Subthalamotomy in parkinsonian monkeys
Behavioural and biochemical analysis


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Summary

Nineteen Macaca fascicularis monkeys were divided into four different groups: Group A (n = 3), control; Group B (n = 3), monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP); Group C (n = 8), animals treated with MPTP in which the subthalamic nucleus (STN) was unilaterally lesioned by kainic acid injection; in Group D (n = 5), the STN was lesioned prior to MPTP administration. Subthalamotomy resulted in a bilateral improvement of tremor, spontaneous activity, bradykinesia (evaluated by a manual motor test) and freezing in Group C. All these monkeys developed hemichorea contralateral to the lesion. The improvement was maintained and the hemichorea continued until death. The monkeys in group D showed severe hemiballism which persisted throughout MPTP administration and developed parkinsonian signs mainly on the side ipsilateral to the lesion. Analysis of the in situ hybridization of the mRNA coding for glutamic acid decarboxylase (GAD) of MPTP monkeys showed a significant increase in the mean density of silver grains over every labelled neuron in the globus pallidum lateralis (56.8% over control) as well as the globus pallidus medialis (GPM) (45.7% over control) and the substantia nigra reticulata (SNR) (35.8% over control). No significant change was observed in the thalamic nucleus reticularis. Subthalamotomy (Groups C and D) produced a significant reduction in mRNA GAD expression on the side of the lesion in the GPM and the SNR (34% and 42.3%, respectively) with respect to the ipsilateral (non-lesioned) side and also when compared with parkinsonian monkeys. These results confirm and expand, at the cellular level, the paramount role of STN hyperactivity in the pathophysiology of parkinsonism. The therapeutic consequences of these findings for surgical treatment of Parkinson's disease are discussed.

Keywords: MPTP treatment; subthalamic nucleus lesion; in situ hybridization (GAD).

Abbreviations: GABA = \( \gamma \)-aminobutyric acid; GAD = glutamic acid decarboxylase; GPL = globus pallidus lateralis; GPM = globus pallidus medialis; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNR = substantia nigra reticulata; STN = subthalamic nucleus; TH = tyrosine hydroxylase

Introduction

Although the pharmacological management of Parkinson's disease has improved considerably in the past decade, motor fluctuations, dyskinesias and psychiatric complications have marred the long-term evolution, increasing patients' disability (Marsden et al., 1982; Bonnet et al., 1987; Obeso et al., 1989). Efforts are being directed at preventing and reducing the complications associated with chronic levodopa therapy. One possible approach consists in revitalizing the use of stereotaxic surgery. This is strongly supported by recent advances in the understanding of basal ganglia functional organization and of the physiological changes associated with the state of parkinsonism (Crossman et al., 1985; Albin et al., 1989; DeLong, 1990).

The nigrostriatal dopaminergic pathway has a dual action on the \( \gamma \)-aminobutyric acid (GABA) output neurons, facilitating the GABA–substance P-dynorphin neurons in the 'direct' circuit and inhibiting the GABA–enkephalin projection in the 'indirect' circuit (Albin et al., 1989; Gerfen et al., 1990). Activation of the 'direct' pathway results in inhibition of GPM and SNR activity and disinhibition of thalamic and brainstem targets, finally leading to movement facilitation (Hikosaka and Wurtz, 1983; Chevalier and

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Deniau, 1990). In contrast, activation of the GABA–
enkephalin neurons in the 'indirect circuit' decreases globus
pallidus lateralis (GPL) inhibitory activity onto the STN,
hence increasing subthalamic excitatory output. The net result
of activation of the 'indirect circuit' should be movement
inhibition. Both pathways are strategically organized to
provide, respectively, facilitatory and inhibitory feedback to
the thalamo-cortical projection. In the parkinsonian state,
dopaminergic depletion removes the excitatory drive on the
GABA–substance P-dynorphin ('direct circuit') pathway and
increases the activity of the GABA–enkephalin neurons
(Augood et al., 1989; Herrero et al., 1995), thus reducing
the striatal inhibition on GPM and increasing the activity of
the GABA–enkephalin output (Gerfen et al., 1990). The
latter results in overinhibition of the GPL which cannot
maintain its normal inhibitory tone on the STN (Crossman
et al., 1988; Mitchell et al., 1989). The neuronal firing rate
in the STN is therefore abnormally increased, producing
overactivity in the GPM (Mitchell et al., 1989; Bergman
et al., 1994). The net effect of lesioning the dopaminergic
pathway is a pathological increment of GPM activity due to
reduced inhibitory input from the 'direct circuit' and excessive
excitatory stimulation from the STN in the 'indirect circuit'
(DeLong, 1990). In accordance with this simplified scheme
of basal ganglia pathophysiology, parkinsonian signs such as
akinesia and bradykinesia would be explained by thalamic
overinhibition of the GPM leading to decreased activity in
the thalamo-premotor cortex projection (DeLong, 1990;
Marsden and Obeso, 1994).

