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REST TREMOR IN RHESUS MONKEYS WITH MPTP-INDUCED PARKINSONISM

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1. ABSTRACT

Rest tremor (RTr) is a typical feature of Parkinson's diseases (PD). Animal models of PD presenting with RTr are indispensable for understanding the pathophysiology of human RTr and the development of new therapeutic agents. In this report we studied the occurrence of tremor on rhesus monkeys rendered parkinsonian by an intracarotid (ICA) infusion followed by 2-4 iv. doses of n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The animals' parkinsonism was assessed using a rating scale, activity monitors and a novel tremor monitor. The animals manifested bilateral parkinsonism with more severe clinical signs on the side of the body contralateral to the ICA infusion. The RTr in these animals had a mean peak frequency of 7.9 Hz (S.E.: 0.12), and a mean amplitude of 5.1/d/s/rtz (S.E.: 0.69). Substantial reduction in RTr amplitude (80.4%) was observed after oral L-DOPA administration. Our results suggest that: 1) RTr is present after the combined administration of ICA and iv. MPTP. 2) The mean RTr frequency in rhesus monkeys may be higher than in parkinsonian patients. However, as in PD, RTr frequency in the monkey was maintained within a narrow band width. 3) As in PD, L-DOPA administration to MPTP-treated monkeys reduced the amplitude of RTr and improved the parkinsonian features. Monitoring and quantifying the RTr in the MPTP-parkinsonian monkeys provide an objective, non invasive way to measure the outcome of therapeutic interventions and, further support the concept that loss of dopaminergic innervation contributes to the occurrence of RTr.

2. INTRODUCTION

One of the typical clinical features of Parkinson's disease (PD) is rest tremor (RTr). Humans accidentally exposed to the neurotoxin MPTP develop a parkinsonian syndrome that replicates all the major symptoms of Parkinson's disease (PD), including a characteristic 4-5 Hz frequency RTr (1, 2). Similar to PD RTr, the tremor observed in MPTP-induced parkinsonism is prominent in some patients and absent in others. Furthermore, it is suppressed by treatment with the dopamine precursor L-DOPA. These observations suggest that studies in monkeys with MPTP-induced parkinsonism might help provide greater insights into the pathophysiology of parkinsonian RTr, however consistent induction of this clinical feature in animal models has been elusive.

In experimental monkeys, MPTP produces a syndrome remarkably similar to PD and is widely regarded as the best animal model of the disease (3, 4, 5). In most of these animals, the tremor induced by MPTP has been reported as either kinetic or postural. However, in the African green, macaca fascicularis and old Java monkeys (6, 7, 8) a tremor reminiscent of a parkinsonian RTr has

been observed. This disparity among the various monkey species remains unclear. It has been suggested that neuronal degeneration caused by MPTP administration depends upon a variety of factors such as the monkey species, the route and dosage of MPTP administration and the animal's age (9, 10).

Unilateral intracarotid (ICA) infusion of MPTP produces degeneration of the nigrostriatal pathway that is almost entirely confined to one hemisphere and a hemiparkinsonian syndrome on the side opposite to the lesioned hemisphere (11) that is stable over time (12). In these primed monkeys, additional intravenous injections of MPTP generate asymmetrical bilateral parkinsonism. The severity of the induced parkinsonian syndrome correlates with the level of damage in the substantia nigra (13, 14, 15, 16, 17, 18). Tremor is a prominent feature of this overlesioned parkinsonian model (17). In this report, we further characterize the tremor induced in rhesus monkeys by a combination of intracarotid and systemic administration of MPTP using a tremor monitor. The tremor recoding device is based on a solid state gyroscope, sensitive to repetitive angular motion (19). This novel non-invasive monitor can be easily wrapped to the dorsum of the hand of the subject, has a high signal to noise ratio and the output is unaffected by gravitational forces, providing a reliable tool to quantify RTr.

