Pedunculopontine nucleus stimulation improves akinesia in a Parkinsonian monkey

Ned Jenkinson,[1,3] Dipankar Nandi,[1,2] R. Chris Miall,[4] John F. Stein[1] and Tipu Z. Aziz[1,2,3,CA]

[1]University Laboratory of Physiology, Oxford University, Parks Road, Oxford; [2]Department of Neurosurgery, Radcliffe Infirmary, Woodstock Road OX2 6HE, Oxford; [3] The Nuffield Department of Surgery, John Radcliffe Hospital, Headley Way, Oxford; [4]Behavioural Brain Sciences, School of Psychology, University of Birmingham, UK

[CA,2]Corresponding Author and Address: tipu.aziz@physiol.ox.ac.uk

Received 4 October 2004; accepted 11 October 2004

We have studied the effects of stimulating the pedunculopontine nuclei through a fully implanted macroelectrode with a s.c. implantable pulse generator whose parameters can be programmed telemetrically, in a macaque before and after inducing Parkinsonian akinesia with MPTP. Our results show that in the normal monkey high frequency stimulation of the pedunculopontine nuclei reduces motor activity while low frequency stimulation increases it significantly over baseline. After making the monkey Parkinsonian with MPTP, unilateral low frequency stimulation of the pedunculopontine nuclei led to significant increases in activity. These results suggest that pedunculopontine nuclei stimulation could be clinically effective in treating advanced Parkinson's disease and other akinetic disorders. *NeuroReport* 15:2621–2624 © 2004 Lippincott Williams & Wilkins.

Key words: Deep brain stimulation; MPTP; Macaque; Parkinson's disease; Pedunculopontine nucleus; PPN

INTRODUCTION

The pedunculopontine nuclei probably play a central role in the generation of movement. Stimulation of the pedunculopontine nuclei increases movement in the rat and cat and inhibition decreases it [1–4]. The region degenerates in akinetic disorders such as Parkinson's disease, multi-system atrophy and progressive supranuclear palsy [5,6]. Uptake of labelled 2-DG in the pedunculopontine nuclei increases in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) exposed Parkinsonian monkey, probably because MPTP causes increased inhibitory afferent activity from the medial pallidum [7,8]. After a lesion of the sub-thalamic nucleus (STN) which produces a reversal of akinesia in Parkinsonian monkeys, such uptake is reduced. Lesioning the pedunculopontine nuclei in normal primates can also induce akinesia [9–11]. In a more recent study we have shown that microinjections of the GABA antagonist bicuculline can alleviate akinesia in the Parkinsonian primate [12]. However, use of bicuculline is not feasible clinically. We have also shown that motor activity can be affected in a frequency dependent manner by direct electrical stimulation of the pedunculopontine nuclei in intact monkeys [13]. However, in those studies an electrode designed for human implantation was used (Medtronic 3389); so the results were probably confounded by stimulation of the closely associated superior cerebellar peduncle and perhaps other tracts and nuclei in this region.

We have studied the effects of stimulating the pedunculopontine nuclei in an intact macaque before and after treatment with MPTP through an implanted custom made macroelectrode (courtesy of Medtronic Inc.) and using an implanted subcutaneous pulse generator (IPG, ITREL III, Medtronic Inc.) that did not restrict the animal's free movements. The pulse generator is capable of being programmed transcutaneously, thus allowing us to alter the stimulation amplitude, frequency and pulse width. Moreover, this system allows a choice of four adjacent ring contacts for bipolar stimulation, to adjust the depth of the site of stimulation.

MATERIALS AND METHODS

Experiments were carried out on a 16-year-old male Rhesus macaque (*Macaca mulatta*) weighing 15 kg. The Monkey was housed in accordance with the United Kingdom Home Office regulations under the Animal (Scientific Procedures) Act 1986. All procedures were performed with the prior approval of the Local Ethics Committee, Oxford University and the Home Office inspectorate. The animal's behaviour was recorded through closed circuit television onto videotape; automated infra-red activity counts were monitored and partially blinded multiple observer clinical motor scoring (PPMRS; [12]) of the monkey's status were taken. A stable baseline score of the animal's motor activity was established over 90 days. Following this, a custom made Medtronic deep brain stimulating electrode (0.7 mm diameter, 4 contacts 2 mm in length and separation of 1 mm) was inserted stereotactically into the left PPN using contrast ventriculography. Surgery was performed under aseptic conditions and under general anaesthetic initiated with a single dose of ketamine and maintained using a continuous i.v. infusion of saffan (Fig. 1). The electrode was anchored