The above-summarized data indicate that hyperactivity of
the STN–GPM pathway plays a paramount role in the
pathophysiology of parkinsonism. Accordingly, reduction of
the STN excitatory drive should improve parkinsonism.
Graham et al. (1990) and Brotchie et al. (1991) described
alleviation of akinesia by the injection of excitatory amino
acid-antagonist kynurenic acid in reserpinized rats and in
two adult marmosets and macaques rendered parkinsonian
by MPTP. The motor benefit was dose-dependent and
unaccompanied by dyskinesias. The value of therapeutic
intervention in the STN–GPM pathway was elegantly
demonstrated in two parkinsonian macaques in which
Bergman et al. (1990) produced a lesion of the STN by local
administration of ibotenic acid. Subsequently, Aziz et al.
(1991, 1992) also reported successful relief of parkinsonism
in monkeys submitted to a thermolitic lesion of the STN.
More recently, high-frequency stimulation applied to the
STN induced an alleviation of rigidity and bradikinesia in
two hemiparkinsonian MPTP-treated monkeys without
dyskinesias (Benazzouz et al., 1993).

In this study, we have analysed, in detail, the impact of
subthalamotomy on motor function during prolonged follow-
up. The major aims of the study were (i) to assess the
changes in the parkinsonian state with particular attention to
signs which are very relevant in Parkinson's disease, e.g.
fine manual activity, freezing, tremor and posture and the
possible interference of chorea/ballism, if present, with motor
function; (ii) to examine the possibility that progressive
dopamine loss could reduce, or abolish a potential therapeutic
action of subthalamotomy in Parkinson's disease (Guridi
et al., 1993)—for this purpose we studied the effect of
repeated MPTP administration following a lesion of the STN;
(iii) to measure GABA activity in the major basal ganglia
output nuclei in parkinsonian monkeys and the effect of
subthalamotomy for which we quantified mRNA GAD
expression by in situ hybridization using cRNA probes.

Material and methods

Animals

Nineteen monkeys (Macaca fascicularis) of both sexes (11
females and eight males) weighing between 2.5 and 5.8 kg
were used in this study. They were housed in approved cages
under standard conditions of humidity (55±5%), temperature
(21±1°C), and light (12 h light/dark cycles) and they had
free access to fruit, water and pellets. Experiments were
carried out in accordance with the Declaration of Helsinki
and with the Guide for the Care and Use of Laboratory
Animals as adopted and promulgated by the National Institute
of Health (USA).

For the purpose of the study, animals were labelled
numerically as CYN (Cynomolgus) 1–19 and were divided
into four groups: Group A (n = 3), control monkeys; Group
B (n = 3), animals in which stable parkinsonism was induced
by MPTP and which received no further pharmacological or
surgical treatment (Table 1); Group C (n = 8), animals which
received MPTP until reaching stable parkinsonism and on
which a subthalamic lesion was subsequently performed;
Group D (n = 5), in which an STN lesion was first made and
and afterwards MPTP was administered.

MPTP administration

MPTP hydrochloride was dissolved in saline (RBI, Natick,
Mass., USA) and injected via the saphenous vein under
anaesthetic conditions (ketamine 10 mg kg\(^{-1}\), i.m.), being
given in individual doses of 0.5–1 mg kg\(^{-1}\) until stable
parkinsonism was reached. MPTP administration (weeks,
doses) and the motor disability of each animal are shown in
Table 1. A period ranging from 8 to 18 weeks (mean 12.8
weeks) was allowed for possible spontaneous recovery before
starting the study (Table 1).

