Lidocaine and muscimol microinjections in subthalamic nucleus reverse parkinsonian symptoms

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Summary

Inactivation of neurones in the subthalamic nucleus (STN) of the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey model of Parkinson’s disease has been shown to relieve parkinsonian motor symptoms. In patients with Parkinson’s disease, neurones in the STN display hyperactive firing rates and rhythmic discharge activity such as tremor-related oscillations (3–8 Hz) and synchronous high-frequency oscillations (15–30 Hz). In this study, microinjections of lidocaine and muscimol, a GABA_A receptor agonist, were performed in the STN of six patients with Parkinson’s disease to determine whether the focal suppression of STN neuronal activity can lead to an improvement in tremor, bradykinesia and rigidity. Microinjections of 10–23 µl of lidocaine produced striking improvements in bradykinesia, limb tremor and rigidity in three out of three patients. These improvements were correlated with good therapeutic effects of subsequent STN deep brain stimulation performed in the same microelectrode trajectories as these injections. The most dramatic observation following lidocaine injections was the appearance of dyskinetic limb movements. In one patient, simultaneous microelectrode recording during an injection of 3.5 µl of lidocaine demonstrated a suppression of neuronal activity at distances of <0.9 mm from the injection site, but no suppression was observed at ≥1.2 mm from the injection site. Microinjections of 5–10 µl of muscimol in a region with tremor-related activity resulted in suppression of limb tremor in two out of two patients. Interestingly, in one of these patients, 4 Hz oscillatory activity was diminished in a neurone recorded 1.3 mm from the injection site, but there was no reduction in the mean firing rate or 20 Hz oscillatory activity. These results demonstrate that inactivation of neuronal activity in the STN of patients with Parkinson’s disease improves motor symptoms. These findings also suggest that a focal block of the STN might alter the oscillatory activity of neurones located beyond the inhibited region.

Keywords: subthalamic nucleus; Parkinson’s disease; microinjections; muscimol; lidocaine

Abbreviations: DBS = deep brain stimulation; GPe = external segment of the globus pallidus; GPi = internal segment of the globus pallidus; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; STN = subthalamic nucleus; UPDRS = Unified Parkinson’s Disease Rating Score

Introduction

Local inactivation of neuronal activity in the subthalamic nucleus (STN) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated non-human primate model of Parkinson’s disease has been shown to ameliorate parkinsonian symptoms (Bergman et al., 1990; Wichmann et al., 1994; Guridi et al., 1996). This therapeutic effect is consistent with the notion of STN hyperactivity or dysfunction in parkinsonism (Miller and DeLong, 1987; Bergman et al., 1994). Intentional lesioning of the STN in patients with Parkinson’s disease has usually been avoided because of the
Acid-A receptor agonist (GABA A). Lidocaine has the effect of injection of substances in the human brain. Deep brain stimulation (DBS) in the STN has also been shown to be highly effective in treating parkinsonian symptoms (Limousin et al., 1995; Kumar et al., 1998; Krack et al., 1998a). To determine the therapeutic and neuronal effects of blocking STN activity in patients with Parkinson's disease, we performed microinjections of the local anaesthetic lidocaine and muscimol, a γ-aminobutyric acid-A receptor agonist (GABA A). Lidocaine has the effect of non-selectively blocking axonal fibres of passage as well as neurones, while muscimol selectively inhibits the cell bodies of neurones.

Elucidating the population behaviour of STN neurones and the role of the STN in the dynamics of the basal ganglia is essential to extending our understanding of the pathophysiology of Parkinson's disease (Ryan et al., 1992; Plenz and Kital, 1999; Magill et al., 2000; Levy et al., 2000). The neuronal population in the STN has been shown to exhibit synchronous oscillatory activity (Plenz and Kital, 1999; Magill et al., 2000; Brown et al., 2001; Marsden et al., 2001), and it has been suggested that increased oscillatory synchronization in the basal ganglia underlies the development of parkinsonian limb tremor (Bergman et al., 1998a; Raz et al., 2000). We recently demonstrated that STN neurones display strongly synchronous 15–30 Hz oscillations ('high-frequency' oscillations), in addition to 3–8 Hz tremor-related oscillations, in tremulous patients with Parkinson's disease (Levy et al., 2000). Synchronized 3–8 Hz tremor-related activity has also been shown in the internal segment of the globus pallidus (GPI) (Hurtado et al., 1999) and in the motor thalamus of parkinsonian patients with Parkinson's disease (Levy et al., 1999). Therefore, if parkinsonian limb tremor is due in part to an elevated level of oscillatory synchronization between basal ganglia subcircuits (Bergman et al., 1998a), it is conceivable that pharmacological blocks, in addition to inactivating local neuronal activity, might influence the oscillatory behaviour of neurones beyond the region directly affected by the block. In theory, invasive surgical therapies could act to reduce limb tremor by desynchronizing pathological oscillations in the basal gangliathalamocortical network (Bergman et al., 1998a; Deuschl et al., 2000). In the present study, we addressed this issue by recording the activity of an STN neurone that displayed both tremor-related and high-frequency oscillations and was located outside a region of pharmacologically blocked tremor cells. This is the first demonstration of the use of simultaneous microelectrode recording to assess the effects of microinjection of substances in the human brain.

