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Recovery from experimental Parkinson's disease in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride treated marmoset with the melatonin analogue ML-23

Gregory L. Willis*, Alan D. Robertson[1]

The Bronowski Institute of Behavioural Neuroscience, Coliban Medical Centre, 19 Jennings Street, Kyneton, Victoria 3444, Australia

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Abstract

A new mechanism has been recently proposed, whereby melatonin may participate in the ongoing process of neuronal degeneration in models of neurodegenerative disorders, such as Parkinson's disease (PD). Antagonism of the melatonin receptor in rats using constant light or pinealectomy induced recovery and reduced the mortality typically associated with dopamine (DA) degeneration. In additional studies, employing ML-23 in the 6-OHDA-treated rat, remission from experimental PD was achieved using this drug in a post 6-OHDA treatment regime. To permit the further assessment of ML-23 as a potential clinical candidate for the treatment of PD, the present study was undertaken to determine the efficacy of ML-23 in the 1-methyl-4-phenyl, 1,2,3,6 tetrahydropyridine (MPTP) model in the common marmoset. ML-23 was administered orally in a dose of 3 mg/kg twice daily to half of the animals, while the other half received vehicle only, in a blinded protocol, for 56 days. The effects of the treatment on positive and negative features of MPTP-induced PD were assessed, including horizontal and vertical movement, head checking, general behaviour and Parkinsonian condition, raisin board performance, the ability to remove a foot label, palatable and dry food intake, water consumption, bradykinaesia, and the positive symptoms of tremor, obstinate progression, and agitation. On all parameters, ML-23 produced a significant remission from MPTP-induced Parkinsonism, and this effect did not abate when ML-23 treatment was withdrawn. In a further pilot study involving a crossover of two animals, one animal treated previously with MPTP plus vehicle showed some remission of negative and positive features, although ML-23 treatment was not commenced until 8 weeks post-MPTP. Conversely, a recurrence of Parkinsonian signs was not observed when ML-23 treatment was withdrawn and substituted with oral vehicle. Dopamine transporter was severely impaired in all marmosets treated with ML-23 or vehicle for the duration of the study. These results suggest that a novel mechanism involving melatonin is involved in the primary aetiology of the chronic aspects of PD, and such a mechanism is not related to the antioxidative function of this hormone. From these preliminary results, it is concluded that ML-23 and other melatonin analogues have an important role to play in the treatment and clinical management of Parkinson's disease.

Keywords: Melatonin antagonism; Parkinson's disease; Melatonin; Dopamine; MPTP

1. Introduction

There has been a resurgence of interest in the function of the pineal gland in the aetiology of neuropsychiatric disease (DeButte et al., 2002; Reiter, 1998; Reiter et al., 1999; Srinivasan, 2002). More specifically, melatonin deficiency in advancing age is postulated to enhance oxidative stress, thereby facilitating the degenerative process of dopamine (DA) neurone death and facilitating the development of disorders such as Parkinson's disease (PD; Reiter et al., 1999). It is for this reason that the administration of melatonin, and other antioxidants, is regarded as being potentially useful in retarding the insidious advancement of this and other neurological disorders (Antolin et al., 2002; Jin et al., 1998; Mayo et al., 1998; Maurizi, 1995).

* Corresponding author. Tel.: +61 3 51 221 412; fax: +61 3 54 226 830.
E-mail address: gwillbro@nex.net.au (G.L. Willis).
[1] Formerly AMRAD Pty. Ltd.

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However, in a series of preliminary studies designed to test the effect of the pineal hormone melatonin on various models of PD, an unexpected phenomenon was observed (Willis and Armstrong, 1999). Slow infusion of melatonin into the CNS, via a slow release pellet, exacerbated many of the symptoms of experimental PD in the 6-OHDA-treated rat, while decreasing the bioavailability of melatonin using pinealectomy (PX) or constant light (LT) enhanced recovery. The results were consistent with experimental evidence describing bradykinaesia after the central or peripheral administration of melatonin or pineal extracts in rats (Reis et al., 1963; Minneman et al., 1976; Bradbury et al., 1985; Chuang and Lin, 1994; Araghi-Nikham et al., 1999; Arushanyan and Ovanesov, 1989; Rodriguez et al., 1984; Willis and Armstrong, 1999; Burton et al., 1991). Further confirmation of this hypothesis is derived from experiments reporting the remission of Parkinsonian symptoms, including bradykinaesia, rigidity, insomnia, and depression after exposure to bright light in man (Artemenko and Levin, 1996; Willis and McLennan, 2001). In additional studies, the melatonin analogue ML-23 was delivered by systemic injection for 3.5 days after 6-OHDA treatment, and an almost complete recovery of behavioural and regulatory function was observed. The usual mortality of 40–50% resulting from the acute effects of DA degeneration was reduced to zero, thereby protracting the life span of DA-deficient rats (Willis and Robertson, submitted for publication). It was concluded from this collective work that melatonin might play a deleterious role in the protracted sequelae of DA-based disease and that some analogues with antagonistic properties may therefore be effective in the treatment and management of PD. While ML-23 was originally selected on the basis of its proposed function as a melatonin receptor antagonist (Anis and Zisapel, 1991; Zisapel and Laudon, 1987), this position has been challenged (Buzzell et al., 1990; Chong et al., 1993; Sugden, 1992). Subsequent work has described it as a partial agonist (Iuvone and Gan, 1994), but in this capacity, it may also function to antagonize the melatonin receptor (Nonno et al., 1999). In addition, in the recent suggestion that more effective drugs for the aged should include those which prolong life (Reiter, 1998), ML-23 has been reported to share this feature with melatonin (Oaknin-Bendahan et al., 1995), and on this basis, it thereby serves as a potential candidate for further examination. The current study was undertaken to confirm the results of these earlier findings using the 1-methyl-4-phenyl,1,2,3,6 tetrahydropyridine (MPTP)-treated marmoset model of PD. On this basis, ML-23 was administered twice daily for 8 weeks (3 mg/kg; orally) to determine if the same effect observed in the 6-OHDA rat model of PD could be reproduced in a higher species, more closely approximating the clinical syndrome. This would shed light on the potential therapeutic utility of ML-23 and of other melatonin analogues in the aetiology and treatment of PD and would aid in better defining the role of the pineal gland in DA-based neuropsychiatric disease.

2. Materials and methods

Six adult male common marmosets ranging in weight from 330 to 480 g at the time of acquisition were used for the study. They were obtained from Monash University Animal Services, Churchill, Victoria, Australia. These animals were obtained, and experimentation was undertaken with the expressed approval of the Animal Experimentation Ethics Committee of The Bronowski Institute of Behavioural Neuroscience. Marmosets were housed individually in wire mesh cages 0.600 (w)×0.600 (d)×2.00 m (h), in pairs of adjacent cages and situated in a room such that visual contact by each pair with the two other pairs was possible. As marmosets are "vertical movers", the cages were specially designed for this purpose and fitted with logs to facilitate vertical movement and provide environmental enrichment for the duration of the study.

3. Injections

3.1. MPTP injections

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-hydrochloride (MPTP) was obtained from Sigma–Aldrich Chemicals and mixed in isotonic saline at a concentration of 2 mg/ml. Solutions were prepared daily for a period of 5 days. Each animal received a daily injection of up to 2 mg/kg (S.C.) beneath the skin on the back to achieve a final dose of between 8 and 10 mg/kg, delivered over a period of 5 days. The dose injected has to be adjusted daily, as idiosyncratic reaction to MPTP injection is not uncommon. While some animals show a very mild response, the same dose can cause death in others. Therefore, the injection regime has to be individualized to insure survival (Smith et al., 2000). This is accomplished by slightly reducing the subsequent injections in the regime to minimize life-threatening reactions. Nevertheless, a minimal total dose of 8 mg/kg of body weight (maximum=10 mg) must be administered over the five injections to insure that an adequate DA depletion and Parkinsonian state is achieved. This approach is in keeping with the method employed by Smith et al. (1997). As shown later in the results section, not only was the acute response to MPTP (Table 1) and reduced DA transporter (DAT; Fig. 9) similar between the two groups, but the total dose of MPTP administered to each group was the same. This method insures a close biochemical and clinical approximation to PD but minimizes trauma to each animal and insures their survival.

For a period of about 1–2 h after MPTP injection, all animals were observed for acute reactions to the drug, and their responses were recorded. The physiological and behavioural responses observed are described in Table 1. This was undertaken to insure that all MPTP-treated animals reacted acutely with a similar degree of impairment in response to the neurotoxic injection.