Motor assessment

The motor state was evaluated by three different tests: (i) a
disability scale developed to assess degree of parkinsonism
(Luquin et al., 1993); (ii) a dyskinesia scale to test the effect of
dopaminergic drugs in parkinsonian monkeys and quantify
the intensity of chorea/ballism following lesion of the STN
(Luquin et al., 1992); (iii) a manual motor task test aimed at
evaluating fine motor activity.
The disability scale ranges from 0 (normal) to 25 (maximal severity) and comprises seven items which include the following; spontaneous activity (0–5), response to external stimulus (0–3), tremor intensity (0–3) and duration (0–3), balance (0–3), posture (0–3), freezing during manual activities such as picking up a piece of food and climbing behaviour (0–3), and feeding (0–2). The dyskinesia scale rated the degree of abnormal movements according to the topography (1, focal; 2, segmental; 3, hemibody; 4, generalized), intensity (1, mild; 2, moderate; 3, severe; 4, very severe) and duration expressed as a percentage of the observation period during 60 min (1, 0–25%; 2, 26–50%; 3, 51–75% and 4, 76–100%). The dyskinetic score ranges from 0 (no dyskinesias) to 12 (generalized, highly incapacitating and continuous dyskinesias). In this study, hemichorea and hemiballism are considered the same type of dyskinesia, only differing in the intensity and amplitude of the movements (parkinsonism) was considered severe when the disability score was higher than 15 and moderate when the score was between 15 and 10. Maximal score was 25.

Lesion of the subthalamic nucleus
Under aseptic conditions, the monkeys were anaesthetized (ketamine 10 mg, i.m.; three to five doses during the procedure) and placed in a stereotaxis frame (David Kopf, 1730 model), and ventriculography was performed. The intercommissural line was measured and the STN coordinates were calculated according to the atlas (Shantha et al., 1968; Szabo and Cowan, 1984). Kainic acid (1 μl 50 mM) was injected through a Hamilton microsyringe into the STN unilaterally. The coordinates for STN lesion in this study were considered to be in the mid-point of the intercommissural line, 1 mm below the third ventricle floor and 5–5.5 mm lateral from the sagittal sinus.

Tissue preparation and neuropathological studies
After ketamine anaesthesia the animals received a lethal dose of sodium pentobarbital (50 mg kg⁻¹, i.p.). Immediately after death, the brains were removed from the skull, dissected in 1.5 cm slabs (containing the areas to be examined) along the frontal plane and rapidly frozen on dry ice reduced to powder and kept at −80°C. Subsequently, 20 μm sections were cut, using a cryostat microtome (Microm HM 500, Heidelberg, Germany). The sections were stained with toluidine blue, 1% phosphotungstic acid or 0.5% cresyl violet.

Table 1 General characteristics of monkeys treated with MPTP

<table>
<thead>
<tr>
<th>Animal</th>
<th>Duration (weeks)</th>
<th>No. of doses</th>
<th>mg/kg</th>
<th>Motor disability</th>
<th>Study period</th>
<th>Lesion side</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B: MPTP treated without surgery—MPTP administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYN 4</td>
<td>15</td>
<td>8</td>
<td>3.20</td>
<td>Severe 16.4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>CYN 5</td>
<td>20</td>
<td>8</td>
<td>2.50</td>
<td>Severe 17.2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>CYN 6</td>
<td>17</td>
<td>13</td>
<td>4.15</td>
<td>Severe 15.8</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Group C: MPTP + subthalamotomy—MPTP administration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYN 7</td>
<td>12</td>
<td>4</td>
<td>2.85</td>
<td>Severe 17.3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>CYN 8</td>
<td>10</td>
<td>4</td>
<td>1.80</td>
<td>Moderate 14.3</td>
<td>18</td>
<td>R</td>
</tr>
<tr>
<td>CYN 9</td>
<td>15</td>
<td>15</td>
<td>8.18</td>
<td>Moderate 13.5</td>
<td>12</td>
<td>L</td>
</tr>
<tr>
<td>CYN 10</td>
<td>15</td>
<td>4</td>
<td>2.26</td>
<td>Severe 20.8</td>
<td>15</td>
<td>R</td>
</tr>
<tr>
<td>CYN 11</td>
<td>16</td>
<td>10</td>
<td>6.10</td>
<td>Severe 15.8</td>
<td>16</td>
<td>L</td>
</tr>
<tr>
<td>CYN 12</td>
<td>16</td>
<td>5</td>
<td>2.33</td>
<td>Severe 16.2</td>
<td>17</td>
<td>R</td>
</tr>
<tr>
<td>CYN 13</td>
<td>20</td>
<td>12</td>
<td>6.24</td>
<td>Moderate 14.7</td>
<td>15</td>
<td>R</td>
</tr>
<tr>
<td>CYN 14</td>
<td>24</td>
<td>29</td>
<td>15.42</td>
<td>Moderate 13.8</td>
<td>8</td>
<td>L</td>
</tr>
</tbody>
</table>

Stable period: time in weeks since last MPTP injection and surgery. R and L = right and left hemisphere. Motor disability (parkinsonism) was considered severe when the disability score was higher than 15 and moderate when the score was between 15 and 10. Maximal score was 25.
In situ hybridization for the mRNA of the glutamic acid decarboxylase isoenzyme, GAD$_{67}$