3. MATERIALS AND METHODS

3.1. Subjects

Three rhesus monkeys (1 male: 4.3 kg; 2 females: 4.2 and 5.2 kg) were single-housed in quarters with a 12 hour light/dark cycle. The animals were fed Purina monkey chow once daily and provided water ad libitum. The study was performed in accordance with federal guidelines of proper animal care and with the approval of the IACUC.

3.2. Drug Administration

3.2.1. MPTP Administration

The monkeys received an infusion (rate of 4 ml/min.) of 60 ml of saline containing 2.5 mg of MPTP-HCl in the left internal carotid artery (ICA; 10, 11, 20), plus 4-6 iv. MPTP-HCl administrations (0.3 mg/kg) in a period of 2 months to induce an advanced bilateral parkinsonian syndrome (13, 14, 15, 16, 17, 18).

3.2.2. L-DOPA Administration

Response to the dopamine precursor L-DOPA was assessed to demonstrate if the parkinsonian features, in particular RTr, responded to dopamine replacement treatment like human PD. The subjects were evaluated in their home cage with a clinical rating scale and personal activity monitors, and in a primate chair (Primate Products, Redwood City, CA) during the tremor recording sessions.

During the course of the study, it became apparent that oral administration of L-DOPA (carbidopa/levodopa (CD/LD, Sinemet) to the subjects in their home cages would be difficult, since it could not be reliably ascertained whether or not they would receive the full dosage. As a result, a 20 mg/kg dose of L-DOPA was administered intramuscularly to approximate the dose of levodopa that would have been received in a 25/250 mg Sinemet tablet (the 20 mg/kg dose of L-DOPA accounts for the levodopa-methyl-ester being comprised of approximately 40% methyl-DOPA and 60% levodopa). Administration of L-DOPA occurred 30 min. after benserazide i.m. administration (2 mg/kg). Each dose of L-DOPA was repeated twice with at least a period of 3 days between testing sessions.

During the tremor recording sessions a dose of 25/250 mg carbidopa/levodopa (CD/LD, Sinemet) was dissolved in 20 ml of orange juice and given orally to the monkeys while they were in the restrainer. Previous to the testing sessions the animals were trained to drink orange juice from a 20 cc. syringe while they were sitting in a primate chair.

3.3. Behavioral Testing

3.3.1. Clinical Rating

A clinical rating scale (PD scale) was utilized to quantify the clinical status of lesioned monkeys using a previously validated measure (15, 16, 17, 18, 21, 22). All the ratings were performed by two trained independent raters blind to the treatment conditions. The scale consists of ratings of tremor (0-3 for each arm), posture (0-2), gait (0-5), bradykinesia (0-5), balance (0-2), gross motor skills (0-4 for each arm), defense reaction (0-2) and freezing (0-2). Occurrence of dyskinesias, psychological disturbances and vomiting was also recorded. The score was obtained as the sum of the features. Out of a total of 32 points, 0 corresponds to normal scoring and 32 to extreme severe disability. A score of more than 10 is related to bilateral parkinsonism. Subjects were assessed with the PD scale prior to MPTP-HCl ICA infusion and thereafter rated weekly. Based on this information, the monkeys were given additional doses of MPTP (4-6 doses) to create a bilateral stable model. Baseline scores were then established and defined as stable PD scores, if the sum was at least 10 points in each weekly rating, during the 6 weeks following the last MPTP iv. administration. During administration of L-DOPA, the monkeys were rated before and 60 minutes after the L-DOPA injection.

3.3.2. Activity Monitoring

Each monkey was tranquilized with ketamine (15 mg/kg, i.m.) and fitted with a primate vest that contained a PAM2 activity monitor (IM Systems, Baltimore, MD) (22) in the inside back pocket. These monitors measure acceleration. Every time a monitor senses an acceleration that exceeds a threshold of 0.1 G, an electrical pulse is generated and recorded. Thus, each pulse represents 234 msec. of acceleration above the 0.1 G threshold. The number of pulses is expressed for a preselected time period (1 min.). After one week period, the animals were again tranquilized with ketamine (15 mg/kg, im.), the jacket was removed, the activity monitor was interfaced with a Macintosh computer and the data was downloaded. The data was expressed as the mean of each 12-hour light/dark cycle, before MPTP administration, approximately 6 weeks after the last iv. administration of MPTP, and during the L-DOPA sessions. L-DOPA activity response was expressed

as a period of 30 minutes, from 45 min to 75 min post L-DOPA administration.