**Methods**

**Patient group**

Studies of muscimol and lidocaine microinjections into the STN were performed in seven Parkinson's disease patients undergoing microelectrode-guided placement of DBS electrodes ($n = 5$) or an STN lesion ($n = 2$) for the treatment of the symptoms of Parkinson's disease. The results for one of these patients are not presented because postoperative MRI revealed that microinjections were performed at the ventral border of the STN. Results for the rest of the patients are presented here. This group consisted of two females and four males who at the time of operation had a mean age of 55 years (range 48–67 years). The average duration of the disease was 12.2 years (±1.8 SE) and all had Parkinson's disease for at least 7 years. All patients gave informed consent and the studies were approved by the Human Experimentation Committee of the Toronto Hospital.

**Injections**

Lidocaine (2% Xylocaine, 20 mg/ml without preservative; Astra Pharma Inc., Mississauga, Canada) and muscimol [1 mg/ml (8.8 mM) in sterile saline, 0.2 µm filter sterilized; Sigma, St Louis, Mo., USA] were injected into the STN through a stereotactically placed 30 gauge stainless steel tube (Small Parts Inc., Miami Lakes, Fla., USA). The injection cannula was connected to a 10–15 cm piece of polyethylene tubing (PE 50, inside diameter 0.58 mm) and sealed with epoxy glue. Substances were preloaded in the cannula and polyethylene tubing and in a 25 µl Hamilton syringe. An air bubble (~1 cm in length) was introduced in the polyethylene tubing to allow the visual determination of the movement of the solutions into the STN. The polyethylene tubing was then friction fitted over the tip of the Hamilton syringe. Substances were injected at a rate $\approx 1.25$ µl/min except in one patient in whom lidocaine was injected at 2.5 µl/min (Patient C). The injection cannula was left in the brain for 20–25 min after microinjection to reduce the likelihood of an injected solution diffusing back up the cannula track. In one case, two injections (saline followed by muscimol) were carried out by employing two fixed parallel injection cannulae (~350 µm apart) with two separate Hamilton syringes. This technique avoided the need to remove a single injection cannula in order to insert a second cannula filled with a different solution, and thereby reduced the likelihood of backflow along the cannula track.

**Determination of the target area for microinjection in STN**

Localization of the STN using microelectrode recording is described in detail elsewhere (Hutchison et al., 1998). Briefly, parasagittal trajectories at either 10.5 or 12 mm from the midline passed through the thalamic reticular nucleus and/or anterior thalamus, zona incerta, STN and the substantia nigra pars reticulata (Schaltenbrand and Wahren, 1977). Single unit microelectrode recording and stimulation mapping allowed the identification of physiological landmarks and cell localization. Exploration of the neuronal activity was carried...
out along the entire dorsal/ventral extent of STN. The main characteristics that were identified in order to localize the motor portion of the STN were: neurones with tremor-related activity, neurones that responded to passive or active movements and microstimulation effects such as tremor reduction or arrest. The anterior–posterior limits of the STN were delimited by regions with sparse neuronal activity and a reduced background noise compared with that observed within the STN. Microinjections were performed in the same microelectrode track that would later be used to insert the DBS electrode (five patients). Microinjection in the one patient undergoing a subthalamotomy was performed at a location that was 3 mm anterior to the centre of a subsequent lesion (see Fig. 1A). Postoperative MRI was carried out in four patients to identify the placement of DBS electrodes or lesion in the STN and to confirm that microinjections were performed in the desired location in the STN (see Table 1). All patients underwent postoperative Unified Parkinson’s Disease Rating Score (UPDRS) assessment of the clinical improvement due to lesion or DBS (in the OFF drug state).