The technical details of the procedure have been described elsewhere (Chesselet et al., 1987; Fontaine et al., 1988; Herrero et al., 1993a). Slide-mounted sections were brought to room temperature, dried under a stream of cold air, postfixed and incubated and sealed under a coverslip. Post-hybridization treatments were performed in order to limit non-specific labelling. Autoradiograms were generated by exposing the slices to X-ray films (Hyperfilm β-max, Amersham, Bucks, UK) for 5 days at room temperature. The sections were then dipped in autoradiography emulsion (NTB-2, Kodak, Technomar, Eaubonne, France) diluted 1/1, air dried and stored at 4°C in lightproof boxes in the presence of desiccant. Following 2 weeks of exposure, the sections were developed in Kodak D-19 for 1.5 min at 4°C, counterstained with thionin, rapidly dehydrated and coverslipped. The experiments were performed in duplicate.

The content of GAD$_{67}$ mRNA was estimated at the cellular level in the GPM, the GPL, the SNR and in the nucleus reticularis of the thalamus. Following hybridization with the antisense GAD$_{67}$ mRNA probe, a specific and reproducible pattern of hybridization was obtained both on film autoradiograms and at cellular level as demonstrated previously (Herrero et al., 1993a). At the cellular level, the antisense GAD$_{67}$ mRNA probe labelled neuronal perikarya (but not neuropil, glia or capillaries) only in those regions of the brain that have also been reported previously to contain GAD$_{67}$ mRNA using in situ hybridization (Chesselet et al., 1987; Herrero et al., 1993a). The specificity of GAD cDNA clone used in this experiment, designed as hGAD 2.7, has been reported previously (Bu et al., 1992) and the specificity of the in situ hybridization signal is supported by the low homogeneous pattern of silver grains obtained with the sense probe, contrasting with the high labelling observed in the GPM, GPL, SNR and reticularis thalamic nucleus with the antisense probe, both in the autoradiograms and on the emulsion-coated sections as already shown (Herrero et al., 1993b). Tissue sections that were incubated in hybridization buffer with the labelled sense probe did not contain any specific labelling. The result of this control procedure indicated that the GAD antisense probe was hybridizing to its target mRNA and that the labelling was not due to chemography artifact.

Sections were analysed at a ×50 magnification under polarized light illumination in order to visualize the silver grains in emulsion. The number of silver grains per neuron was assessed from the optical density, according to the method of Bisconte et al. (1968), using computer-base image analysis (Histo 200, Biocom, Les Vlis, France) as previously described (Javoy-Agid et al., 1990). Since the number of grains per cell is correlated to the cell surface (Strada et al., 1992), this factor was taken into account in the calculation of grain density (number of grains μm$^{-2}$). Thus, the grain density was taken as an index of mRNA expression in the labelled cells. Background silver grain density was estimated at a distance from labelled neurons and was subtracted from the mean grain density in the labelled cells to obtain a measurement of the specific grain density in the labelled neurons. The mean grain density ±SEM per neuron was calculated over 50 labelled neurons.

Statistics
The stability of motor function before lesioning the STN was assessed with Friedman's test. The effect of subthalamotomy on the motor scores and manual activity were analysed using the Mann–Withney U test. Student's $t$ test with two tails was used to compare optic density values for mRNA GAD expression in the areas of interest in the four groups of animals.

Results

Effect of subthalamic nucleus lesion in MPTP treated monkeys: motor behaviour

Induction of parkinsonism

MPTP was administered to 11 previously normal monkeys. Following repeated MPTP treatment, all the animals developed marked parkinsonian signs: a loss of spontaneous activity; bradykinesia for volitional movements such as eating and grabbing objects; freezing of walking; flexor posture of the trunk and conspicuous postural tremor (Table 1). The parkinsonian syndrome in the three control animals, Group B, remained stable throughout the observation period of 12 weeks. Group C had no significant variation ($P < 0.05$) in either the disability scale or in the motor manual test prior to surgery.

Effect of the subthalamic lesion in MPTP monkeys (group C)

Lesion of the STN was carried out uneventfully in the eight animals. Two monkeys died within the 2 weeks after surgery.
Subthalamotomy in MPTP monkeys

Fig. 1 (A) Reduction in the disability score after subthalamic lesion in three macaques previously treated with MPTP. Maximum score was 25. (B) Improvement in spontaneous activity in the same parkinsonian monkeys submitted to subthalamotomy. The spontaneous activity referred to spontaneous movement, relation with other monkeys, jumping, etc. and was derived from 2×30 min observation periods every day. Maximum score was 5.