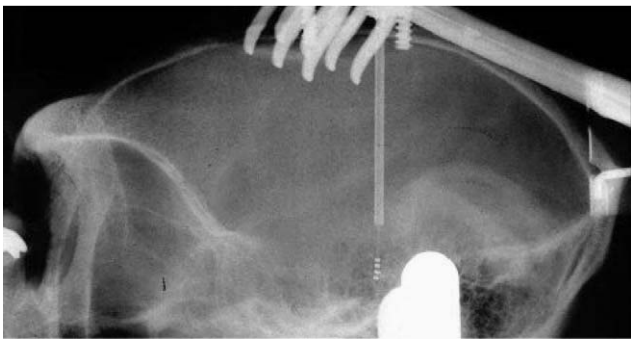

Fig. 1. A lateral venticulogram taken in the stereotactic frame, with the cannula in place during implantation of the deep brain stimulator electrode into the pedunculopontine nucleus. The four ring electrodes of the stimulating electrodes can be clearly seen.

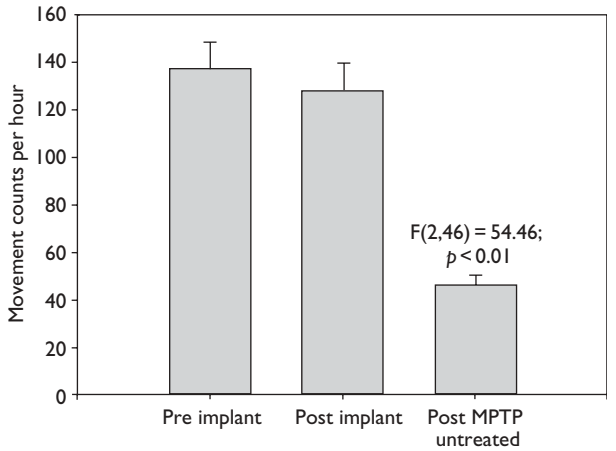

Fig. 2. Activity counts per hour for the monkey before and after the implantation of the deep brain stimulating electrode, and after treatment with MPTP.

in situ with dental acrylic and then connected to the s.c. implanted pulse generator (Itrel 3, Medtronic). The surgical wounds healed well within a week. The animal was then allowed to recover completely from the operation before stimulation studies were performed 14 days later. The stimulator could be turned on and off, and the stimulation parameters altered remotely using an implantable pulse generator programmer (Medtronic 7432 Programmer) by placing the small transmitter head of the programmer against the s.c. implanted pulse generator. The animal was trained to allow us to do this in the home cage without the necessity of sedation, or restraint.

Baseline studies 2 weeks after surgery confirmed the animal's post-operative motor activity was unchanged compared with pre-operative scores (Fig. 2). The deep brain stimulator was then tested at different parameter settings and electrode contacts till we found the settings that brought about consistent results with the lowest possible voltage amplitude. The final settings chosen were stimulation amplitudes of 3.5 and 4.5 V and a pulse width of 120 μs.

Motor activity was then recorded during stimulation of the pedunculopontine nuclei changing only the frequency of stimulation. Three conditions were tested; stimulation off, 5 Hz and 100 Hz. Each condition was tested 5 times with a minimum of 24 h between the start of each session. All observations were performed with the animal in its home cage in the presence of other monkeys in adjacent cages.