**Simultaneous microinjection and microelectrode recording of neuronal activity**

In two patients (Patients A and F), simultaneous microelectrode recording of neuronal activity during microinjection was performed using a second independently driven microelectrode that was inserted in parallel with the injection cannula at a centre to centre distance of ~600 μm. Both the microelectrode and the cannula occupied separate guide tubes (23 gauge stainless steel tubes) and the guide tubes were positioned medial–lateral to one another. Microelectrode recordings were performed posterior–ventral to the injection site (i.e. microelectrode was advanced ahead of the injection site). Spectral analysis of single-neurone discharge activity has previously been described in detail (Levy et al., 2000). Briefly, the event times of neuronal discharges were converted to waveforms (with a final sampling rate of 1000 Hz) representing the discharge density over time (10 ms bins) using standard software (Spike2; Cambridge Electronic Design, Cambridge, UK). Subsequent Fourier analysis was used to determine the oscillatory modulation of this discharge. Five hundred and twelve spectral estimates were made between 0 and 500 Hz thereby yielding a frequency resolution of 0.98 Hz. Statistical significance of spectral peaks was assessed by comparing spectra with a white noise signal with the same mean power in the range 0–40 Hz (i.e. mean spectral ‘noise’). Spectral estimates were deemed significant if they were equal to or greater than the upper bound of a \( 100(1 - \alpha)\% \) confidence interval about this white noise signal. The upper bound was given by \( 2Nf(\omega)/\chi_{2N,1-\alpha/2}^2 \) where \( f \) is the power, \( \omega \) is the frequency, \( 2N \) is the effective degrees of freedom and \( N \) is the number of sampling windows (Chatfield, 1996). A plot of frequency versus time was constructed by analysing data in consecutive non-overlapping 20-s windows. This plot displayed normalized spectra where each spectral estimate was divided by the spectral noise in the respective window.

**Assessment of clinical changes due to microinjections**

All injections were performed with the patients in the OFF state (12–14 h after last anti-parkinsonian medications). In order to rate the clinical effect of microinjections, patients underwent a partial UPDRS assessment of bradykinesia (item 24), rigidity (item 22, arm/wrist) and tremor (items 20 and 21, arm/wrist). Tremor, bradykinesia and dyskinesias were assessed from camera tapes by one of the investigators (A.E.L.). Changes in rigidity were assessed by either of two investigators (A.E.L. or A.M.L.). When possible, bradykinesia was also assessed using quantitative tasks measuring movement time and amplitude such as wrist pronation/supination or repetitive pointing with the index finger from the patient’s chest to a target placed ~50 cm in front of the patient (total trial lengths ~10 s). In one patient, rigidity was quantified using a commercially available device (Prochazka et al., 1997). This device allows the accurate determination of rigidity by quantifying the impedance provided by a limb to an applied force imposed by the examiner.

**Results**

**Localization of injections**

Figure 1 displays digitized parasagittal plates (Schaltenbrand and Wahren, 1977) of the STN (standardized to the patient’s anterior–posterior commissural distance) to show the location of the microelectrode trajectories and to provide information about the neuronal activity and the location of the individual microinjections. In all patients, microinjections were performed in regions of the STN populated by neurones with movement-evoked activity, tremor-related activity or neurones characteristic of those found in the motor portion of the STN (Hutchison et al., 1998). As shown in Table 1, STN DBS in five patients and the subthalamotomy in the remaining patient (Patient A) resulted in a dramatic improvement in contralateral bradykinesia, tremor and rigidity. Postoperative MRI was available in four patients and confirmed that the interventions were performed in the STN.

**Lidocaine injections**

The diffusion of lidocaine was examined in Patient A by simultaneously recording neural activity close to the injection cannula. These data are shown in Fig. 2. After 3.5 μl of lidocaine was injected over a 5 min period, there was a dramatic decrease in neural activity recorded 0.6 mm from the injection site. When the recording electrode was moved
Simultaneous recording of neuronal activity during microinjection was performed in Patients A and F (see Methods). STN = subthalamic nucleus; Thal = thalamus; SNr = substantia nigra pars reticulata; H2 = fields of Forel; rTh = reticular nucleus of the thalamus; Voa = nucleus ventralis oralis anterior; Vop = nucleus ventralis oralis posterior; Vim = ventrointermedius. The dashed line represents the anterior–posterior commissural line; RF = receptive field (a neurone that responded to passive or active limb movement).

Fig. 1  Sagittal sections from the Schaltenbrand and Wahren stereotactic atlas at 12 mm from the midline displaying the microelectrode trajectories, neuronal activity and location of microinjection in the six patients (definitions of the symbols used are at the bottom of the figure). DBS electrodes were placed in the same microelectrode trajectory as the microinjection in Patients B–F. The lesion in Patient A was placed 3 mm posterior to the location of the lidocaine microinjection. The diameter of the injection cannula was 0.3 mm, but is drawn as 0.2 mm so as not to obscure data in the figure. Simultaneous recording of neuronal activity during microinjection was performed in Patients A and F (see Methods). STN = subthalamic nucleus; Thal = thalamus; SNr = substantia nigra pars reticulata; H2 = fields of Forel; rTh = reticular nucleus of the thalamus; Voa = nucleus ventralis oralis anterior; Vop = nucleus ventralis oralis posterior; Vim = ventrointermedius. The dashed line represents the anterior–posterior commissural line; RF = receptive field (a neurone that responded to passive or active limb movement).