Table 1
The effect of five daily MPTP injections on several physiological and behavioural parameters (The final dose achieved per animal is 8–10 mg/kg in the Common Marmoset)

	PUPIL DIAL	DOCILITY	AGG/ HYPER	BRADYKIN	SLEEP/ DROW	MUSC RIG B	MUSC RIG HL	MUSC TWITCH	UNCO/ FALL	TREMOR/ BDY	TREMOR/ HD	TAIL CURL	BREATH/ LAB	DYSTONIA	EYE CLOSURE	CHEWING	SHIVERING	PENILE EREC	OTHER
Day 1																			
762	*	*	*	*	*	*		*											
16	*	*		*															
78				*															
92				*															
40	*			*															
76	*	*																	
Day 2																			
762	*				*					*		*	*		*				
16				*	*							*		*	*				
78				*								*	*		*				
92				*	*							*	*		*	*			
40				*								*					*		
76				*								*					*		
Day 3																			
762			*		*										*				
16								*	*			*							
78			*	*		*		*			*								
92				*									*						
40			*		*		*	*				*	*					*	
76			*		*		*	*									*		
Day 4																			
762					*	*	*					*	*						
16					*	*	*					*	*						
78					*	*	*					*	*						
92					*	*	*					*	*				*		
40						*	*					*	*				*		
76						*	*					*	*				*		
Day 5																			
762					*	*						*	*						
16					*	*						*	*						
78						*						*	*						
92					*	*						*	*				*		
40						*							*				*		
76						*						*	*						

PUPIL DIAL=pupil dialation; AGG/HYPER=aggitation/hyperactivity; BRADYKIN=bradykinaesia; SLEEP/DROW=sleepiness/drowsiness; MUSC RIG B=muscular rigity of the body; MUSC RIG HL=muscular rigidity of the head and limbs; MUSC TWITCH=muscular twitching; UNCO/FALL=uncoordination/falling; TREMOR/BDY=tremor of the body; TREMOR/HD=tremor of the head; BREATH/LAB=laboured breathing; PENILE EREC=penile erection.
The asterisks (*) indicate those animals that displayed those features during the 1- to 1.5-h period after each daily S.C. MPTP injection.

Immediately following the first MPTP injection, vertical dividers were placed in the cages of each animal to prevent falls that might result in severe injury. These dividers were left in place until about the fifth week post MPTP injection. Blankets were placed on the surface of the dividers to prevent body heat loss known to occur after MPTP treatment.

3.2. ML-23 and vehicle administration

The melatonin analogue ML-23 was prepared at a final concentration of 3 mg/ml. The dose of ML-23 employed and the times of injection was chosen on the basis of previous work (Oaknin-Bendahan et al., 1995; Merle et al., 2000; Willis and Robertson, submitted for publication). The powder was first dissolved in dimethylsulfoxide (DMSO) and then brought up to volume with soy bean oil for oral administration. The final concentration was delivered in a volume of 1 ml/kg. Control solution was prepared using yellow food coloring (Tarterazine), with the final solution color matched to the ML-23 solution. The experimenters undertook the study in a single blind design and were not aware of which of the two solutions contained vehicle or ML-23. The solutions were identified only as "solution A" or "solution B". Solutions were administered via gavage onto the back of the tongue twice daily, with a specially modified syringe to avoid trauma to the mucosa. The assignment of animals to drug groups and solutions to animals was done by two persons outside the lab not having access to information regarding the condition of any of the marmosets prior to the commencement of the study. In respect to the age and body weight of the marmosets assigned to each group, both factors were equally represented between the two groups. Drug administration was commenced 1 day after MPTP treatment was concluded and was continued until day 55. The implementation of a crossover study required that one marmoset that received solution A in the first study receive solution B for 2 weeks after the first study was completed. Conversely, one marmoset that received solution B during the main study received solution A during the 2 weeks of crossover.

3.2.1. Artificial feeding

Within 2 days after MPTP administration, artificial feeding was necessary, as all marmosets became severely aphagic and adipsic. Although they were given ad libitum access to specially formulated pellets, fresh fruits, vegetables and water, they had to be force fed daily to maintain their body weight and to ensure survival. Assisted feedings consisted of a highly nutritious solution made from egg white, sucrose, infant formula, banana, banana essence, multivitamins, powdered marmoset pellets and water. Each animal received 6–12 ml of this solution twice daily. Water or a commercially available vitamin fortified black current drink was administered twice daily in a volume of 6–12 ml per feed, with all animals receiving a similar volume of food per day. Feeding was continued until day 42 post-MPTP, by which time all animals were capable of maintaining food and water intake on their own. At the conclusion of the study, the identity of the solutions was revealed, with solution A containing ML-23 and solution B containing vehicle and Tarterazine.

On day 13 post-MPTP, one marmoset was lost from the ML-23-treated group due to an undiagnosed condition prior to entry into the study. A postmortem examination indicated that the death was not related to treatment.

4. Dependent variables

4.1. Behavioural measures

4.1.1. Vertical and horizontal movement

For the assessment of vertical and horizontal movement, a wire cage similar in size to the home cage was constructed and fitted with laminated safety glass in the top and side panels. It stood 1.2 m in height and had two wooden perches fitted 400 and 800 mm off the floor. Video cameras were fitted approximately 1 m from the top and side glass surfaces, with leads connecting the side video camera to a VCR, and leads connecting each camera to a computer that permited recording and evaluation in an isolated, adjacent laboratory.

Computer software was constructed to permit the recording of the number of beams broken during each session by cameras overlooking the activity cage. The number of vertical and horizontal movements during each designated test period were recorded.

Each animal was placed in the apparatus and left to habituate for 3 to 5 min after the experimenter left the room. The clock was then started and the animal was observed for a 30-min period. Activity measurements were made on days 2, 4, and 8 prior to MPTP administration and days 4, 9, 10(N), 39, 45, 47(N), 52, 54(N), 59, 61(N), 66, and 68(N) post-MPTP. The days followed by (N) indicate that the measurement was made at night under low intensity (<50 lux) red light.

4.1.2. Food and water intake

Dry marmoset pellets (Monash Animal Services) and fresh fruits and vegetables (palatable) were made available ad libitum from dishes attached to the front of the cage throughout the study, but the voluntary intake of palatable food and pellets was measured daily at approximately 900–1000 h, commencing at day 43 post-MPTP for five consecutive days. The daily food intake was measured from this time because this is when all assisted feeding ceased, as MPTP injected animals were able to maintain themselves from this time onward. Water was measured daily from calibrated plastic cylinders attached to the front of each cage.

4.1.3. Raisin board test

A Perspex board (300×150 mm) with 10 indentations confined to one area on the board were outlined in blue

marking pen to enhance the visualization of the area containing the raisins when viewed by the overhead camera. Each marmoset was placed in the experimental chamber for a period of 10 min immediately after the 30-min activity session. The time to mount the board, time to consume each raisin piece, the total number of raisins consumed, and time to dismount the board were recorded from the adjacent laboratory. If all raisins were not consumed, the board was removed from the cage after 10 min and that time was recorded as the time required to remove and consume each of the remaining raisins. This test was similar to that reported previously for use in *Macaca fasicularis* (Willis et al., 1987). This test was performed on days 4, 9, 39, 45, 52, 59, and 66 post-MPTP.

4.1.4. Head checking

Head position was estimated for a period of 2 min and the position recorded at 2-s intervals, giving a total number of 60 position readings during each session. This test was run immediately following the raisin board test with top and side view cameras providing views for this test from the adjacent laboratory. The head position was recorded using the beat of a metronome to signal the moment of evaluation (Annett et al., 1994). If a clear view of the head was obscured during this test, then recording was suspended until the head position became unobscured. The number of changes in head position during each 120-s session was calculated from these records. This parameter was measured during test sessions held on days 4, 9, 39, 45, 52, 59, and 66 after MPTP.

4.1.5. Label removal test

Consistent with previous reports (Annett et al., 1994) to determine the severity of sensory-motor deficits, a 15×50 mm self-adhesive label was wrapped around each foot between the ball of the foot and the heel. The sticky surfaces of the label were joined at the ends, and the fit was snug, but not too tight. Natural cutaneous oils were wiped from the foot prior to placing the label to avoid premature detachment of the label. After applying the labels to both feet, the animal was returned to the experimental chamber for 10 min. The time required to commence removal and time required to remove each label and successful removals were recorded. The method of removal (biting, scratching, etc.) was also recorded. If the animal did not attempt to remove or did not remove the label within 10 min, then that score was assigned for each phase of the task. This parameter was measured on days 4, 9, 39, 45, 52, 59, and 66 after MPTP.

4.1.6. Clinical assessment scale

Clinical assessment was undertaken in line with the parameters described previously (Treseder et al., 2000). Various measures of motor function, behavioural status and physiological state were assigned values based on normality (0) and severe Parkinsonism (25) (possible total minimum and maximum scores). Assessment was based on observations made during daily handling and routine laboratory inspections and were recorded during experimental sessions. Parkinsonian and normal behaviour were judged on a 24+ point scale, which required the assessment of episodes of freezing, tremor, checking, eye movement, oral movement, head twitches, wet dog shakes, balance, posture, vocalization, alertness/reactivity, and obstinate progression/escape. This parameter was measured on days 4, 9, 39,45, 52, 59, 61(N), 66, and 68(N) post-MPTP. The occurrence of positive symptoms was assessed on the basis of data taken from this evaluation and from the data expressed in Table 2.

4.1.7. General behavioural assessment scale

Each animal was rated on several behavioural parameters at 3-min intervals during the course of several 30-min sessions in the experimental chamber (giving 10 ratings per session). The behaviours generally ranged from normal behaviour [climbing (+2), jumping (+2), bark chewing and stripping (+2), grooming/scratching (+2), playing (+2), hiding (+2), checking (+2), and looking (+1)], to intermediate behaviour [i.e., could be regarded as slightly Parkinsonian or normal and was rated as stationary (0), or Parkinsonian features including freezing (−2), tremor (−2), and obstinate progression/escape (−2)]. This point system was based on the principle that each rating represents degrees of severity for each behaviour exhibited and is expressed in the parenthesis next to each behaviour with normal behaviours given a plus rating, intermediate behaviour given a rating of zero, and Parkinsonian features rated with a minus score. The location in the cage and body position was also recorded. General behaviour rating scales were done on days 47(N), 52, 54(N), 59, 61(N), 66, and 68(N) after MPTP administration.