In order to test the effect of pedunculopontine nuclei stimulation on Parkinsonian akinesia 0.3 mg/kg MPTP was slowly administered i.v. under ketamine sedation (10 mg/kg, i.m.). We waited until the animal had recovered from the acute effect of MPTP before the animal's motor activity was recorded using the infra-red counter starting at between 08.00 h and 12 noon and ending 6 h later. Motor activity was recorded each day until it was seen to be stable. At this point the animal was making considerably fewer spontaneous movements in its home cage compared to the counts seen before MPTP treatment (Fig. 2). To test whether direct stimulation of the pedunculopontine nuclei would alter the motor activity of the Parkinsonian animal the stimulator was turned on in the morning just before the activity monitoring started. In the on condition, we used stimulation frequencies of 2.5, 5.0 and 10 Hz. We found that at higher frequencies it required less voltage amplitude to produce consistent changes in the animal's motor behaviour. Hence we used 6, 4 or 1.5 V, respectively. Shortly after stimulation commenced we started recording activity, which continued for at least a 6 h period. Only one condition was tested per day, and each week on at least one day the stimulator was not turned on so that control unstimulated activity could be sampled over a similar period, this was to test that the animal's base line unstimulated motor activity did not change over time. We also tested the effectiveness of L-dopa in alleviating the Parkinsonian symptoms. The animal was given an equivalent to human dose of Madopar (187.5 mg; 150 mg levodopa, 37.5 mg benserazide, 12.5 mg Madopar/kg) on nine occasions, at a similar time in the morning to when the stimulator would have been turned on, and the animal's movements were recorded over the same amount of time. At least 24 h separated a dose of L-dopa and subsequent stimulator response trial.

RESULTS

Pedunculopontine nuclei stimulation before MPTP: Low frequency stimulation at 5 Hz at either 3.5 or 4.5 V significantly increased the normal monkey's activity compared to the off state (Students separate variance *t*-test $t(4.3)=-4.28$; $p<0.05$), whereas high frequency stimulation (100 Hz) significantly decreased it ($t(8)=16.68$; $p<0.01$; Fig. 3). In all cases in we used bipolar stimulation between the middle two ring contacts. In other respects the behaviour of the animal did not show appreciable change, either on the clinical rating scores and review of the video recordings. No abnormal changes in the manner or range of the motor behaviour were seen. We observed no specific increases in any particular stereotypic motor behaviours, nor any involuntary movements: no tremors, dyskinesias or dystonic posturing. Interaction with other monkeys in adjacent cages appeared normal. The effects of stimulation on activity were completely and immediately reversible.

After MPTP: The animal's baseline activity decreased significantly after the animal was given MPTP (comparing all pre MPTP scores with movement scores on untreated control sessions after MPTP treatment, ($t(26.6)=9.4$; $p<0.001$; Fig. 2). Switching on the stimulator increased the

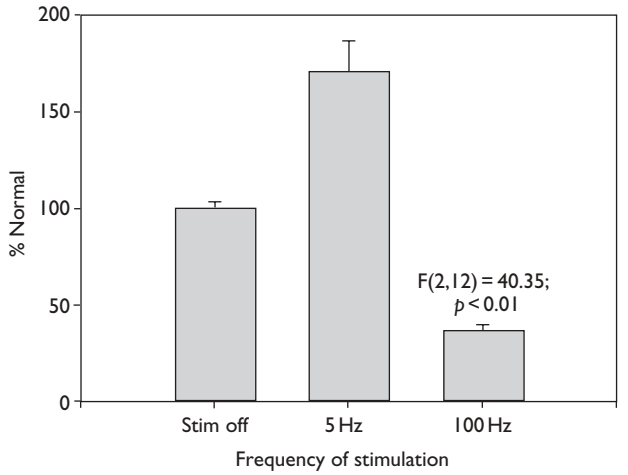

Fig. 3. The effect of stimulus frequency on movement counts per hour in the animal.

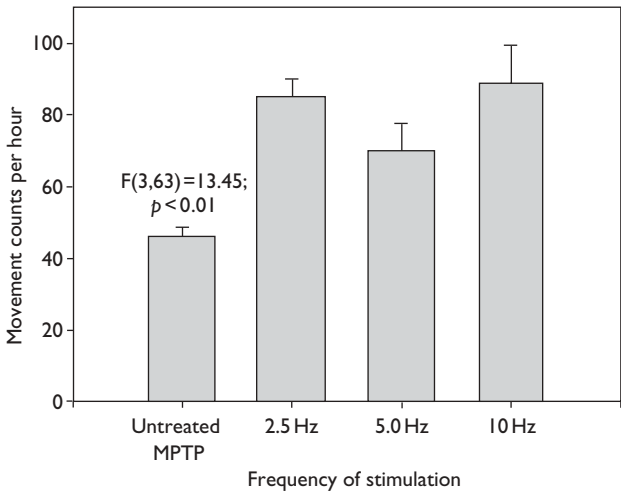

Fig. 4. The effect of stimulus frequency on movement counts per hour after treatment with MPTP.

