to flexion torque pulses ($n = 5$ before and $n = 4$ after MPTP treatment, $n = 5$ after STN lesion).

fashion is consistent with evidence that neurons in STN are somatotopically arranged (DeLong et al. 1985; Wichmann et al. 1994) and that the circuits through the basal ganglia and thalamus are functionally highly segregated and specific. Parkinsonian motor signs may develop in specific parts of the body as result of activity changes in selected portions of the basal ganglia–thalamocortical motor circuit. Previous studies on the behavioral effects induced by radio frequency or excitotoxic lesions of STN (Carpenter et al. 1950; Hamada and DeLong 1992a; Hammond et al. 1979) failed to show any site specificity of such lesions, likely because of the fact that in these studies large portions of the nucleus were inactivated when movement effects developed (Carpenter et al. 1950), similar to our experience with ibotenic acid injections into STN in parkinsonian animals (Bergman et al. 1990; present study).

In a previous study on the effects of excitotoxin lesions of STN in normal monkeys (Hamada and DeLong 1992a), dyskinesias appeared with a significant delay after the ibotenic acid lesions and lasted for only 5 h. In contrast, the

involuntary movements in our animals appeared almost immediately after the ibotenic acid lesion and were evident for weeks. Several methodological differences between the two studies may account for these discrepancies. The previous study used normal macaque monkeys, whereas our study was carried out in parkinsonian African green monkeys. Furthermore, as also shown by the results presented in Fig. 4 of the present study, the neurotoxic effects of ibotenic acid injections vary widely between individual experiments, possibly because of differences between batches of ibotenic acid or different susceptibilities of individual monkeys to the toxin. Even after multiple injections of ibotenic acid, the final (histological) size of the lesions in the study by Hamada and DeLong (1992a) was rather small, possibly preventing the development of long-term dyskinesias. Some of the dyskinesias seen acutely in that study may have been caused by partial conversion of ibotenic acid to muscimol (Nielsen et al. 1985), which may have affected a larger portion of the nucleus than that ultimately lesioned.

It is of interest that the amelioration of parkinsonian motor signs and the expression of dyskinesias can be distinguished. In our experiments, as well as in some studies in MPTP-treated primates, using radio frequency lesions (Aziz et al. 1991, 1992; Guridi et al. 1993) or high-frequency inactivation (Benabid et al. 1993; Benazzouz et al. 1993) of STN, and in the report of a patient whose parkinsonian motor signs were ameliorated after a hemorrhage involving STN (Sellal et al. 1992; Yamada et al. 1992), dyskinesias tended to subside, whereas the beneficial effect of STN destruction on parkinsonian motor signs remained. Both the mechanism resulting in dyskinesias after STN lesions as well as the compensatory process that subsequently reduces these dyskinesias are still functional in the parkinsonian state. The exact localization of these mechanisms remains unclear.

The generalized behavioral activation observed after STN lesioning in our study was also reported by Aziz et al. (1991) after unilateral radio frequency lesions of STN. Although this group reported a striking bilateral reversal of akinesia, this was less apparent in our animals. In subsequent studies we have also observed bilateral motor activation after unilateral GPi inactivation (Baron et al. 1992). In recent studies in parkinsonian patients undergoing pallidotomy, bilateral effects have also been observed (Baron et al. 1993). Theses studies suggest that the output from the basal ganglia in one hemisphere can strongly influence motor function bilaterally. Bilateral influences may result from pathways through the brain stem or through precentral motor fields including the supplementary motor area (SMA) (Brinkman 1984).

The lack of a significant behavioral effect of microinjections of bicuculline into STN of our severely parkinsonian animals was not surprising. Bicuculline presumably blocked transmission via the GPe-STN pathway, which is the primary GABAergic input to STN. Because this input is underactive under parkinsonian conditions (Filion and Tremblay 1991; Miller and DeLong 1987), further blockade of this input may have had little additional impact on the neuronal activity in STN. In previous reports, injections of bicuculline into STN of normal monkeys (Crossman et al. 1980, 1984) or rodents (Feger et al. 1991) often paradoxically led to dyskinesias or continuous abnormal movements, probably by inducing a dose-dependent depolarization block (Crossman 1987; Feger et al. 1991). We have not observed signs suggesting depolarization block in our animals. Methodological differences, e.g., between species or between normal and MPTP treated monkeys, as well as the effects of anesthesia used in the earlier studies may all have contributed to these discrepancies.

It is of interest that local bicuculline injections into STN in the present study as well as in that of Féger et al. (1991) resulted in the emergence of burst discharges. This suggests the possibility that STN might also play a role in the pathogenesis of parkinsonian tremor. The blockade of GABAergic input induced by bicuculline might lead to bursting secondary to the resulting depolarization of STN neurons.

Effects of permanent inactivation of STN in MPTP-treated primates on neuronal activity in GPi

The reduction of both the frequency and the magnitude of the early excitatory responses of pallidal cells to torque application after STN lesion was predicted because lesions of STN deprive GPi of its major excitatory afferents. The majority of increases in discharge of GPi cells after torque application probably arise from this excitatory STN input. A similar finding has previously been documented in normal macaques after ibotenic acid lesioning of STN (Hamada and DeLong 1992b).

Although the reversal of tremor by STN lesions in our monkeys makes it likely that some aspect of the activity in STN-GPi pathway is responsible for the development of tremor, it cannot be said with certainty whether tonic or phasic changes are more important. From previous experiments in nontremulous macaques (Filion and Tremblay 1991; Miller and DeLong 1987), with very prominent alterations of tonic firing rates after treatment with MPTP, it is unlikely that tremor is entirely due to changes in tonic firing in GPi. On the other hand, the persistence of MPTP-induced oscillatory activity in GPi after STN lesions in our monkeys, even after tremor had been greatly reduced by STN lesion, argues against the sole responsibility of changes of the phasic discharge of GPi neurons for the development of tremor. Similarly, burst activity persisted also in substantia nigra pars reticulata (SNr) neurons in 6-hydroxy-dopamine-treated rats after STN lesions (Burbaud et al. 1992). From these considerations it appears that membrane properties of neurons in the basal ganglia output nuclei, GPi and SNr, favor the development of rhythmic burst discharges at the parkinsonian tremor frequency independent of proprioceptive input reaching GPi via the corticosubthalamic route. As pointed out in the previous paper in this series (Bergman et al. 1994), the development of rhythmic discharges in GPi may be the result of loss of dopamine in that structure. However, rhythmic discharges in GPi are apparently not sufficient to induce overt tremor in parkinsonism. Rather, it appears that a particular constellation of increased tonic firing and oscillatory bursts in GPi is needed to induce tremor. The former may lead to hyperpolarization of thalamocortical cells, a condition that has been shown to be burst-promoting (Buzsaki et al. 1990; Jahnsen

and Llinas 1984a,b), and may allow oscillatory discharges in pallidal cells to manifest themselves in tremor. Alternatively, the corticosubthalamopallidal pathway may synchronize the oscillatory activity in GPi in parkinsonism; subsequent destruction of STN may prevent tremor by desynchronizing periodic oscillatory activity in GPi. A distinction between these possibilities could not be adequately made in our experiments because the sample of simultaneously recorded GPi cells was not sufficient to assess the degree of synchrony between neighboring GPi cells after STN lesions.

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