to another cell 0.78 mm away (relative to the tip of the injection cannula), neural discharge at this site decreased ~30 s later. However, neural activity 1.2 mm away was not blocked at 5 min after the end of the injection period. No change in contralateral arm rigidity, tremor or bradykinesia was observed at 13 min after the injection (data not shown).
There was a significant decrease in baseline resting tremor of Patient D at all times. Accelerometer traces at the bottom of Fig. 3 demonstrate that muscimol but not saline (both injected at a rate of 1 µl/min) into a region with tremor-related activity in Patient E caused a dramatic reduction in contralateral resting tremor within 5 min after the start of injection. This was also observed using an accelerometer placed on the index finger of the contralateral hand (bottom traces in Fig. 4). The patient was asked to perform mental arithmetic throughout the sampling period to enhance spontaneous resting tremor. Muscimol also improved the performance of wrist pronation/supination movements during this period (11 min after initial injection).

**Muscimol injections**

A marked anti-parkinsonian effect following muscimol injections was observed in Patients E and F. Figure 4 demonstrates that muscimol but not saline (both injected at a rate of 1 µl/min) injected into an area of the STN with tremor-related activity in Patient E caused a dramatic reduction in contralateral resting tremor within 5 min after the start of injection. At 9 min following injection, myoclonic jerking movements were also observed. Dyskinetic movements in the foot were also observed when the patient performed wrist pronation/supination movements during this period (11 min after initial injection).

Following lidocaine injections, dyskinesias were observed in all three patients. Patient B developed low amplitude choreoathetotic movements of the ipsilateral foot (indicated by dashed arrow in Fig. 3). Patient C developed dystonic contralateral wrist movements at 15 min after injection. These movements interfered with the patient’s ability to perform hand-gripping movements (filled squares in Fig. 1, middle panel, UPDRS item 24). Patient D displayed dystonic dyskinesias (i.e. a sustained posturing) of the contralateral foot at 6 min following injection. At 9 min following injection, myoclonic jerking movements were also observed. Dyskinetic movements in the foot were also observed when the patient performed wrist pronation/supination movements during this period (11 min after initial injection).

### Table 1: The clinical effects of microinjections and STN, DBS or subthalamotomy

<table>
<thead>
<tr>
<th>Patient</th>
<th>STN side</th>
<th>Clear therapeutic benefit of injection?</th>
<th>Procedure*</th>
<th>Contralateral UPDRS tremor†</th>
<th>Contralateral UPDRS rigidity‡</th>
<th>Contralateral UPDRS bradykinesia§</th>
<th>Postoperative MRI targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Left, 3.5 µl lidocaine</td>
<td>No</td>
<td>Left STN lesion (subthalamotomy) (1 week)</td>
<td>6/1</td>
<td>5/1</td>
<td>13/5</td>
<td>Centred in left STN</td>
</tr>
<tr>
<td>B</td>
<td>Right, 10 µl lidocaine</td>
<td>Yes</td>
<td>Bilateral DBS (1 month)</td>
<td>2/0</td>
<td>5/3</td>
<td>14/4</td>
<td>Not available</td>
</tr>
<tr>
<td>C</td>
<td>Right, 23 µl lidocaine</td>
<td>Yes</td>
<td>Bilateral DBS (3 months)</td>
<td>3/0</td>
<td>6/3</td>
<td>11/7</td>
<td>Effective contacts in STN</td>
</tr>
<tr>
<td>D</td>
<td>Left, 10 µl lidocaine</td>
<td>Yes</td>
<td>Unilateral DBS (3 weeks)</td>
<td>7/0</td>
<td>Not available</td>
<td>14/10</td>
<td>Effective contacts in STN</td>
</tr>
<tr>
<td>E</td>
<td>Left, 10 µl muscimol</td>
<td>Yes</td>
<td>Bilateral DBS (1 month)</td>
<td>4/2</td>
<td>6/5</td>
<td>8/5</td>
<td>Effective contacts in STN</td>
</tr>
<tr>
<td>F</td>
<td>Left, 5 µl muscimol</td>
<td>Yes</td>
<td>Bilateral DBS (3 weeks)</td>
<td>4/1</td>
<td>4/0</td>
<td>5/1</td>
<td>Not available</td>
</tr>
</tbody>
</table>

All patients were assessed in the OFF drug condition (following an overnight drug holiday). For Patients B–F, data are reported as the UPDRS score for DBS OFF/DBS ON (DBS at optimal parameters and contacts). For Patient A, data are reported as the UPDRS for preoperative score/postoperative score. Scores are reported for the body side contralateral to the side of injection in the STN. *Stimulation used and postoperative time of assessment is indicated in parenthesis. †Tremor is calculated as the sum of UPDRS items 20 and 21 (unilateral scores; maximum possible score of 12). ‡Rigidity is calculated from UPDRS item 22 (unilateral scores; maximum possible score of 8). §Bradykinesia is calculated as the sum of UPDRS items 23–26 (unilateral scores; maximum possible score of 16).
Fig. 2 Microelectrode recording of neuronal activity during the microinjection of lidocaine in Patient A. Lidocaine was injected at the volumes (µl, bold numbers in grey boxes) and times indicated by the grey boxes to the left side of vertical timeline. The relative distance from the tip of the injection cannula to the tip of microelectrode is indicated by the numbers on the left hand side of the figure. Neuronal recordings were sampled at the time indicated by position of traces with respect to timeline.