3.3.3. Tremor Recording

The animals were trained to be removed from their cages and placed on a primate chair by using a pole and collar handling system (Primate Products, Redwood City, CA) (23). The monkeys were trained to remain restrained in a primate chair for 120 minutes. The method used to objectively record tremor was based on a solid state gyroscope, sensitive to repetitive angular motion. This novel non-invasive monitor has a high signal to noise ratio and the output is unaffected by gravitational forces.

During a regular testing session, the angular rate sensor (weight 56 g) from a tremor monitoring device (Motus I movement monitor, Motus Bioengineering, Benicia, CA) (19) was mounted on the dorsum of the hand contralateral to the side of the MPTP ICA infusion and secured by an elastic bandage. The opposite arm was immobilized temporarily. The sampling time for each tremor recording was 12 seconds. The tremor monitor performed a fast Fourier transform yielding a frequency power spectrum and calculated "Q" (mean frequency/width of frequency band at 50% maximum) representing the constancy of frequency (the higher the Q, the more constant the frequency). The tremor amplitude was expressed as degrees/seconds/root-hertz (d/s/rtz) units. During the tremor recording, a dose of 25/250 mg carbidopa/levodopa (CD/LD, Sinemet) was dissolved in 20 ml of orange juice and given orally to the monkeys while in the restrainer. Tremor recordings were made at 15 min intervals beginning 45 min before and ending 45 min following the administration of CD/LD or until the amplitude dropped to a value under 1.5 d/s/rtz and the tremor was not evident to the observer.

4. RESULTS

4.1. MPTP-induced Parkinsonism

Prior to MPTP administration the young normal animals score 0 on the PD scale, and manifested high general activity measured by personal monitors (mean ± S.E: 27.34 ±1.7). Intracarotid infusion followed by iv. infusions of MPTP-HCl induced a stable bilateral parkinsonian syndrome characterized by bradykinesia, freezing, stooped posture and tremor. The use of the arm contralateral to the MPTP lesion was severely decreased in the three animals (gross motor skills score: 3-4). The total scoring of these parkinsonian features for the three animals was of 13.9±2.2. The observed hypokinesia measured with general activity monitors recorded a significant decrease for the day period (7.7±1.3; ANOVA (2,4) $F=17.33$. $P<0.01$; Post hoc Bonferroni/Dunn $P<0.013$) .

4.2. Clinical Effects of L-DOPA

Administration of 20 mg/kg im. of L-DOPA significantly improved the parkinsonian features as indicated by a decrease of 33.53% in the PD score (9.24± 3.45; t (1, 4)= 3.4;$P<0.0271$) and an increase in the general activity of 487% from the MPTP baseline (45.2±6.56; ANOVA (2,4) $F=17.33$. $P<0.01$; Post hoc Bonferroni/Dunn $P<0.0006$). Gait, bradykinesia and tremor improved in the three animals, while balance, defense reaction and freezing showed differences between individuals. The asymmetric dopaminergic impairment was evident with the presence of circling contralateral to the side of the ICA MPTP infusion after the L-DOPA injection.

4.3. Tremor Monitoring

As described previously, tremor in the upper limbs was observed clinically in all the animals (17). While in the home cage, the animals displayed tremor while walking and reaching for treats. This postural tremor was most frequent on the side ipsilateral to the site of MPTP ICA administration. Episodes of head tremor during rest were observed in all monkeys. A continuous tremor was seen in the arm contralateral to MPTP-HCl ICA administration while the animal was at rest, therefore RTr measurements were obtained in that arm. In the primate chair, the monkey's arms were held in a resting position with the tremor monitor wrapped to the dorsum of the hand contralateral to the ICA injection. The baseline rest tremor recorded in these animals had a mean peak frequency of 7.9 hertz (SE: 0.12), a mean maximum amplitude of 5.1 d/s/rtz (SE: 0.69) and mean Q of 9.5 (SE: 0.17) (table 1, figure 1).