motor activity scores in the Parkinsonian monkey, compared to similar periods when the stimulator was not turned on. All three frequencies of stimulation used (2.5, 5.0 and 10 Hz) significantly increased the number of movements/h made by the animal ($t(>34)>=3.19$; $p<0.05$; Fig. 4). It should be noted that in the Parkinsonian animal we found that the most consistent stimulation results were gained from bipolar stimulation between the deepest two ring contacts in the stimulating electrode. The effect of administering L-dopa on the animal's movement counts was not significantly different from the effect of stimulation of the pedunculopontine nuclei ($t(45)=-0.63$; $p>0.5$; Fig. 5). We did not test 100 Hz stimulation on the animal after it had been given MPTP as we did not want to decrease the amount of movement made by an animal that already showed a severe reduction in motor activity.

DISCUSSION

This study is the first to reliably demonstrate an increase in motor activity driven by low-frequency deep brain-stimulation. Stimulating the pedunculopontine nucleus in a Parkinsonian monkey reversed akinesia as effectively as L-dopa treatment. Increased movement was seen during low frequency deep brain-stimulation both before and after MPTP treatment without incurring abnormal involuntary movements or altering general behaviour, other than increasing motor activity. But even though both pedunculopontine nuclei stimulation and treatment with L-dopa produced significant increases in motor activity, neither pedunculopontine nuclei stimulation, nor L-dopa treatment returned the animal's activity entirely back to normal (Fig. 5).

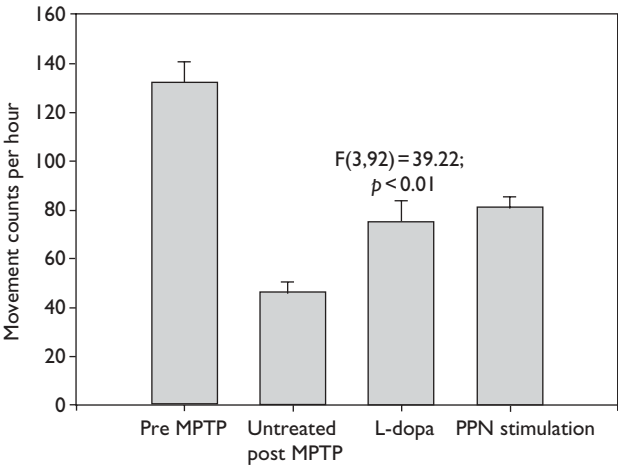

Fig. 5. Activity counts per hour before treatment with MPTP (left) and after MPTP, in the untreated state, with PPN stimulation, or with L-dopa administration.

High frequency (100 Hz) deep brain-stimulation decreased activity in the normal animal. This confirms the findings from our earlier study [13] with the refinement that we were able to use a much smaller electrode that therefore affected a much smaller volume of tissue. We did not observe any effects, such as tremor or nystagmus that would be expected if we had inadvertently stimulated the superior cerebellar peduncle.

That low-frequency deep brain-stimulation of the pedunculopontine nuclei increased movement in the Parkinsonian monkey, and inhibition of the pedunculopontine nuclei using high frequency stimulation decreased movement in the normal animal is consistent with the idea that Parkinsonian akinesia is, in part, caused by over-inhibition of the pedunculopontine nuclei by descending afferents from the basal ganglia. This overinhibition can be overcome by direct stimulation of the pedunculopontine nucleus, either electrically as demonstrated here, or chemically as we have previously demonstrated [13].