Discussion

**Inactivation of the STN improves parkinsonism**

This is the first report of microinjections of muscimol and lidocaine in the STN of humans. This study demonstrates that local inactivation of the STN using pharmacological blocking agents results in a transient improvement in akinesia, rigidity and limb tremor in patients with Parkinson’s disease. These results support the current model of basal ganglia pathophysiology, which predicts that a reduction of excessive activity in the STN of patients with Parkinson’s disease should produce a therapeutic benefit (DeLong, 1990). These results in humans are consistent with previous observations made in the non-human primate MPTP model of parkinsonism (Bergman et al., 1990; Aziz et al., 1991, 1992; Guridi et al., 1994; Wichmann et al., 1994).

**Suppression of cell bodies and axons versus suppression of only cell bodies**

Although subthalamotomy and STN DBS in patients with Parkinson’s disease improves all parkinsonian cardinal motor signs (Limousin et al., 1995; Gill and Heywood, 1997; Obeso et al., 1997; Alvarez et al., 2001), it is unknown if these effects are due solely to inactivation of STN neurones rather than an action on fibres passing through or near the affected region. The anti-parkinsonian effect of muscimol in this study confirms that suppression of STN sensorimotor-related neuronal activity, as opposed to possible alterations in nearby pallidofugal fibres, results in a therapeutic benefit.

Since lidocaine acts on fibres passing through the blocked volume of cell bodies, it can indirectly affect other STN regions by preventing transmission through both inhibitory and excitatory afferent inputs and excitatory efferent projections. Although inhibition of GABAergic afferent fibres...
The effects of lidocaine injections in the STN of three patients. The three plots show the effects of lidocaine injection on the UPDRS measures of tremor, rigidity and hand grips and the rigidity meter readings (see box at bottom right for distinction of graph symbols, values for the body side contralateral to the injected STN). The stippled bars show time period over which lidocaine was injected, the volume of lidocaine in µl is given by the numbers in the bars. Lidocaine resulted in a decrease in rigidity in all three patients and the rigidity in two of these patients (Patients B and C) was observed to recover to baseline values. Lidocaine reduced limb tremor in Patients B and D (Patient C did not have limb tremor during the procedure). Traces at the bottom of the figure are accelerometer traces of 90 s of resting tremor of Patient D (traces were sampled starting at the times indicated to the left). The bar graph at the top right shows that lidocaine injections in Patient B resulted in improved performance on a movement time task (see Methods, *P < 0.05, ANOVA on ranks). The traces at the bottom right show the improvement in repetitive wrist pronation/supination movements (WPS) in Patient D following lidocaine injections. The time of appearance of dyskinetic movements is indicated by downward dashed arrows. Following lidocaine injections, all three patients developed dystonic or dyskinetic movements (see Results).
The involvement of the STN in the pathogenesis of dyskinesias is supported by many studies (Crossman, 1990). Dyskinesias and dystonic postures are observed in normal monkeys following excitotoxic cell-specific lesions (Hamada and DeLong, 1992) or electrical stimulation of the STN (Beurrier et al., 1997). Dyskinesias can also be induced by injection of the GABA antagonist bicuculline in the GPe (Matsumura et al., 1995) and disinhibition of the GPe should lead to a decrease in STN activity. In MPTP-treated monkeys, inactivation of the STN results in transient dyskinesias in addition to improving parkinsonism (Bergman et al., 1990; Aziz et al., 1991, 1992; Guridi et al., 1994; Wichmann et al., 1994). In patients with Parkinson’s disease, choreic or dystonic dyskinesias are sometimes observed during penetration of the STN target by microelectrodes or DBS electrodes or during lesion making (Benabid et al., 2000; Alvarez et al., 2001).