After the completion of the study, a method for double blind assessment of the severity Parkinsonism in MPTP plus ML-23 versus MPTP plus vehicle was undertaken. Ten video clips, 10 to 20 s in length, were selected to illustrate normal versus Parkinsonian behaviour from the library of tapes collected during the course of the study. One assessor was an experienced scientific researcher involved in the assessment procedure during the course of the formal study. The second assessor had no scientific background and was unaware of the object of the experiment. The assessors viewed representative clips of a normal marmoset and of a marmoset displaying severe Parkinsonism. The assessors were given the task of independently rating the 10 video clips on a scale of 1 to 10 with normal=0 and severe PD=10. No communication between assessors was permitted during the assessment. At the end of the assessment, the naive assessor was debriefed, and the results were discussed and then statistically analyzed.

Table 2
The effect of ML-23 (3 mg/kg BD O*) on the obstinate progression syndrome, tremor, and agitation of MPTP-induced PD in marmosets

Date	Day	Obstinate progression syndrome						Tremor/agitation					
		762*	16	78*	92	40*	76	762*	16	78*	92	40*	76
1/10	08	BV											
2/10	09	B		BV									
3/10	10	2BO											
4/10	11	O											
5/10	12	2B		B				A					
6/10	13	–						–					A
7/10	14	–		2B				–	T		T		AT
8/10	15	–		3B	BOI			–				AT	A
9/10	16	–						–	T		T		A
10/10	17	–						–				AT	A
11/10	18	–	B	B				–					
12/10	19	–	2B					–					
13/10	20	–	2B	B				–					
14/10	21	–	2B					–					
15/10	22	–	2B					–					
16/10	23	–	2B		O	O		–			T		
17/10	24	–	2B					–					
18/10	25	–	2B					–	T		T	T	
19/10	26	–	B					–					
20/10	27	–	BO		O		O	–			T		
21/10	28	–	B		O			–					
22/10	29	–	3B		BI			–					
23/10	30	–	B					–					
24/10	31	–						–	T		T		T
25/10	32	–	BO					–	T		T		T
26/10	33	–						–	T		T		
27/10	34	–						–					
28/10	35	–			O			–	T		T		
29/10	36	–						–					
30/10	37	–						–					
31/10	38	–						–	T		T		
01/11	39	–	I°		O			–	T		T		
02/11	40	–	BO		BO	O		–					
03/11	41	–	B		3O			–					
04/11	42	–	2B		O			–					
05/11	43	–						–					
06/11	44	–	B					–					
07/11	45	–	B		O			–	T		T		
08/11	46	–						–					
09/11	47	–	O		O		O	–	T		T		T
10/11	48	–						–					
11/11	49	–			O			–					
12/11	50	–						–					
13/11	51	–						–					
14/11	52	–	O		O			–	T		T		
15/11	53	–						–					
16/11	54	–	O		O			–					
17/11	55	–						–					

B=stage 1: rustling of bedding; O=stage 2: progressive and forward, forceful movement/escape; I=injury resulting from stage 2 behaviour; V=vomiting; (°)=injury first noticed on this date, but was an old scar.
Any code preceded by a number indicates the frequency of occurrence during any given day.
(–) Indicates death due to causes unrelated to any treatment imposed during the experiment.
A=agitation; T=tremor; day=no. of days post MPTP treatment.
* (3 mg/kg BD O).

4.1.8. Assessment of positive feature of MPTP induced PD

During the post-MPTP period, several changes in behaviour were noted during the daily exposure to the animals during routine care and formal observations. Many exhibited a bizarre syndrome characterized in the less excessive form as pronounced agitation and frantic activity with flailing of the arms and legs. The occurrence of this stage was indirectly detected by ruffled bedding in the home

cage (score=1). The more excessive form of this was characterized by positioning their bodies into a corner of the cage and pushing relentlessly with their head against the cage. This included prolonged periods of scratching and biting at the cage and flailing the upper arms along the glass. This would persist for 1 to 5 min (score=2). In the most severe stage, some animals showed relentless forward progression with resulting injury to the head or limbs (score=3). For the purpose of scoring these behaviours on a daily basis, the basic components of increasing intensity were scored as indicated with the number in brackets. In addition, agitation (score=1) and tremor (score=2) were often observed during routine handling and observation periods, and these were also assigned the values indicated to permit quantification. The frequency of occurrence of these features was recorded on a daily basis and are a common component of DA degeneration and PD after MPTP administration in the marmoset (Smith et al., 2000).

4.1.9. Crossover study

At the completion of the study on day 56 post MPTP treatment, two animals were used in a further pilot study implementing a crossover design. One marmoset, maintained in the first study on solution A (ML-23) and presenting with few Parkinsonian features, was administered vehicle orally for an additional 14 days. The adjacent marmoset, maintained on Solution B (vehicle) for the first 56 days post-MPTP and displaying the most severe Parkinsonian features with no remission, was administered oral ML-23 for 14 days. Each parameter described earlier was monitored at the times indicated during the additional 2 weeks of observation: Horizontal and Vertical Movement, days 59, 61(N), 66, and 68(N); Raisin Board test, days 59 and 66; Adhesive Label Test, days 59 and 66; Clinical Assessment Scale, days 59, 61(N), 66, and 68(N); Head Checking, days 59 and 61; and General Behavioural Assessment Scale on days 59, 61(N), 66, and 68(N) post-MPTP. Positive Parkinsonian features and food and water intake were monitored each day for the duration of the crossover study up to day 68 post-MPTP.

4.2. Design and analysis

As noted in the section describing the independent variables explored in this study, a repeated measures design was employed. This design permits the generation of multiple data points per treatment group over several test sessions and is commonly used in drug efficacy testing (Smith et al., 2000). Testing all animals at time points close to and then distant from the time of neurotoxin administration would provide a basis of comparison to determine if, and to what degree, spontaneous recovery might be wrongly attributed to an observed therapeutic effect. Each animal in the ML-23 or vehicle-treated groups was measured on several occasions during the course of the "acute phase" (days 5–10 post-MPTP), the "long-term phase " (days 39–56 post-MPTP), or during the crossover of treatments (days 57–68). These time periods were also chosen to elucidate the effects of ML-23 treatment on the process of degeneration per se (acute) versus the effects of the drug on the consequences of progressive DA loss (long term), which more closely approximates the clinical syndrome of PD. In this regard, any progressive improvement observed with chronic administration of the drug might suggest a therapeutic effect, removed from the acute degenerative effects characterizing the models of PD. The time of drug administration commenced 1 day after the completion of the MPTP regime to minimize the possibility of interference with the primary neurotoxic action of MPTP by ML-23.

The parametric statistics used for analysis of the data collected during the main study included independent *t*-tests, ANOVA with Tukey's HSD for multiple comparison, and the Pearson correlation. Nonparametric analysis was performed using the Mann–Whitney test. ANOVA with Tukey's HSD was used for analysis of data for the crossover study. Microsoft Excel and SPSS for Windows were used for statistical analysis. The confidence levels were chosen pre-hoc and set at a minimum of 5% to depict a minimal significant effect, while confidence levels ranging from 0.06 to 0.09 depicted a significant trend.

4.2.1. DA transporter (DAT) histochemical analysis

Briefly, the method of DAT histochemistry involved euthanasing the marmosets with an overdose of sodium pentobarbitone (0.35 mg/g) and perfusing each animal with 30 ml of heparinised phosphate buffered saline, followed by paraformaldehyde, followed by picric acid. Brains were removed from the cranium and then placed in 30% sucrose for at least 12 h. DA terminals in the caudate were labeled in the caudate putamen (CPU) so that stereological counts could be made and the density of DA terminals could be ascertained. This was achieved by sectioning the brain at 50 μm and then mounting the sections onto chrome/alum/gelatinized slides. After fixing to the slides, sections were incubated with primary and then secondary anti-DAT serums. A series of washed slides were then dehydrated with graded alcohol and then cover slipped. The density of the DA terminals in the CPU was estimated using a fractionator sampling design and a grid program utilizing a Leica DML microscope fitted with a Micro bright-field stereo investigator. Photographs were taken of representative areas for comparison in standard controls. For a more detailed description of the technique employed, refer to Parish et al. (2002).

5. Results

Table 1 illustrates the acute effects of MPTP administration (⩽2 h post injection). As all marmosets received similar doses in line with earlier reports (Jenner et al., 1984; Smith et al., 2000), all animals showed similar behavioural

and physiological responses after each of the five injections, during the five consecutive days of drug administration. The most commonly observed features were bradykinaesia, hypoactivity, drowsiness, muscular rigidity, twitching, tail curling, labored breathing, eye closure, and shivering. On the basis of these observations, it is clear that all animals were affected, more or less, equally by the injection regimen.