CYN-3 was in a severe state of parkinsonism (disability scale 20.8) and developed lower limb chorea without neurological improvement and CYN14 improved clearly, but died 2 weeks later. Three monkeys (CYN 7, 8 and 9) did not show any long-lasting modification in their motor state after surgery.

In the three remaining animals (CYN 11, 12 and 13) the disability scale showed a significant ($P < 0.01$) reduction post-surgery (Fig. 1A) for each animal. This effect was immediate in the moderately parkinsonian monkey (CYN 13) and was noticed over the next 4 weeks in the other two severely affected monkeys (Fig. 1A). The three monkeys remained markedly improved with respect to the pre-surgery period during the next 3–4 months until sacrificed. The scores for all seven items included in the global disability scale were reduced. However a posteriori analysis revealed significant improvement only in the items: postural tremor, spontaneous activity and freezing. The tremor was completely abolished in the limbs contralateral to the STN lesion and reduced in the ipsilateral side. The effect on spontaneous activity ($P < 0.02$) (Fig. 1B) and freezing behaviour was dramatic in all monkeys. Balance and posture were alleviated in the follow-up period but did not reach statistical significance. Hemichorea was present and persisted chronically in the three monkeys, with a mild tendency towards reduction in monkeys 11 and 12 and no change in CYN 13, who had a hemiballism. The monkeys adapted extremely well to the hemichorea which was not a real source of motor disability.

Evaluation of the manual motor task test disclosed a significant improvement in the three animals ($P < 0.02$). Two macaques (CYN 12 and 13) improved bilaterally, and in CYN 11 the effect was only for the limb contralateral to the lesion (Table 2). In this latter animal, hemichorea interfered partially with test performance during the first 10 days postoperatively, but reached normal values in the ensuing weeks. All monkeys continued to perform the test with normal speed until sacrifice.

Effect of subthalamotomy before MPTP administration (Group D)

Five monkeys (CYN 15–19) were operated on in order to lesion the STN prior to MPTP administration. The animals developed contralateral hemiballism ($n = 4$) and hemichorea ($n = 1$) within the first 24 h. The severity of the dyskinesia was, in general, much higher than in the parkinsonian monkeys (Group C). The animals did not use the dyskinetic

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Time (in seconds) employed in performing a manual activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Controls</td>
<td></td>
</tr>
<tr>
<td>CYN 1</td>
<td>2.80±0.23*</td>
</tr>
<tr>
<td>CYN 2</td>
<td>3.12±0.26</td>
</tr>
<tr>
<td>CYN 3</td>
<td>3.81±0.33</td>
</tr>
<tr>
<td>Preoperative</td>
<td>Postoperative</td>
</tr>
<tr>
<td>CYN 11</td>
<td>Contralateral hand</td>
</tr>
<tr>
<td>CYN 11</td>
<td>Ipsilateral hand</td>
</tr>
<tr>
<td>CYN 12</td>
<td>Contralateral hand</td>
</tr>
<tr>
<td>CYN 12</td>
<td>Ipsilateral hand</td>
</tr>
<tr>
<td>CYN 13</td>
<td>Contralateral hand</td>
</tr>
<tr>
<td>CYN 13</td>
<td>Ipsilateral hand</td>
</tr>
</tbody>
</table>

Each value represents the average of 100 trials over several weeks. *The data shown for control monkeys represent the mean of the right and left hand, since there were no differences between sides.
Table 3 mRNA GAD expression in various brain regions in the four experimental groups of monkeys

<table>
<thead>
<tr>
<th>Group</th>
<th>GPM</th>
<th>GPL</th>
<th>SNR</th>
<th>RTN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 3)</td>
<td>0.555±0.02**</td>
<td>0.596±0.02**</td>
<td>0.940±0.14*</td>
<td>0.830±0.03</td>
</tr>
<tr>
<td>Group B (n = 3)</td>
<td>0.808±0.02</td>
<td>0.938±0.03</td>
<td>1.220±0.14</td>
<td>0.848±0.04</td>
</tr>
<tr>
<td>Group C+D (n = 7)</td>
<td>1.311±0.04***</td>
<td>1.019±0.04</td>
<td>1.198±0.04*</td>
<td>1.115±0.04</td>
</tr>
<tr>
<td>Non-operated side</td>
<td>0.860±0.04</td>
<td>0.839±0.04</td>
<td>0.696±0.02</td>
<td>1.317±0.04</td>
</tr>
<tr>
<td>Operated side</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GPM = globus pallidus medialis; GPL = globus pallidum lateralis; SNR = substantia nigra reticulata; RTN = reticularis thalamic nucleus. Group A, control animals; Group B, MPTP monkeys; Group C, MPTP monkeys with subthalamotomy; Group D, monkeys with subthalamotomy prior to MPTP administration. Asterisks indicate statistically significant differences between group A and B and between operated and non-operated side for group C+D: *P < 0.05; **P < 0.01; ***P < 0.001.