Limousin and colleagues demonstrated that STN DBS at higher voltages than those required to control parkinsonism could also induce dyskinesias in patients with Parkinson’s disease (Limousin et al., 1996). It was suggested that because the anti-parkinsonian effect and dyskinetic effect occurred with different stimulation parameters, the mechanisms responsible for these two effects might be distinct. In the present study, $\geq 10 \mu l$ of lidocaine reduced parkinsonian effects.
Fig. 5 Effects of muscimol injection in the STN of Patient F. (A) The top trace shows the accelerometer (Acc) recording of the hand/wrist tremor (accelerometer was placed on index finger). The histogram shows the firing rate (20 s bins) of an STN neurone recorded during the injection and located 1.3 mm from the cannula tip. The grey bar represents the time period over which the 5 µl of muscimol was injected. (B) Power spectra of oscillatory neuronal discharge recorded before (left panel, 0–3 min) and after (right panel, 9–12 min) muscimol injection (0.24 Hz resolution). The inset in each plot shows the frequency spectra of the accelerometer over each time period (0.39 Hz resolution). (C) Time-frequency analysis of oscillatory neuronal discharge (0.98 Hz resolution × 20 s bins). The colour legend indicates those spectral estimates with signal-to-noise ratios (green to light green) that were greater than a 99.8% confidence interval about the mean spectral noise. These plots demonstrate that the lower frequency oscillatory activity of the recorded neurone, but not firing rate or 20 Hz oscillatory activity, was reduced concurrent with a reduction in hand tremor.
symptoms and produced dyskinesias in three patients, while in the remaining patient (Patient A), no motor benefit was obtained and no dyskinesias were observed following an injection of 3.5 µl of lidocaine. These results suggest that, in parkinsonian patients, the dyskinetic effect requires a greater degree of STN inactivation than does the anti-parkinsonian effect. This would explain why injections of muscimol did not produce dyskinesias in this study but did produce an anti-parkinsonian effect if the volume of the STN inactivated by muscimol produced only a threshold anti-parkinsonian effect, but was not large enough to produce dyskinesias. It is also of interest that both choreic and dystonic dyskinesias were observed in the present study. These types of dyskinesias were typical of those seen during levodopa-induced dyskinesias (Fahn, 2000) and support the finding that the neuronal activity of the STN in patients with Parkinson’s disease is reduced during apomorphine-induced dyskinesias (apomorphine is a non-selective D₁- and D₂-dopamine receptor agonist) (Levy et al., 2001).

**Location and spread of microinjections**

The location of microinjections into the STN in this study was determined using microelectrode techniques and postoperative MRI verification of DBS electrode or lesion position relative to the STN. In addition, DBS in the same microelectrode tracks as the injections resulted in an improvement in tremor, rigidity and bradykinesia. The simultaneous recording of neuronal activity in two patients was also used to confirm that injections were performed in the STN. This technique is the most direct way to assess the effectiveness and spread of inactivating agents (Malpeli, 1999), and was especially important with regard to Patient A because it demonstrated that the lidocaine injection was well targeted and that the injection effectively inactivated neuronal tissue although no clinical effect was observed.

By recording neuronal activity at multiple distances from the injection site, we were also able to assess the spread of lidocaine and the size of the effective block. It took ~5 min for 3.5 µl of lidocaine to block neuronal activity within ~1 mm of the injection site in the posterior–ventral direction (i.e. below the cannula, see Fig. 1). Our results are comparable with those of Martin and Ghez (Martin and Ghez, 1999). They demonstrated, using autoradiographic monitoring of glucose uptake/metabolism, that following an injection of 1 µl of lidocaine a reduction in glucose uptake within ~1 mm of the injection centre was attributable to drug spread. Other observations of diffusion distances in rat thalamic and spinal tissue have demonstrated that a 1 µl injection of lidocaine would result in a block of neuronal activity in a spherical region of radius 0.8 mm at 10 min after injection (Myers, 1966; Sandkuhler et al., 1987). Although we injected 3.5 µl and these studies injected 1 µl of lidocaine, there are many factors that could account for variability in diffusion distances. For example, it is assumed that the distribution of lidocaine is spherical in shape in nuclear and cortical regions (Myers, 1966; Martin and Ghez, 1999), but there is significant anisotropy in the direction and extent of diffusion from the injection site in regions containing fibres of passage (Sandkuhler et al., 1987). Some of the injected lidocaine might have also preferentially diffused up along the cannula shaft and therefore reduced the diffusion distances observed with our simultaneous microelectrode recording set-up. Hupe and colleagues demonstrated that with pressure injections, inactivation is more efficient above than below the pipette tip and that the volume of inactivation has an ellipsoidal form centred above the tip of the pipette (Hupe et al., 1999). Other factors contributing to the observed differences in diffusion distances include: the volume of the extracellular space, the diffusibility of the drug through the extracellular space, the homogeneity of the medium of diffusion (white matter versus grey matter), the vascularization of the tissue surrounding the injection cannula (which would affect the washout of the drug), how fast the drug is degraded and the rate of drug delivery (Sandkuhler et al., 1987; Malpeli, 1999).