These findings have very significant implications for the treatment of Parkinson's disease and related disorders. Akinesia is the most disabling symptom of these conditions and may be the predominant one in many cases. In the later stages drug resistant akinetic symptoms, such as gait ignition failure and gait freezing, become crippling, and are not relieved by deep brain stimulation of conventional targets such as the subthalamic nucleus. In such patients stimulation of the pedunculopontine nuclei could alleviate these akinetic symptoms. Syndromes such as progressive supranuclear palsy and multisystem atrophy cause early

akinesia which is disabilitating and resistant to any medical therapy. Low frequency stimulation of the PPN may therefore help to alleviate these conditions at an early stage. Moreover stimulation at such a low frequency would reduce the costs of deep brain-stimulation surgery for akinetic disorders. Conventional STN or globus pallidus stimulation requires continuous 180 Hz pulses. Stimulating the pedunculopontine nuclei at 5–10 Hz, would mean dramatically reduced power consumption, which would greatly extend battery life, thereby facilitating transcutaneous recharging with simpler programming requirements. Such cost reductions would have immediate impact on the availability of deep brain-stimulation therapy for akinetic disorders.

CONCLUSION

Low frequency stimulation of the pedunculopontine nuclei region in the MPTP-treated Parkinsonian monkey partially reversed its akinesia. Thus pedunculopontine nuclei stimulation may be useful clinically, to alleviate dopa-resistant akinetic symptoms in late stage Parkinson's disease, progressive supranuclear palsy or multi-system atrophy.

REFERENCES

1. Brudzynski SM, Houghton PE, Brownlee RD and Mogenson GJ. Involvement of neuronal cell bodies of the mesencephalic locomotor region in the initiation of locomotor activity of freely behaving rats. *Brain Res Bull* 1986; **16**:377–381.
2. Garcia-Rill E, Houser CR, Skinner RD, Smith W and Woodward DJ. Locomotion-inducing sites in the vicinity of the pedunculopontine nucleus. *Brain Res Bull* 1987; **18**:731–738.
3. Milner KL and Mogenson GJ. Electrical and chemical activation of the mesencephalic and subthalamic locomotor regions in freely moving rats. *Brain Res* 1988; **452**:273–285.
4. Mogenson GJ and Wu M. Differential effects on locomotor activity of injections of procaine into mediodorsal thalamus and pedunculopontine nucleus. *Brain Res Bull* 1988; **20**:241–246.
5. Jellinger K. The pedunculopontine nucleus in Parkinson's disease, progressive supranuclear palsy and Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 1988; **51**:540–543.
6. Zweig RM, Jankel WR, Hedreen JC, Mayeux R and Price DL. The pedunculopontine nucleus in Parkinson's disease. *Ann Neurol* 1989; **26**:41–46.
7. Crossman AR, Mitchell IJ and Sambrook MA. Regional brain uptake of 2-deoxyglucose in N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism in the macaque monkey. *Neuropharmacology* 1985; **24**:587–591.
8. Gnanalingham KK, Milkowski NA, Smith LA, Hunter AJ, Jenner P and Marsden CD. Short and long-term changes in cerebral [^{14}C]-2-deoxyglucose uptake in the MPTP-treated marmoset: relationship to locomotor activity. *J Neural Transm Gen Sect* 1995; **101**:65–82.
9. Aziz TZ, Davies L, Stein J and France S. The role of descending basal ganglia connections to the brain stem in parkinsonian akinesia. *Br J Neurosurg* 1998; **12**:245–249.
10. Kojima J, Yamaji Y, Matsumura M, Nambu A, Inase M, Tokuno H *et al*. Excitotoxic lesions of the pedunculopontine tegmental nucleus produce contralateral hemiparkinsonism in the monkey. *Neurosci Lett* 1997; **226**:111–114.
11. Munro-Davies L, Winter J, Aziz TZ and Stein J. Kainate acid lesions of the pedunculopontine region in the normal behaving primate. *Mov Disord* 2001; **16**:150–151.
12. Nandi D, Aziz TZ, Giladi N, Winter J and Stein JF. Reversal of akinesia in experimental parkinsonism by GABA antagonist microinjections in the pedunculopontine nucleus. *Brain* 2002; **125**:2418–2430.
13. Nandi D, Liu X, Winter JL, Aziz TZ and Stein JF. Deep brain stimulation of the pedunculopontine region in the normal non-human primate. *J Clin Neurosci* 2002; **9**:170–174.

Acknowledgements: We are grateful to the Medical Research Council, UK and the Wellcome Trust for financial support on this project. We thank the staff of the Biological Services Unit for their continuous support and assistance.