There was no correlation between the degree of neuronal loss and disability scores (Herrero et al., 1993b). No morphological alterations were observed in the surviving neurons in the midbrain of all MPTP-treated animals and consisted mostly of pyknotic changes. No distinct morphological abnormalities at the level of the striatum and globus pallidum were observed.

Lesion of the subthalamic nucleus
A lesion of the STN was established in five monkeys (CYN 9 and 11–14) from Group C and four monkeys from Group D (CYN 16–19). Thionin staining showed severe neuronal loss and reactive gliosis of the STN. The lesion destroyed about 80–90% of the nucleus in all animals. There was no sign of damage to nearby structures. The topography and extension of the lesion was similar for monkeys in Group C and D. Monkey CYN 9 developed choreatic movements transiently for a few hours, and no modification in his motor state subsequently, but had a lesion similar to the animals with marked improvement.

In situ mRNA GAD67 expression
GAD67 mRNA expression in control monkeys (Group A)
Autoradiographs and emulsions were developed after a short exposure time (2 weeks) to avoid saturation of the emulsion which would obscure potential differences in the RNA levels.

As previously reported, (Herrero et al., 1993a), labelled neurons were observed in the reticularis thalamic nucleus, SNR, GPL and GPM in sections processed for in situ hybridization with the 35S-RNA GAD and emulsions autoradiography. In control animals, the intensity of labelling per neuron was similar on the two sides of the brain in all the structures. Not all of the pallidal and/or nigral neurons contained GAD67 mRNA. This unexpected finding could be due to the sensitivity of the hybridization parameters used, since all pallidal neurons (at least in the rat) express GAD mRNA (Mercugliano et al., 1992) (Table 3).
GAD mRNA expression in parkinsonian monkeys (Group B)

In MPTP lesioned monkeys, the mean density of silver grains over every labelled neuron was increased in the GPL with respect to the control (56, 87%, \( P < 0.005 \)) as well as in the GPM (45, 76%, \( P < 0.006 \)) and SNR (35, 88%, \( P < 0.04 \)) (Table 3 and Fig. 2). In the thalamic nucleus reticularis, the average amount of GAD_{67} transcript per neuron was not significantly increased.

Effect of subthalamic nucleus lesion

At the end of the behavioural study, animals from Groups C and D showed an identical clinical and pathological picture. Both groups are, therefore, pooled for analysis. A significant reduction in the intensity of labelling per neuron in GPM (34%, \( P < 0.001 \)) and SNR (42%, \( P < 0.02 \)) on the side of subthalamotomy was observed when compared with the contralateral side (Table 3 and Fig. 2). The intensity of labelling over the GABAergic neurons of the GPL was decreased but the difference did not reach statistical significance. The RNT was unchanged. These findings were consistent in all the animals studied.

The comparison of GAD_{67} mRNA expression in the hemisphere with a STN lesion (Groups C and D) with group B also revealed a statistically significant decrease in SNR (\( P < 0.002 \)) but not for GPM.

Discussion

This study confirms and extends previous observations (Bergman et al., 1990; Aziz et al., 1991, 1992) indicating that lesioning the STN can induce a substantial amelioration of parkinsonism in MPTP-treated monkeys. Bergman et al. (1990) described a marked improvement in the contralateral side in two monkeys with a unilateral STN lesion induced by ibotenic acid injection. Hemichorea was present in both animals, disappearing gradually within 24 h in one, and persisting attenuated in the other until death 3 weeks after surgery. Aziz et al. (1992) reported successful relief of parkinsonism in six monkeys submitted to a thermolitic lesion of the STN. The lesion was bilateral in two monkeys. Improvement occurred bilaterally in four monkeys, three treated with unilateral subthalamotomy, particularly regarding spontaneous movements, facial expression and posture, and it was mainly unilateral for rigidity and tremor. Two monkeys developed hemiballism, which was permanent in one, and a third monkey had mild hemichorea (Aziz et al., 1991, 1992), despite which, the animals preferred to use the dyskinetic limb. Administration of apomorphine did not enhance the hemichorea/ballism but reversed the residual symptoms. Our observations are basically in agreement with the above. The analysis performed in this study offers further details as to the effect of subthalamicotomy in parkinsonian monkeys and provides further insight into the potential therapeutic action of this approach for Parkinson's disease. First of all, we documented a bilateral, though asymmetrical, effect on bradykinesia, as shown by the manual tests employed in this study, which allowed objective measurement of a fine motor task. The previous publications had not analysed freezing in particular, which is a common source of disability in Parkinson's disease. We observed a bilateral abolition of freezing, both during spontaneous behaviour and whilst performing the manual task. We confirmed a marked improvement in spontaneous activity, facial expression and degree of attention to external stimuli as indicated previously (Aziz et al., 1992; Wichmann et al., 1994a) but our monkey did not recover a completely normal erect posture after subthalamotomy, persisting in a tendency to flex the trunk.