Fig. 1 illustrates vertical (left) and horizontal movements (right) during the control period prior to MPTP treatment and during the acute and long-term phases of the study. During the control measurements, horizontal movement was similar for both groups, as an average of about 400 beams were broken during each 30-min test. During the acute period, 5–10 days after MPTP treatment, vertical movement decreased to about 30% of control performance, and this was similar between the ML-23 and vehicle-treated animals, and they were not significantly different (Mann–Whitney test; N=15; p=1.0). In the long-term phase, marmosets maintained on oral ML-23 became more active compared to those maintained on vehicle, and this was significant (Mann–Whitney test; N=25; p=.02).

Similarly, the performance of marmosets in either group on horizontal movement during their pre-MPTP period was similar. The response of both groups during the acute phase after MPTP treatment was about 20% of their control performance, but they were not significantly different from each other at this time (Mann–Whitney test; N=15; p=1.0). Evaluation of movement performed during the long-term phase revealed that the two groups were differentially affected. Long-term exposure to the drug into the long-term phase revealed an improvement in their performance, with a significant trend toward increased horizontal movement in animals treated with ML-23 compared to those receiving oral vehicle (Mann–Whitney test; N=26; p=.08). An additional comparison was made between both groups for the last 3 days of the long-term phase for this parameter (end phase). Testing at this time revealed that animals treated with ML-23 engaged in more horizontal movement than did those treated with vehicle, and the difference was significant (Mann–Whitney test; N=15; p=.04).

When food and water consumption was measured for the 10-day period immediately following the cessation of artificial feeding, differences in both parameters were observed (Fig. 2; left trace). Dry food intake was significantly increased for animals treated with ML-23 compared to that of those treated with vehicle (independent t-test; t=3.36; df=3; p<.05). Conversely, there was a significant trend for vehicle-injected marmosets to drink more compared to those treated with ML-23, and this may well represent enhanced prandial drinking (independent t-test; t=−2.49; df=3; p<.1).

The number of raisin consumed during the raisin test (Fig. 2; right trace) was similar between the ML-23- and vehicle-treated marmosets prior to MPTP injection during control session measurement. During the acute phase of testing, the number of raisins eaten by animals treated with ML-23 was similar to their control performance, but this was significantly greater than the number eaten by those treated with vehicle (Mann–Whitney test; N=10; p=.05). During the long-term phase, the number of raisins eaten by both groups was not significantly different (Mann–Whitney test; N=15; p=.858).

Fig. 3 (left traces) depicts the time required to eat 10 raisins, and ANOVA revealed a significant main effect (ANOVA; df=2,3; F=30.3, p<.01); Tukey's HSD for Multiple Comparisons revealed that ML-23 control versus

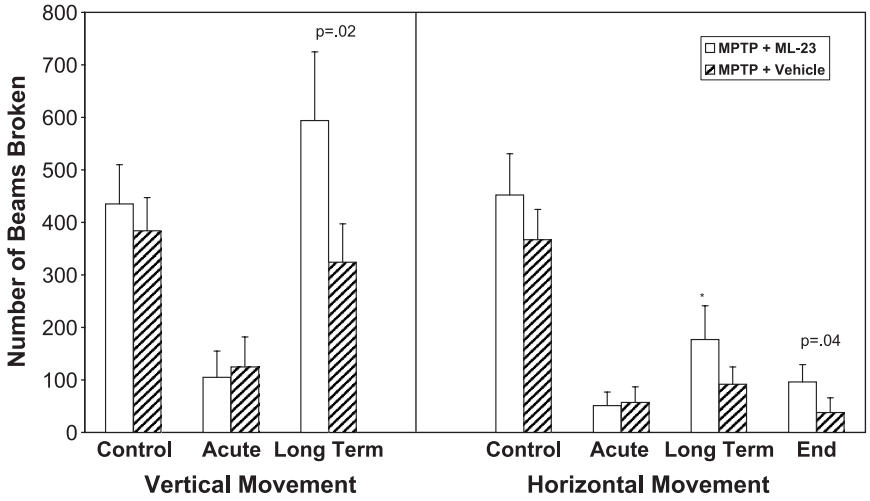

Fig. 1. The effect of ML-23 treatment on vertical (left) and horizontal (right) movement in marmosets with experimental PD. Each bar represents the mean of several measurements taken 1–7 days prior to MPTP treatment (control), 5–10 days (acute phase) post-MPTP, or 39–54 days (long-term phase) after MPTP. An additional comparison for horizontal movement was performed for the last 3 days of the long-term phase (End). The open bars represent the performance of marmosets treated twice daily with oral 3 mg/kg ML-23 (twice daily) after MPTP, while the diagonal bars represent the performance of animals receiving oral vehicle delivered twice daily. The statistical significance achieved is expressed above each comparison. The asterisk indicates that a significant trend was detected. The T-bars represent the standard error of the mean.

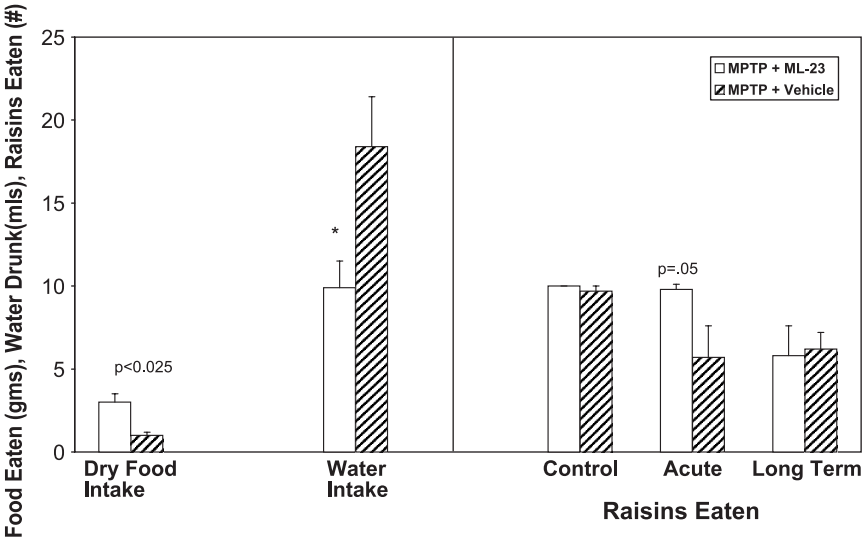

Fig. 2. The effect of ML-23 treatment on dry food and water intake (left trace) and the number of raisins eaten during a 10-min test (right trace) in MPTP-treated marmosets. The food and water intake values depicted represent the mean daily intake occurring over a 10-day period after tube feeding was discontinued on day 42 post-MPTP. The open bars represent the mean of animals treated with oral ML-23 (3 mg/kg), while the diagonal bars represent those marmosets treated with 3 ml/kg of oral vehicle. Statistical comparisons were performed, and the achieved level of significance is expressed above each comparison. The asterisk indicates that a significant trend was detected. The T-bars represent the standard error of the mean.

vehicle control were not significantly different. However, during the acute phase of testing, the time required to eat 10 raisins remains similar to that during control performance for the ML-23-treated animals, while those treated with vehicle were significantly impaired. In the long-term phase, the time required to eat each of 10 raisins was not significantly different between the two groups.

Fig. 3 (right traces) also depicts the latency to remove a sticky label from the foot in ML-23 versus vehicle-treated marmosets during the acute versus the long-term phase after MPTP. ANOVA revealed a main effect (df=1, 3; F=12.276, p<.05), with Tukey's HSD revealing a significant difference between the ML-23-treated animals during the acute phase compared to those receiving vehicle. While both groups showed further improvement during long-term phase testing, they were not significantly different at this time.

Fig. 4 illustrates the mean number of changes in head positioning (checking) during a 2-min test at various times during the course of the study. During the acute and chronic phases of testing, animals treated with ML-23 were not significantly different from vehicle-treated marmosets (ANOVA, df=3, 3; F=5.557, p>.05).

In Fig. 5, the severity of Parkinsonian features, based on the clinical rating scale, was judged to be almost three

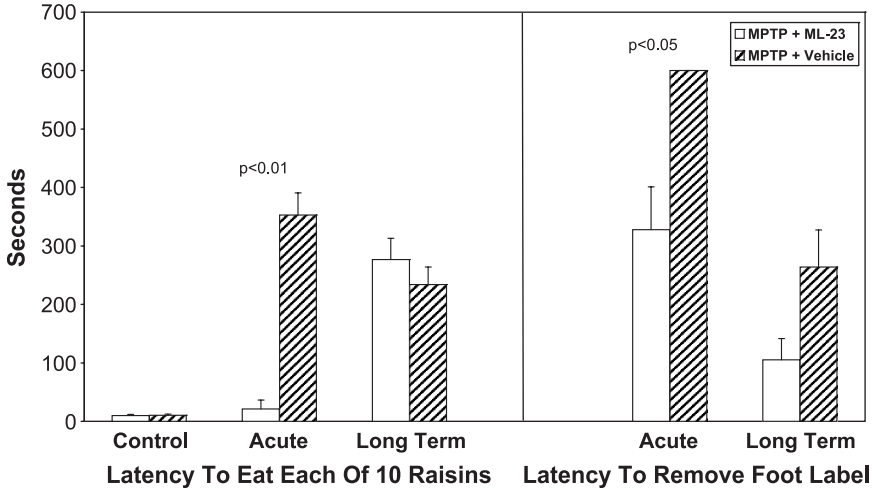

Fig. 3. The mean latency to consume 10 raisins (left) or to remove a sticky foot label (right) in a series of sessions in the test cage, assessed during the 7 days prior to MPTP (control) or 5–10 (acute phase) or 39–54 days (long-term phase) after MPTP treatment. The open bars represent the mean of animals treated with oral ML-23 at 3 mg/kg, while the diagonal bars represent those treated with 1 ml/kg of oral vehicle. Statistical comparisons were made between ML-23 and vehicle groups at the times designated, with the level of significance expressed above each comparison. The T-bars represent the standard error of the mean.