One caveat of this study is the inability to completely rule out that the observed effects were not due to diffusion of lidocaine or muscimol into neighbouring structures (especially when simultaneous recording was not performed), most notably the substantia nigra pars reticulata. The effects of these agents on this nearby structure might have a similar effect to STN inactivation since increased neuronal activity and abnormal patterning of the substantia nigra pars reticulata is observed in the MPTP monkey model of Parkinson’s disease (Wichmann et al., 1999).

**Rates of injection and volume effects**

Although the injection rates used in this study (1–2.5 µl/min) were greater than those used in animals (0.1–2 µl/min) (Myers, 1966; Demer and Robinson, 1982; Duncan et al., 1993; Wichmann et al., 1994; Burbaud et al., 1998), there are several factors that indicate that volume effects did not produce the observed clinical effects. (i) Simultaneous recording of neuronal activity was quite stable during the period of injection and indicates that tissue was not being deformed at distances ≥0.6 mm from the injection site when substances were injected at a rate of ~1 µl/min. (ii) It is unlikely that the effects observed were due to a volume effect since there was a delay in the anti-parkinsonian effect produced by these injections; e.g. in Patient C (23 µl injected at 2.5 µl/min), a marked decrease in rigidity did not occur until 20 min following injection. In contrast, the subsequent insertion of the DBS electrode in this patient resulted in an immediate improvement in rigidity (not shown) [NB if the maximum length of the DBS electrode (1.27 mm in diameter) in the STN is ~7 mm (see Fig. 1), the volume of the STN tissue displaced by the DBS electrode is ~9 µl]. Benabid and co-workers have also reported that a significant decrease in akinesia and rigidity, along with the emergence of ballistic or choreoballistic movements, occurs at the time of insertion of the DBS electrode in patients with Parkinson’s disease
(Benabid et al., 2000). However, Demer and Robinson demonstrated that injections of >12 μl and/or at rates >1 μl/min produced irreversible damage in a region within 0.7 mm from the injection site, which was marked by gliosis and fibre loss (Demer and Robinson, 1982). Therefore, we cannot rule out that tissue damage did not occur due to higher rates of injection (especially in Patient C). Yet, it is unlikely that the observed clinical effects were due to permanent tissue damage because there was a return to baseline parkinsonism in all patients in whom recovery data were available.

**Time course**

The time course of the clinical effects of the lidocaine and muscimol injections, beginning 5–10 min following injection and lasting 30–50 min, closely matched those seen in animals (Sandkuhler et al., 1987; Wichmann et al., 1994; Martin and Ghez, 1999). In contrast, recovery to baseline tremor following a 5 μl muscimol injection (Patient F) was observed ~5 min after the end of the injection. This time course is similar to the time course of tremor suppression observed following injections of a similar volume of muscimol in the thalamus of patients with essential tremor (Pahapill et al., 1999). These results are consistent with studies using GABA which demonstrate that the duration of neuronal inactivation is proportional to the volume of substance injected (Hupe et al., 1999). These data suggest that the potential clinical application of microinjections to aid in the determination of the optimal target for lesions or DBS is limited. A significant advantage of the use of stimulation techniques over the use of microinjections as described in the present work is that intraoperative stimulation decreases tremor or rigidity with a very short latency (<1 min) and is highly reproducible (Rodriguez et al., 1998).

**Inhibition of tremor-related neuronal activity suppresses limb tremor**

Following MPTP-treatment in monkeys, there is a prominent increase in the number of STN neurones that display oscillatory activity (Bergman et al., 1994), and limb tremor reduction has been demonstrated following muscimol injections in the STN (Wichmann et al., 1994). In the present study, injections of muscimol into regions of the STN with tremor-related activity caused a reduction in limb tremor. Our results support the view that oscillatory activity in the STN is important in the mediation of parkinsonian limb tremor (Bergman et al., 1994).

It is interesting that the latency of the effect of muscimol or lidocaine on limb tremor was short. Five minutes after the start of a 5 μl lidocaine injection, we observed a reduction of limb tremor in Patients B and D, and in Patients E and F after 5 μl of muscimol. Although we did not wait for a long period after the saline injection in Patient E, it was observed that saline did not affect tremor. The effect of muscimol occurred immediately after 5 μl of muscimol was injected, while there was no immediate effect of saline (both were injected at 1 μl/min). Since tremor suppression occurred with small volumes of inactivating agents and with a short latency, our results suggest that tremor suppression can be accomplished by silencing the activity of a small region of STN containing tremor cells. Evidence from simultaneous microelectrode recording techniques has indicated that potential tremor-generating circuits in the basal ganglia can occupy a relatively small volume (<1 mm³) (Hurtado et al., 1999; Levy et al., 2000). Our results are also consistent with those of Rodriguez and colleagues who demonstrated that microstimulation in STN regions containing tremor cells reduces tremor with a very short latency (Rodriguez et al., 1998). It has been suggested that microstimulation affects a small volume of tissue at currents ≤100 μA, the maximum generally possible with microelectrodes (Dostrovsky et al., 2000).