Subthalamotomy also induced a definite, albeit not dramatic, antiparkinsonian effect on the side ipsilateral to the lesion. The explanation for this observation is not apparent. Lesion or blockade of the STN reduces excessive neuronal driving onto the GPM and SNR and very likely also tends to normalize the afferent activity on the region of the pedunculo-pontine nucleus, which is abnormal in MPTP monkeys (Mitchell et al., 1989). All these structures are well interconnected bilaterally (Hazrati and Parent, 1991) so as to explain the effect of subthalamotomy but further studies are needed in order to specify which circuits mediate the bilateral improvement. Equally difficult to explain is the absence of motor manifestations in one of our monkeys even when the STN was perfectly lesioned. In humans, it is well known that vascular lesions of the STN and other basal ganglia nuclei may produce transient hemichorea, indicating some type of compensatory capacity. A similar, albeit obscure, mechanism could have occurred in that monkey.

The tremor present in our monkeys was of the postural type and affected the four limbs. Abolition of tremor on the side contralateral to the lesion and marked attenuation on the other side was the first and most consistent indication of a successful surgical procedure in this study. This is in keeping with the recent suggestion (Wichmann et al., 1994a) that parkinsonian tremor originates as a consequence of increased activity in a loop involving the STN, GPM and motor thalamus.

Following subthalamic lesion, the presence of hemichorea and even hemiballism was constant and persistent in monkeys against the prevailing opinion which minimizes its occurrence. Such a difference might be due to the large size of the lesions induced by kainic acid injections in our study. In accordance with Aziz et al. (1991, 1992) and Bergman et al. (1990), subthalamotomy-induced dyskinesia did not interfere greatly with motor performance. This is actually emphasized by our observation with the manual motor test which the monkey carried out much faster, and accurately, despite the presence of chorea or ballism. The data from the previous investigations (Bergman et al., 1990; Aziz et al., 1991, 1992; Wichmann et al., 1994a) and the present report, lead one to conclude that subthalamotomy can substantially ameliorate all the major motor signs induced by lesion of Parkinson's disease.
the substantia nigra pars compacta in the primate. The presence of dyskinesia is a drawback which may possibly be minimized, or even avoided completely, by accurate positioning of the lesion in discrete zones of the motor STN (Hamada and DeLong, 1992; Wichmann et al., 1994d).

An original aspect of the present study consisted in the effect of MPTP administration in monkeys with a previous subthalamotomy. The aim of this experiment was to attempt to ascertain whether progressive substantia nigra pars compacta degeneration could override the antiparkinsonian action of subthalamotomy. In all four animals included in this group, repeated MPTP treatment caused severe parkinsonism on the side ipsilateral to the STN lesion, but the contralateral side remained dyskinetic and only mildly parkinsonian. In fact, these monkeys were indistinguishable from monkeys belonging to Group C discussed above. These results suggest that the changes induced by subthalamotomy on neuronal activity in the major basal ganglia output nuclei persist despite progressive dopaminergic depletion. Strominger and Carpenter (1965) had also found in monkeys that a unilateral electrolytic lesion of the substantia nigra pars compacta did not modify hemiballism.

Hyperactivity of the STN appears to be a fundamental factor in the pathophysiology of parkinsonism in primates (Crossman et al., 1987). In MPTP monkeys, electrophysiological studies have shown a pathological increase in neuronal activity both in the STN and GPM (Miller and DeLong, 1987; Filion and Tremblay, 1991; Bergman et al., 1994). Metabolic studies using the 2-deoxyglucose mapping technique have also provided evidence indicating hyperactivity in the STN–GPM pathway in monkeys treated previously with MPTP (Mitchell et al., 1989, 1992). In amine-depleted rats, Robledo and Feger (1991) found a significant increase in spontaneous neuronal activity of the STN compared with control animals, and enhanced response in the SNR following bicuculline-induced stimulation in the STN. The present study using in situ hybridization for the expression of GAD mRNA provides the first molecular approach to this topic. Changes in GAD67 mRNA levels are thought to reflect modifications in GABA concentration, turnover rate and transmission (Litwak et al., 1990). GAD67 may provide the constitutive level of transmitter GABA required to support tonic synaptic transmission (Martin and Rimvall, 1993). GAD67 is highly sensitive to small changes in GABA synthesis and changes in its expression are probably reflecting metabolic modifications of GABA activity (Rimvall and Martin, 1994).