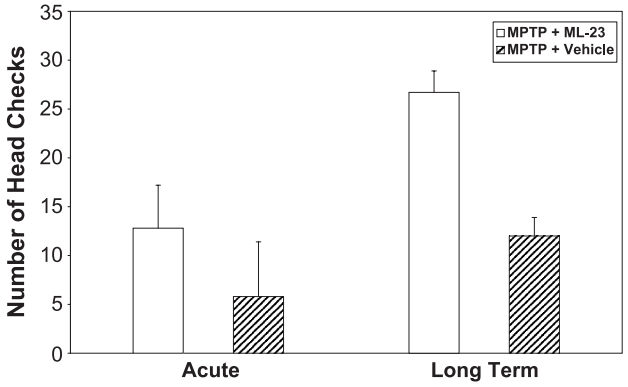

Fig. 4. The mean number of head checks during several 2-min test sessions in the experimental chamber assessed during the acute (5–10 days) and the long-term phases (39–54 days) following MPTP treatment. The open bars represent the mean of animals treated with oral ML-23 at the dose of 3 mg/kg, while the diagonal bars represent those treated with 1 ml/kg of oral vehicle. Statistical comparisons were made between the means of each group for ML-23 and vehicle at the times designated. The T-bars represent the standard error of the mean.

times more severe in the vehicle-treated animals when compared to those treated with ML-23 for the acute phase extending for days 5–10 days after MPTP treatment. Analysis implementing the Mann–Whitney test revealed a significant trend for reduced activity in reduced Parkinsonian features for the ML-23-treated group when compared to those treated with vehicle (N=5; p=.08). While some improvement was observed in tests carried out for both groups during the long-term phase, the ML-23-treated animals were near normal in their behaviour, while those injected with vehicle remained significantly Parkinsonian (Mann–Whitney test; N=15; p=.01).

When the mean number of Parkinsonian features displayed by each marmoset, when applying the behavioural assessment scale, is expressed for all post-MPTP times from 5 days post-MPTP to day 56 (Fig. 6, left trace) marmosets treated with ML-23 displayed fewer Parkinsonian features than did those treated with vehicle (independent t-test; t=7.6; df=3; p<.025). Similarly, when the number of positive Parkinsonian features for the course of the study is separately represented in Fig. 6 (right trace), there is a significant trend for these to occur more frequently in marmosets treated with vehicle than in those treated with ML-23 (independent t-test; t=−2.4; df=3; p<.1). The occurrence of positive Parkinsonian features occurring for the duration of the study embraces a broader period of observation and is expressed in Table 2. These signs occurred about four times more frequently for the vehicle-treated animals than for those treated with ML-23 for the entire 8-week period of observation. The occurrence of self-induced injury is a commonly encountered even when experimental PD is induced with MATP in marmosets. This occurred in two of the three animals treated with vehicle but was not observed in any of the marmosets maintained on ML-23.

Interrater reliability for the assessment of Parkinsonian versus normal behaviour undertaken during the course of the study was found to correlate highly between the two raters (Pearson correlation: ρ=0.990; N=20; p=.01).

5.1. Crossover study

The effect of ML-23 treatment on vertical movement in the severely impaired animal treated previously with vehicle

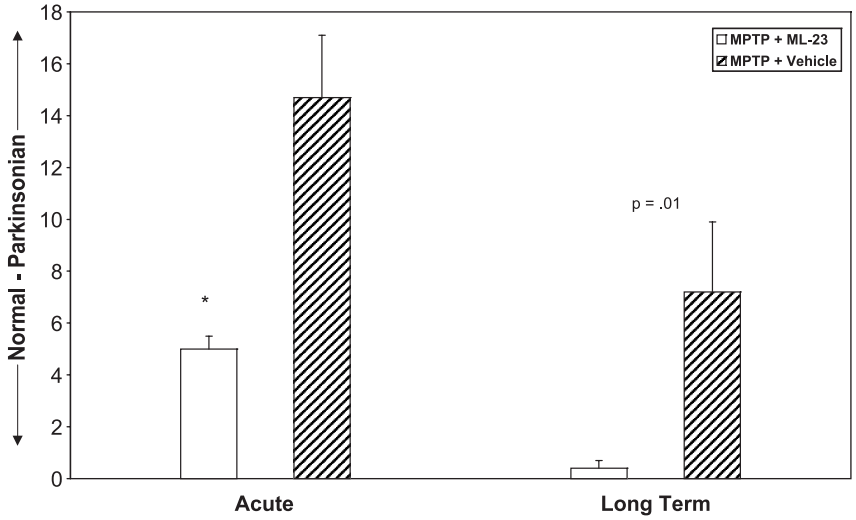

Fig. 5. The mean number of Parkinsonian features versus normal behaviours exhibited by MPTP-treated marmosets treated with oral ML-23 at 3 mg/kg (open bars) compared to those treated with 1 ml/kg of vehicle (diagonal bars). All animals were observed daily for several days during the acute (5–10 days) and the long-term phases (39–54 days) following MPTP treatment. Assessment was based on observations made during daily handling, routine laboratory inspections, and experimental sessions. Parkinsonian features were in keeping with the clinical assessment scale employed, which categorized behaviour on a 24+ point scale and included episodes of freezing, tremor, various movement parameters, balance, posture, vocalization, alertness/reactivity, and obstinate progression/escape. Statistical comparisons were made between the means of each group treated with ML-23 and vehicle at the times designated, with the level of significance expressed above each comparison. The asterisk indicates that a significant trend was detected. The T-bars represent the standard error of the mean.

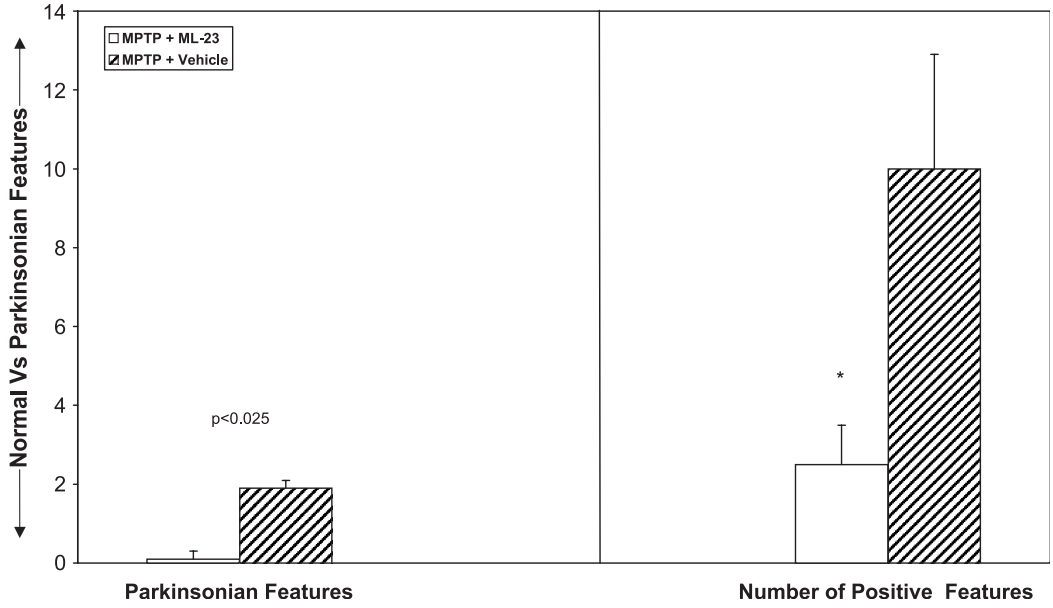

Fig. 6. The Parkinsonian versus normal behaviours displayed during several 30-min periods of observation in the test chamber in MPTP-treated marmosets (left trace). The behavioural assessment scale assessed performance on normal behaviours, including climbing, bark chewing and stripping, grooming, playing, hiding, checking, and sitting, as well as Parkinsonian features, including freezing, tremor, obstinate progression, and time stationary. Behavioural parameters were assessed every 3 min for each 30-min test session. The mean number of positive Parkinsonian features (tremor, agitation, obstinate progression, etc.) displayed each day during a 56-day period of observations following MPTP administration are expressed in the right trace. This assessment was based on observations made during daily handling and routine laboratory inspections (see Table 2). Positive features were assigned the following scores: agitation=1 and tremor=2. In addition, the obstinate progression syndrome observed after MPTP treatment was scored as follows: pronounced agitation and frantic activity with flailing of the arms and legs (1). In the absence of direct observation of this phenomenon, ruffled bedding in the home cage indicated that it had occurred. The more excessive form was characterized by positioning the bodies into a corner of the cage and pushing relentlessly, and this was often accompanied by vomiting. When in the experimental chamber, positive symptoms included prolonged periods of scratching and biting at the cage and flailing the upper arms along the glass and persisted for 1 to 5 min (2). In the most severe stage, this was characterized by obstinate progression, which often induced injury (3). Marmosets treated with oral ML-23 at 3 mg/kg are represented by the open bars, while the diagonal bars represent those treated with 1 ml/kg of vehicle. Statistical comparisons were made by comparing the means for ML-23- versus vehicle-treated marmosets tested on several occasions during the acute (days 5–10) or long-term phase (days 39–54) after MPTP treatment. The level of significance obtained is expressed above each comparison. The asterisk indicates that a significant trend was detected. The T-bars represent the standard error of the mean.

was not dramatically changed by the end of the extended 2 weeks of treatment with ML-23. While any change in this parameter could be regarded as minor, the number of counts recorded on the last day of observation (day 68) was the highest recorded during the extended 2-week period. Similarly, horizontal movement appeared to show slight but delayed improvement on the last of four sessions of observation when compared to the four previous sessions prior to crossing over to ML-23 treatment, but this was not remarkable.