Following administration of CD/LD, the tremor amplitude gradually decreased (table 1) in two of the animals (A94/7 and A94/11), while in the third (A94/6), the amplitude showed a temporary increase at 15 and 30 minutes before decreasing. Overall, there was a substantial reduction in tremor amplitude (80.4%) by the time the last measurement was taken post-CD/LD administration (table 1; figure 1).

5. DISCUSSION AND CONCLUSION

Rest tremor is considered to be a characteristic feature of PD, although it is not present in all patients. In MPTP- treated monkeys the existence of RTr is a controversial issue. Nearly all monkeys with MPTP-induced parkinsonism have been reported to exhibit a postural or kinetic tremor (2, 10). Although RTr has been noted in a few animals (6, 7, 8), it is unclear why RTr has not been observed in most animals. Perhaps the fact that these animals are rarely at rest makes such an observation unlikely or maybe the individual variations observed in the models are more similar to what happens to humans exposed to environmental toxins than researchers expect. Also, the monkey's unique physical characteristics (i.e.: relative arm length, body posture, hand and finger position) could influence the character of the tremor; the typical "pill rolling" tremor exhibited in many patients with PD may not be observed in the monkeys because they lack the ability to oppose the thumb. Interestingly, the type of tremor observed seems to vary among species. Whereas squirrel monkeys administered systemic MPTP manifest an action tremor, the African green monkeys develop a tremor more reminiscent of parkinsonian RTr (1, 2, 6). Bergmann and colleagues (8) have even referred to MPTP-induced RTr in vervet, and the lack of it in rhesus monkeys as the tremulous and non tremolous variants of PD.