We have shown increased expression of GAD mRNA in the GPM of parkinsonian monkeys compared with control animals, confirming at the cellular level the data discussed above. The same analysis in SNR also revealed an increment in the parkinsonian animals. This original finding suggests a very important role for the SNR in the pathophysiology of the parkinsonian syndrome. In keeping with this notion, subthalamotomy in our parkinsonian monkeys subsequently induced a very significant reduction in GAD mRNA expression in the SNR, even more than in the GPM. Substantia nigra reticulata neurons in normal monkeys were found to discharge mainly in relation to orofacial and swallowing movements (Mora et al., 1977; DeLong et al., 1983). It is tantalizing to hypothesize that increased activity in the SNR may be mainly responsible, not only for the abnormalities of saccadic eye movements present in Parkinson’s disease, but also for axial parkinsonian symptoms and signs such as hypomimia, hypophonia, flexor posture, walking problems, etc., while limb bradykinesia and rigidity might depend mainly upon abnormal GPM output.

The problems associated with chronic drug treatment in Parkinson’s disease have led to a renewed interest in stereotaxic surgery of the basal ganglia. Thalamotomy and postero-ventral pallidotomy are the techniques currently accepted for surgical treatment of Parkinson’s disease (Baron et al., 1992; Laitinen et al., 1992). The possibility of considering the STN as a surgical target in Parkinson’s disease is highly attractive (Guridi et al., 1993) but has been approached with extreme caution (Kulisevsky et al., 1994; Inzelberg and Korczyn, 1994) and even pessimism (Iacono et al., 1994). The accumulated experience in monkeys clearly indicates that subthalamotomy induces a marked improvement in parkinsonism associated with hemichorea of variable intensity and duration. The fear of provoking severe hemiballism in parkinsonian patients is certainly justifiable (Dierssen et al., 1961). However, two parkinsonian patients with a subthalamic lesion of vascular origin (Selall et al., 1992; Vidakovic et al., 1994) showed motor improvement without hemichorea/ballism. Chronic electrical stimulation of the STN in two hemiparkinsonian MPTP monkeys (Benazzouz et al., 1993) and in five patients with electrodes implanted bilaterally into the STN (Benabid et al., 1993; Limousin et al., 1995) resulted in a dramatic benefit in motor performance with mild or absent dyskinesias. Our own experience with monkeys suggests that the tendency to develop hemiballism is reduced when the degree of parkinsonism is severe. This is possibly because the excessive activity in the GPM/SNR associated with the parkinsonian state is, in part, secondary to low inhibitory activity in the ‘direct’ GABA–substance P striato-pallidal projection (Gerfen et al., 1990). In severely parkinsonian monkeys, the metabolic activity of this circuit is much reduced, thus counteracting the fall in excitatory drive to the GPM provoked by lesioning the STN (Obeso et al., 1994).

There are some important theoretical reasons for considering the STN as a target for Parkinson’s disease. First, inactivation of the STN will reduce the excessive inhibitory output from both the GPM and SNR and also midbrain tegmentum (Granata and Kitai, 1991), possibly having a much wider regularizing effect on neuronal activity (Ryan and Sanders, 1993) than pallidotomy and thalamotomy. This increases the probability of achieving a greater overall improvement in motor function. Secondly, inactivation of the STN leaves the ‘direct’ striato-pallidal pathway intact and GPM efferent activity is functionally preserved, reducing the possibility of disturbing motor control. Finally, electrophysio-
logical identification of the sensorimotor area of the STN is easier than for the GPM (Wichmann et al., 1994b). On the other hand, parkinsonian patients with levodopa-induced dyskinesias as a major problem will not, in principle, be good candidates, since subthalamotomy, in contrast with pallidotomy, will not abolish the dyskinesias.

In conclusion, we have found as a result of this study, that subthalamotomy in parkinsonian monkeys improves all major features of parkinsonism and has a profound impact at the functional level of the major basal ganglia output nuclei. We believe that surgery of the STN for Parkinson’s disease should receive attention in the immediate future.

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