As illustrated in Fig. 7, the consumption of dry food (left trace) before the crossover was significantly greater in the marmoset maintained on ML-23 compared to the one on vehicle (Mann–Whitney test; N=6; p=.01). However, when the marmoset on ML-23 was switched to vehicle and the marmoset on vehicle was switched to ML-23, the latter increased its food intake and their intake was not significantly different after the switch (Mann–Whitney test; N=8; p=.4). Prior to the crossover, the vehicle-injected animal showed depressed water intake compared to the animal maintained on ML-23 (Mann–Whitney test; N=6; p=.02). After the crossover, the water intake of both animals was not significantly different (Mann–Whitney test; N=8; p=.1).

Fig. 8 illustrates that the vehicle-treated animal, prior to the crossover, displayed severe Parkinsonian features for the three proceeding sessions. This was evident during the general behavioural assessment for all 30-min periods of observation from day 47 to the end of the study. When this was compared to this animals' performance after the crossover, when ML-23 was administered, a significant improvement was seen (Mann–Whitney test; N=70; p=.0005). The marmoset maintained on ML-23 showed few Parkinsonian signs prior to the crossover and did not deteriorate when vehicle was substituted for ML-23 (Mann–Whitney test; N=70; p=.19).

As illustrated in Fig. 9, the effect of MPTP treatment on the density of DAT-labeled terminals in the caudate of marmosets treated with MPTP was similar after ML-23 or vehicle treatment. The mean density of the terminals for marmosets treated with MPTP and ML-23 was 1.48×10^{-3} (±0.0004) μm^3, while the mean density of those treated with vehicle was 1.67×10^{-3} (±0.0004) μm^3. The degree of damage for the ML-23 or vehicle-treated groups was not significantly different (ANOVA with Tukey's Multiple Comparisons; df=2,3; F=60.464; p<.01), while both groups

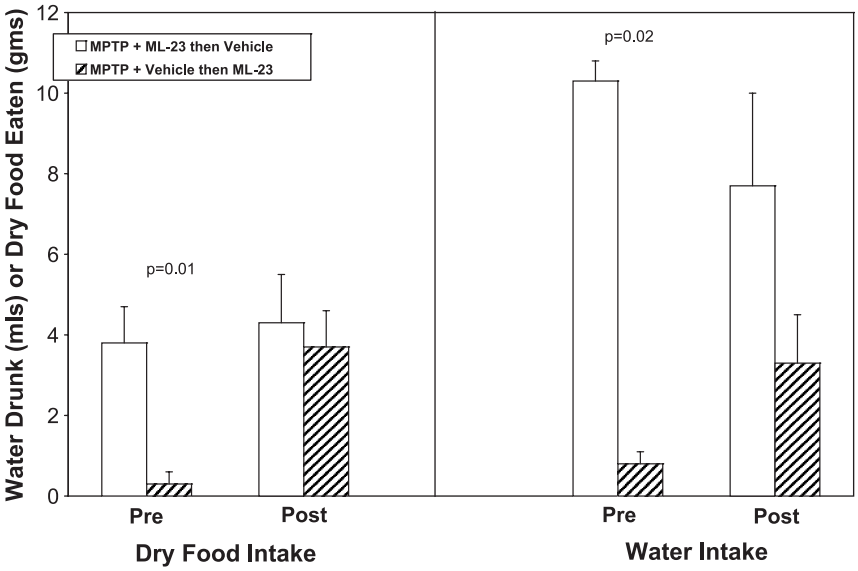

Fig. 7. The mean number of grams of dry food eaten (left trace) and milliliters of water consumed (right trace) for one marmoset crossing over from 56 days of vehicle treatment to 2 weeks of ML-23 treatment (1 ml/kg vehicle twice daily to 3 mg/kg ML-23 twice daily, diagonal bars) compared to that of a marmoset crossing over from 56 days of ML-23 treatment to 2 weeks of treatment with vehicle (3 mg/kg ML-23 twice daily to 1 ml/kg vehicle twice daily, open bars). The mean of several measurements taken before the crossover are label "Pre", while the mean of several measurements taken after the crossover are marked as "Post". The level of significance achieved is expressed above the comparisons, and the asterisk indicates that a significant trend was detected. The T-bars represent the standard error of the mean.

showed a decrease in DA transporter density which was significantly less than that of controls.

Fig. 10 depicts the effects of MPTP treatment on DAT status in the substantia nigra of marmosets treated with vehicle or ML-23. In control animals (plate A), a dense dendritic proliferation is seen to surround normal nigral cells. In one animal treated with vehicle after MPTP (plate B), the background density of dendrites is dramatically decreased, while the remaining cell bodies became swollen, while others in the later stages of degeneration

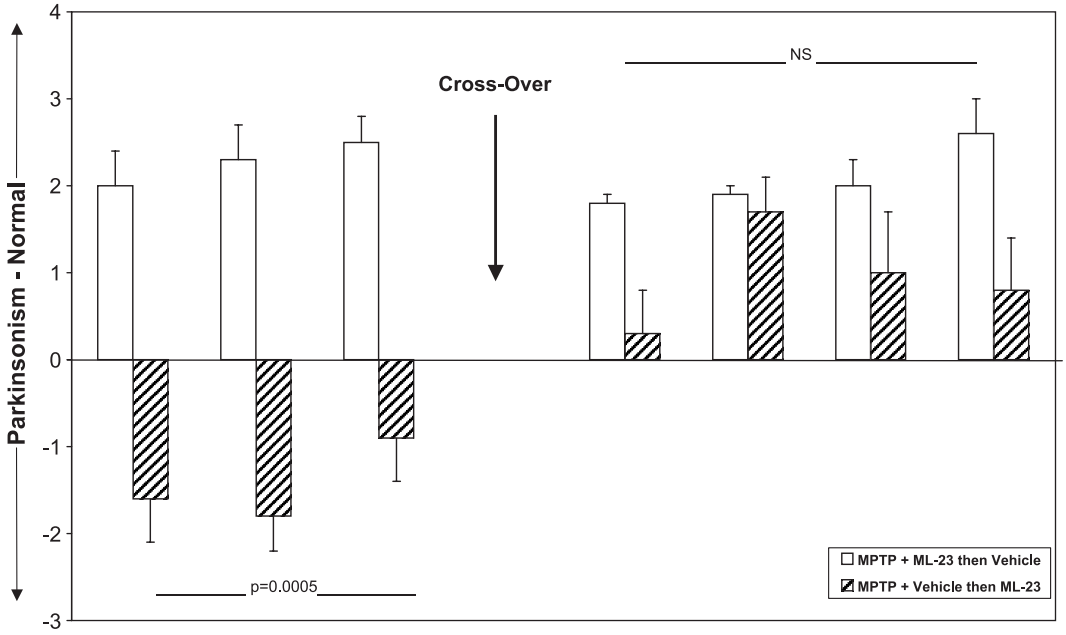

Fig. 8. The mean number of Parkinsonian versus normal behaviours displayed during each 30-min test session for one marmoset crossing over from 56 days of vehicle treatment to 2 weeks of ML-23 treatment (1 ml/kg vehicle twice daily to 3 mg/kg ML-23 twice daily, diagonal bars) compared to that of a marmoset crossing over from 56 days of ML-23 treatment to 2 weeks of treatment with vehicle (3 mg/kg ML-23 twice daily to 1 ml/kg vehicle twice daily, open bars). Statistical comparisons were made between the means for several pre- versus post-crossover measurements taken 3 days before and 4 days after the crossover (n=10 per session). The level of significance achieved is expressed above the comparisons, with NS indicating that the difference between the that groups was not significant. The T-bars represent the standard error of the mean.