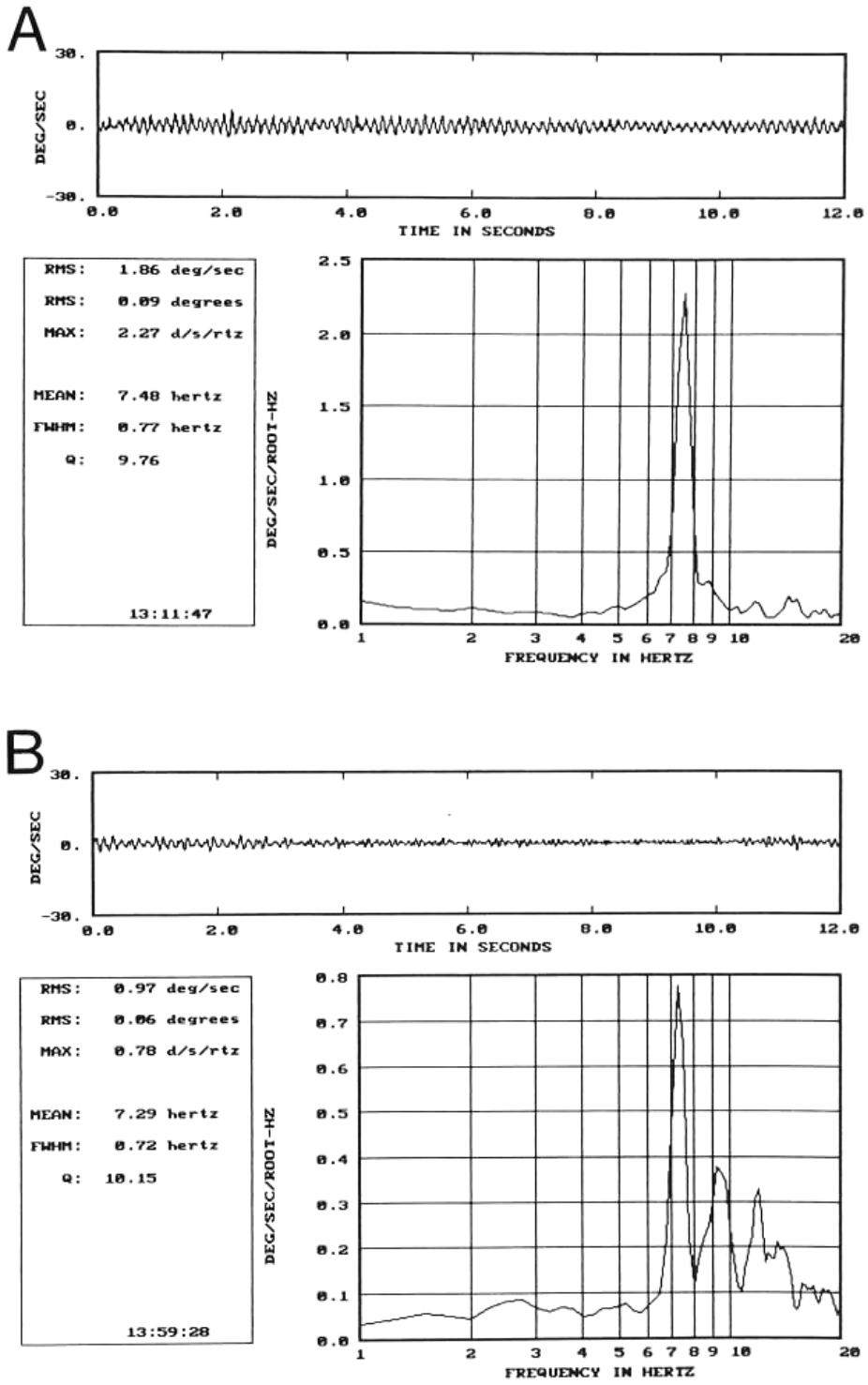

Figure 1. Tremor recording of the monkey A94/6 on the hand contralateral to the ICA MPTP infusion measured by the angular rate sensor of a tremor monitor device. **A.** Baseline recording; **B.** Recording at 45 min. after oral administration of 25/250 mg carbidopa/levodopa (CD/LD, Sinemet). Note that the frequency expressed as MEAN remains between 7-8 hertz (htz), while the maximum amplitud expressed as MAX decrease after CD/LD administration. Abbreviations: RMS, root mean square; MAX, peak amplitude; d/s/rtz, degrees/seconds/root hertz; MEAN, mean peak frequency; FWHM: frequency width at half maximum; Q, mean peak frequency/FWHM.

Table 1. Resting tremor amplitude before and after oral L-DOPA administration measured by the angular rate sensor of a tremor monitor device

ANIMAL #	TREMOR PARAMETERS	Pre CD/LD	Post CD/LD		
		(Mean+ S.E.)	15 (min.)	30 (min.)	45 (min.)
A94/6	Amplitude (d/s/rtz)	2.4+0.2	5.4	5.6	0.8
	Frequency (Htz.)	7.4±0.1	8.1	8	7.3
	Q	8.9±0.5	6.9	7.5	10.2
A94/7	Amplitude (d/s/rtz)	2.5±0.3	2	1.4	1.8
	Frequency (Htz.)	8.7±0.1	8.5	8.7	8.3
	Q	9.5±2.2	7	5.3	6.9
A94/11	Amplitude (d/s/rtz)	9.0±1.6	5.8	0.4	
	Frequency (Htz.)	7.8±0.2	7.7	7.5	
	Q	10.7±1.2	7.5	4.9	
TOTAL	Amplitude (d/s/rtz)	5.1±1.2	4.4+1.2	2.4+1.6	1.3+0.5
(Mean+ S.E.)	Frequency (Htz.)	7.9±0.2	8.1+0.2	8.1+0.3	7.8+0.5
	Q	9.5±0.8	7.1+0.2	5.9+.8	8.5+1.6

Abbreviations: CD/LD: carbidopa/levodopa; d/s/rtz : degrees/seconds/root-hertz; Q: mean peak frequency/FWHM (Frequency Width at Half Maximum).