**Changes in tremor-related oscillations beyond the blocked region of tremor cells**

There is now much evidence from single-unit microelectrode recordings indicating the involvement of the STN in the pathogenesis of parkinsonian limb tremor (Bergman et al., 1990, 1994; Hutchison et al., 1998; Krack et al., 1998b; Rodriguez et al., 1998; Magarinos-Ascone et al., 2000). However, increasing our understanding of the pathophysiology of tremor and expanding the current model of Parkinson’s disease has required the use of novel experimental techniques. To this end, simultaneous recording of tremor-related activity at multiple sites has proved invaluable (Nini et al., 1995; Bergman et al., 1998a, b; Hurtado et al., 1999; Levy et al., 1999, 2000; Raz et al., 2000). In the present study, simultaneous recording of neuronal activity during microinjection allowed us to directly assess the time course of the effect of a pharmacological block in an area not directly deactivated by the block. We demonstrated that the 4 Hz discharge oscillation in a neurone located outside a blocked region of tremor cells was reduced concurrent with a reduction in limb tremor. However, the firing rate and 20 Hz oscillatory activity remained unchanged. This result suggests that a block of neuronal activity can modify the neuronal discharge of cells located beyond the blocked area. There are several possible ways that this result might be interpreted.

First, if the recorded neurone received proprioceptive input from the tremulous contralateral hand/wrist, reduction of limb tremor could account for a reduction in the lower frequency oscillatory activity of the recorded neurone (Krack et al., 1998b). However, at no time was the oscillatory activity of the neurone coherent with wrist flexor EMG or the accelerometer signal, which would be expected if its firing was dependent on sensory input. Secondly, if the
recorded neurone received 4 Hz oscillatory input directly from cells at the injection site, inactivation of these cells would also lead to a reduction in the lower frequency component of the recorded neurone (Martin and Ghez, 1999). Yet, although intrinsic axon collaterals in the STN are frequent in the rat (Kita et al., 1983), they are rare in primates (Yelnik and Percheron, 1979; Sato et al., 2000). Furthermore, coincident discharge between pairs of STN neurones in patients with Parkinson’s disease is not observed, suggesting that STN tremor cells do not directly drive the activity of other STN tremor cells (Levy et al., 2000). Thirdly, muscimol may have affected the activity of the recorded neurone by acting on a dendrite extending within the blocked region. For example, it has been shown that the dendritic fields of neurones located centrally in the STN can extend over nearly two-thirds of the structure (Sato et al., 2000). However, this would probably result also in a decrease in the overall spontaneous discharge of the neurone, and this was not observed. Fourthly, both parkinsonian limb tremor (Scholz and Bacher, 1995) and tremor-related activity in the basal ganglia (Bergman et al., 1998b; Hurtado et al., 1999; Levy et al., 2000; Raz et al., 2000) have been shown to exhibit fluctuations in rhythmic activity. However, it is unlikely that both the decrease in 4 Hz oscillations of the recorded neurone and a decrease in 5 Hz limb tremor are merely due to spontaneous variability, because these oscillations decreased and reappeared with a similar time course.

Lastly, suppression of limb tremor could arise as a result of, in addition to direct inactivation of tremor-related discharge, the ‘desynchronizing’ effect of a small block of the STN upon neighbouring subcircuits in the corticobasal ganglia–thalamic loop (Bergman et al., 1998a; Deuschl et al., 2000). This concept is based on the observation that increased tremor-related oscillatory synchronization in the basal ganglia underlies the development of limb tremor in MPTP-treated monkeys (Bergman et al., 1998a; Raz et al., 2000). It has also been hypothesized that the STN might play a role in synchronizing oscillatory activity in the GPi, because oscillations in the tremor frequency range within the GPi persist in spite of a reduction in limb tremor following lesions of the STN (Wichmann et al., 1994). The reduction of oscillatory activity in the tremor-frequency range of a neurone located beyond a deactivated region, as demonstrated in the present study, supports these hypotheses. It is interesting to note that high-frequency oscillatory activity was not suppressed during the effect of the muscimol block, and this further supports the notion that the underlying mechanisms of the tremor and high-frequency oscillations in the STN are different (Levy et al., 2000).

Summary
This study demonstrates that microinjections of pharmacological blocking agents in the STN of patients with Parkinson’s disease results in a transient anti-parkinsonian effect, similar to results from animal models of Parkinson’s disease. Consistent with predictions of the current model of Parkinson’s disease (DeLong, 1990), inactivation of the STN also produces dyskinesias. In addition to suppressing oscillatory STN output, inactivation of neurones with tremor-related activity possibly results in a reduction of limb tremor due to the desynchronizing effect of the focal block on pathological oscillations in the basal ganglia (Bergman et al., 1998a; Deuschl et al., 2000).

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