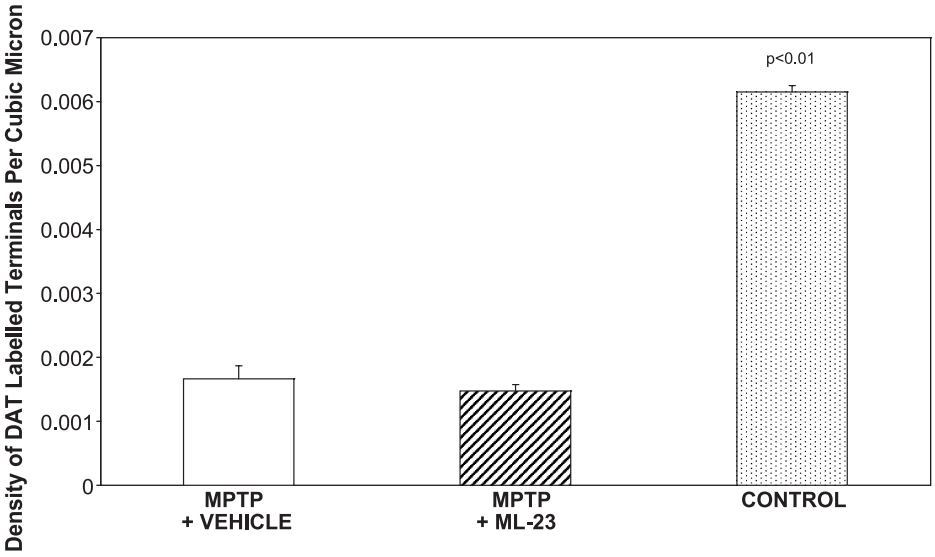

Fig. 9. Dopamine transporter (DAT) terminal density in the caudate putamen in marmosets treatment with oral ML-23 or vehicle following subcutaneous injection of MPTP. The mean density of DAT-labeled terminals was decreased in both groups of marmosets treated with MPTP compared with that of control tissue (stippled bar), but the density was not significantly different in vehicle-treated animals (diagonal bar) compared to those marmosets receiving oral ML-23 (open bar). The level of significance achieved is expressed above the comparisons. Control animals were drug-free, and the T-bars represent the standard error of the mean.

were dramatically reduced in size. In an animal treated with ML-23 after MPTP, the cells disappeared completely, with the dendritic background not visible on this side of the brain.

6. Discussion

These results demonstrate that the melatonin receptor antagonist ML-23 can reverse the bradykinaesia, rigidity and positive features associated with experimental PD in the MPTP-treated marmoset. While our previous work in other species has shown that ML-23 can reverse the negative features of the disease (Willis and Robertson, 2004), there is considerable evidence from the present findings that the negative and positive features (i.e., agitation, tremor, and the obstinate progression syndrome) respond equally well to ML-23. The quite surprising characteristic of this drug is that, unlike other palliative treatments such as DA replacement therapy, when the drug is withdrawn, the Parkinsonian state does not worsen (Nomoto et al., 1998; Smith et al., 1997). The potential of this drug as an anti-Parkinsonian therapy was first discovered in the rat model (Willis and Robertson, 2004), and the present work extends this finding to the marmoset. Given that ML-23 is a melatonin analogue with the activity as a partial agonist (Iuvone and Gan, 1994), it could be argued that ML-23, like melatonin itself, might be acting as an antioxidant, thereby halting the progression of the disease. However, this is unlikely for three reasons. First, ML-23 administration did not commence until 24 h after the MPTP injection regime was complete. The efficacy of melatonin to halt the progressive degeneration depends on the administration of this compound before or during the process of neurotoxin administration (Antolin et al., 2002; Jin et al., 1998; Mayo et al., 1998), and the time chosen for commencing ML-23 administration in the present study was too late for this purpose. Second, results from the crossover study indicate that even in severe, long-term Parkinsonism, long after MPTP induced DA degeneration is complete, ML-23 was effective in reversing some aspects of the experimental syndrome. Third, DAT activity was severely reduced to a similar level in ML-23- and vehicle-treated marmosets. The attenuation of DA degeneration would be predicated in the ML-23-treated group if it was acting as an antioxidant, but this did not occur. Alternatively, in its capacity as a partial agonist, it may be antagonizing the melatonin receptor (Nonno et al., 1999) and this is consistent with other reports demonstrating that treatments that block melatonin receptors or reduce the bioavailability of melatonin can provide relief from various signs and symptoms of PD, in animals and man. Exposure to bright or constant light (Artemenko and Levin, 1996; Willis and Armstrong, 1999; Willis and McLennan, 2001), pinealectomy (Willis and Armstrong, 1998), treatment with ML-23 (Willis and Robertson, 2004), or Atenolol (Barajas Garcia-Talavera et al., 1985; cf. Stotchitzky et al., 1999) have all been shown to be effective in alleviating symptoms of PD. The efficacy of the melatonin receptor antagonist, luzindole, has been reported to improve depression in models of this disease (Dubocovich et al., 1990), which supports the contention that the therapeutic effects of bright light works via reducing melatonin secretion (Rosenthal et al., 1986) and would explain why bright light may be efficacious for treating depression specifically associated with PD (Willis and McLennan, 2001).

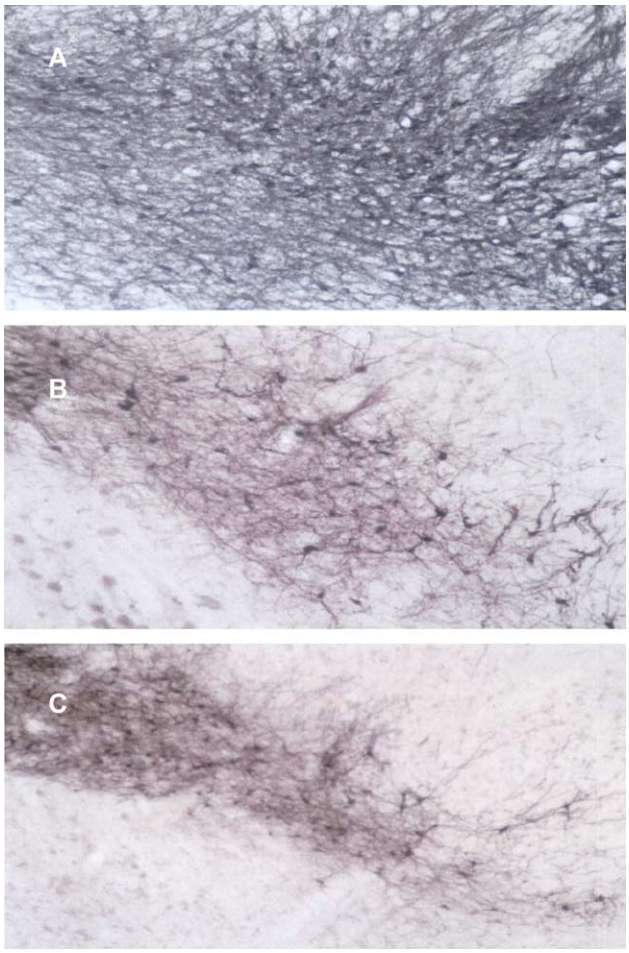

Fig. 10. Dopamine transporter (DAT) density in the substantia nigra of marmosets treated for with oral vehicle or ML-23 after neurotoxic insult with MPTP. Plate A shows normal cell body size and dense dendritic proliferation in the surrounding neuropile in a drug-free control tissue. Plate B shows that many of the cells remaining in the nigra of one marmoset treated with MPTP plus vehicle appear swollen and the fine dendritic elements in the background neuropile are not present as in plate A. The faint outline of many cell bodies also appear in the background neuropile. In plate C, the cell bodies have almost disappeared completely, with only remnants of cell bodies remaining, while the dense dendritic profile seen in plate A is gone. This animal received oral ML-23 after MPTP (magnification ×60).

One possible limitation of the present study is the effect of ML-23 on horizontal movement. Improvement in this parameter was only marginal (about 50% of control performance), and it reached significance at the end of the 8 weeks post-MPTP period compared to those injected with vehicle. With bradykinaesia being a key feature of PD, consideration of this aspect of our finding in vacuo would suggest that ML-23 might have limited application in treating this aspect of PD. However, given the fact that marmosets are "vertical movers", the present findings may have to be considered from a more comparative perspective, and their overall motility may have to be evaluated in a broader context, which typifies this species. In addition, during the evaluation process in the experimental chamber, it was discovered that the animals spent more time on the cage floor after MPTP treatment. From the aspect of the overhead motion detector, which monitors horizontal movement, movement and position were often obscured by the perches when marmosets were on the floor of the experimental chamber (about 40% of the floor area). These limitations should be factored in when considering the impact of the present results. Nevertheless, ML-23 did significantly improved performance on many parameters other than horizontal movement, which collectively support the contention that it does possess anti-Parkinsonian characteristics. The parallel between the protracted time required to improved bradykinaesia using bright light therapy (Willis and McLennan, 2001), and the present findings with horizontal movement would suggest that the therapeutic effect is slow to achieve and requires prolonged exposure to treatments, which antagonize melatonin. These issues are the theme of ongoing research.

While positive features appear to be the most dramatically affected shortly after treatment is commenced (i.e., agitation and tremor), negative symptoms respond slower and may take up to 3 to 4 weeks of treatment before remarkable improvement is seen. This is evidenced from two findings: First, during the initial 3-week period immediately following MPTP treatment, both ML-23- and vehicle-treated animals showed similar bradykinaesia, particularly in respect to horizontal and vertical movements. Improvement in these parameters was not seen until the fifth to eight weeks after ML-23 treatment was commenced. However, in the short term, positive signs diminished well before 2 to 3 weeks after ML-23 therapy was commenced (see Table 2). Similarly, in the crossover study, the severely impaired marmoset, maintained on vehicle up to the time of the crossover, displayed only minimal improvement in horizontal and vertical movement for the extended 2-week period of observation. This was confirmed in the general behavioural assessment and clinical assessment scores as the most remarkable improvement in these parameters was observed toward the end of the extended observation period. However, positive symptoms, including tremor, agitation, and the obstinate progression syndrome, improved shortly after crossover to ML-23 in the animal maintained for 8 weeks on vehicle.