The method of MPTP administration affects the characteristics of the PD model. For many years our groups have used the unilateral ICA infusion of MPTP to produce a stable hemiparkinsonian syndrome (11, 12). When these primed monkeys receive additional intravenous injections of MPTP, an asymmetrical bilateral parkinsonism can be observed, in which tremor is a prominent feature (13, 14, 15, 16, 17, 18). The unilateral ICA MPTP infusion along with sequential systemic doses of MPTP produces near complete degeneration of the nigrostriatal pathway on the side of infusion and partial damage on the contralateral side. In this severe model of PD, RTr has been associated with nigrostriatal dysfunction manifested by a decline of [^{18}F] 6-fluoro-L-m-tyrosine uptake by positron emission tomography (17) and a decrease in the number of dopaminergic nigral neurons (18). In the current study, 3 rhesus monkeys that received the combined ICA plus iv. MPTP injections developed features of PD that were responsive to L-DOPA treatment. In these animals RTr was recorded and measured using a novel tremor device, which confirmed that RTr can be induced in rhesus monkey by combined administration of ICA and iv. MPTP. Further, similar to PD RTr , the RTr observed in these overlesioned animals was also suppressed by L-DOPA administration. Transient increase of tremor amplitude in one monkey following L-DOPA administration may have been a result of heightened excitability (30).

It can be argued that a limitation of this report is the small number of animals used. Previous reports about RTr in non human primates exposed to MPTP have also used limited number of animals. For example, Gomez-Mancilla and colleagues (7) reported their findings in only one old Java monkey. In our study, like other experiments that require acclimation to a restrainer, the multiple labor intensive training sessions to a primate chair before performing the tremor recording limited our ability to use larger number of subjects. However, the similarity of the syndrome induced by ICA and iv. MPTP (as observed with the clinical rating scores and personal activity monitors data) between the cases described in this study and our previous reports (13, 14, 15, 16, 17, 18) indicate that RTr can be induced in rhesus monkeys by MPTP administration and its occurrence is related to the severe loss of dopaminergic nigrostriatal function.

Although the tremor in these animals closely resembled parkinsonian RTr, the mean peak tremor frequency of 7.9 Hz (range 7.2 to 8.9 Hz) was higher than that typically seen in human PD patients (range 4 to 6 Hz), but similar to that observed in African green monkeys (4 to 8 Hz) (24). This disparity could be due to a lighter limb or other anatomical differences, but might also be the result of a slightly higher discharge rate in the pallidal and/or thalamic circuitry that likely plays a role in the production of tremor (25, 26, 27). In this regard, Fillon and Tremblay (28) studying the electrophysiology of tremor in rhesus monkeys rendered parkinsonian by iv. administration of MPTP, found spontaneous discharges of GPi to be 91.5 to 95 spikes/sec, while the discharge rate of these neurons in humans has been reported to be 58.52 spikes/sec (29).

The method used to record tremor in this study was based on a solid state gyroscope (SSG) (19) that appears to have distinct advantages over electromyography (EMG), accelerometry or mechanographic methods. The SSG is very sensitive to repetitive angular motion and has a high signal to noise ratio compared to accelerometers. In contrast to accelerometers, the output is unaffected by gravitational forces, a potential source of artifact. Further, it is a non invasive device that could easily be used by investigators interested on reliably quantifying RTr. One potentially problematic feature of the SSG used in this study is the weight (56 grams). While we do not rule out the possibility that this weight could have influenced tremor frequency, our results seem to be in agreement with those of other investigators.

In conclusion , the combination of intracarotid and systemic administration of MPTP in rhesus monkeys induces the characteristic clinical features found in humans with PD, including a typical parkinsonian rest tremor as measured with an objective non invasive recording device. Thus, the MPTP-induced model of PD in species such as

the Rhesus and the African green monkey may provide valuable insights into the pathophysiology of parkinsonian rest tremor.

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