One remarkable observation on the effect of ML-23 on positive symptoms was seen a few minutes after the first oral administration of ML-23. In the later stages of the MPTP injection regime, agitation, aggression, and irritability are often observed, and most of the marmosets displayed these features at some time during or after the injection regimen was completed. On the day of the first administration of ML-23, two of the marmosets in the ML-23 group were particularly agitated and aggressive. Approach or contact by an experimenter would set this reaction into motion, but the animal would continue for several minutes, even if the experimenter moved to a more remote part of the laboratory. Within a few minutes of the

first ML-23 administration, two of the three ML-23-treated animals showing this reaction became docile and did not display this behaviour again for the remainder of the study. The reason for this phenomenon is unclear, but the present study suggests that ML-23 may be more effective at suppressing positive symptoms than reducing melatonin bioavailability using noninvasive methods, such as bright light. This is based on results from clinical studies whereby light therapy exerted its main effect on rigidity, bradykinaesia, insomnia, and depression but had a minimal effect upon tremor (Artemenko and Levin, 1996; Willis and McLennan, 2001).

An alternative explanation as to the antioxidative effect of melatonin and its analogues may pertain to the effect that melatonin exerts on cytoskeletile organization and axoplasmic transport. Given that all neuropsychiatric conditions, including PD, have been characterized by such neuropathological changes (Appel, 1981; Gajdusek, 1985; Goldman and Yen, 1986; Price et al., 1987) and that melatonin and other mitosis inhibitors produce similar changes (Cardinalli and Friere, 1975; Matsui and Machado-Santelli, 1997; Meléndez et al., 1996; Schmid, 1993; Willis and Armstrong, 1999), it is possible that ML-23 might be blocking this effect of melatonin and thereby induce remission. Whether the melatonin (M1) receptor subtype provides direct access to the subcellular compartment and subcellular organization (Bordt et al., 2001) or that melatonin and ML-23 may be acting directly on the cytoskeletile structure as it passes readily through cellular membranes and can gain access to intracellular compartments are two possibilities (Finocchiaro and Glikin, 1998; Melchiorri et al., 1995; Reiter et al., 1999). The present work, in conjunction with recent reports, confirms that melatonin may play a deleterious role in neurones already compromised by the process of DA degeneration, and that its antagonism may enhance recovery from such a condition. From the broader perspective, and taking into account the ubiquitous nature of melatonin, its dwindling functional importance in the aging organism (Reyes, 1982), and that PD and dementia are marked by cytoskeletile and cellular transport pathology, aberrant melatonin function may play a role in the aetiology of neuropsychiatric disease.

The increased intake of dry food in ML-23-treated marmosets is an interesting effect in consideration of the consequences of MPTP, 6-OHDA, and lesions of the lateral hypothalamus (LH) or nigrostriatal (NS) system on food intake in other species. A large body of work describes rodents, ruminants, and primates with lesions of the LH as being "finicky" and becoming prandial drinkers after recovery from these lesions, confirming that the nigrostriatal DA system serves a role in processing the sensory qualities of food (Baile and McLaughlin, 1987; Berridge and Robinson, 1998; Myers and Martin, 1973; Teitelbaum et al., 1969). With water intake occurring mainly in conjunction with food intake, such animals have a preference for palatable foods. The significant increase in water consumption observed in the MPTP+vehicle-treated marmosets may represent increased prandial drinking, which would be predicted in more severely impaired animals. Recovery from debilitating DA lesions can be facilitated if palatable foods are made readily available in the acute phase after MPTP administration. Swallowing is often impaired with impaired salivation and facial/masticatory muscular function, and similar changes are routinely reported in PD. It is remarkable that ML-23-treated marmosets in the present study recovered spontaneous food intake quicker than did those treated with vehicle and consumed more dry food each day. However, the two groups did not consume different amounts of palatable foods. This may be a function of the time of measurement, as all animals were required to reach the criterion of being capable of self-maintenance before food intake assessment commenced. Perhaps, earlier measurement of palatable food ingestion would reveal any differences in this parameter. Further to this, it is interesting to note that ML-23-treated marmosets consumed more raisins quicker during the acute phase of testing, but this did not extend into the long-term phase. Given that all animals could self-regulate food intake in the long term, the higher intake of raisins in the early stages by the ML-23-treated animals may represent enhanced intake of more palatable foods earlier than those treated with vehicle, which is consistent with the enhanced palatability hypothesis.

The differential response of various motor parameters to ML-23 treatment provides an interesting challenge in understanding how to refine methods of treatment for PD and in understanding what systems might be involved. For example, while vertical movement was uniformly better in ML-23-treated animals after several weeks of treatment, horizontal movement remained depressed, even after several weeks of ML-23 administration. Even in the presence of a robust recovery of other behavioural parameters, horizontal movement did not improve with ML-23. This may have been attributable to several factors. First, restricting their movement with a cage divider for 5 weeks after MPTP treatment, to prevent injury, may have reduced their propensity to move. The restriction of this parameter in the home cage could have influenced movement in the test chamber during their exposure during intermittent testing. When the dividers were removed after several weeks, their vertical movement in the test chamber increased only marginally, while horizontal movement did not increase at all. Results from other motor tests employed (i.e., head checking and time required to remove a foot label) were found to be significantly better at all times after MPTP treatment in the ML-23-injected group, indicating that the effect of restricting movement may have exerted its influence mainly on activity measures of locomotion. At best, activity in the open field is a highly variable phenomenon susceptible to extremely high within-group variability. Treatments aimed at increasing motor function in diseases such as PD must take into account a wider range of motor parameters and strive to return motor-based behav-

iour to near normal levels rather than simply exaggerate highly variable, species-specific measures, such as open-field activity. This is a problem with DA replacement therapy, as the typical criterion in preclinical studies is to increase DA levels several fold, with the consequence of exaggerating open-field activity.

While the present results provide strong evidence that ML-23 may be useful in the treatment of most negative and positive features of PD, it seemed to have little effect on the parameter depicting sensory neglect, which often typifies this syndrome (Abbruzzese and Berardell, 2003; Marshall et al., 1976), and which, at least at the preclinical level, typically responds to DA replacement therapy. As revealed by the removal of the label test, marmosets treated with ML-23 regained the motor capacity to remove the label faster than did those treated with vehicle, but the tendency to "sense" the presence of a foreign body on the skin surface was not different from those treated with vehicle. We cannot account for this difference at the present time, other than the possibility that ML-23 may have differential effects on specific features of PD. However, that the ML-23 group increased their intake of dry food during the food and water intake test suggests that ML-23 selectively improved only some of the sensory neglect seen in models of PD (Marshall et al., 1976). That the response to cutaneous stimulation was not improved while oral-facial sensation was enhanced by ML-23 treatment suggests that these drugs may be anatomically or functionally selective in its effect. Work with other analogues may help to define what systems and symptoms may best respond to ML-23 and related analogues.

One of the most interesting aspects of the present finding using marmosets is the effect that ML-23 exerted on the positive features of the disease in this model. In the rat and mouse model of PD, positive features are not accurately manifested, and the efficacy of anti-PD drugs is usually based on the reversal of bradykinaesia and rigidity as depicted mainly by open-field activity shortly after MPTP treatment in these models (Antolin et al., 2002; Donnan et al., 1987; Zuddas et al., 1992). When new candidates enter into the clinical arena, temporary remission of positive and negative symptoms are observed, but this is often short lived, and side effects, including TD, involuntary movement, agitation, and psychosis, commonly occur, and the treatment itself becomes debilitating (Cotzias et al., 1972; Mendis et al., 1999). However, with the demonstrated tendency for ML-23 to possess anti-psychotic features as well, it is conceivable that it may be an advance on DA replacement therapy in this regard. Even as an adjunct therapy, it may serve a valuable function by increasing motor activity, reducing rigidity, and minimizing the side effects of traditional DA replacement therapy.

In conclusion, the findings in the present report confirm previous reports on the anti-Parkinsonian effects of ML-23 in lower species (Willis and Robertson, submitted for publication) and extends the therapeutic effects to include a broader spectrum of symptoms occurring in PD. Furthermore, it is possible that the side effects associated with the treatment of this disorder and caused by DA replacement therapy itself may also be ameliorated with ML-23. On a theoretical basis, the current findings provide support to the contention that PD is a multidimensional disease involving many neurochemical systems within the human brain (Jellinger, 1999) and that novel treatments with therapeutic efficacy do not exclusively involve DA replacement (Eltze, 1999). While the current study requires confirmation in larger numbers of animals, ML-23 shows sufficient promise to warrant further investigation of its potential as an anti-Parkinsonian drug and for other CNS disorders. ML-23 is an interesting compound that may help to elucidate the role of the pineal gland in neuropsychiatric disease and to significantly decrease the morbidity of PD and related disorders (Bruguerolle and Simon, 2002; Ghaemi et al., 2001).

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