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Recovery of experimental Parkinson's disease with the melatonin analogues ML-23 and S-20928 in a chronic, bilateral 6-OHDA model: a new mechanism involving antagonism of the melatonin receptor

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Abstract

Over the past 10 years, there has been a resurgence of interest in examining the role of melatonin in health and disease. While the brunt of research in this area has portrayed melatonin in a favorable light, there is a growing body of evidence suggesting that melatonin may possess adverse effects contributing to the development of various neuropsychiatric disease states. In preclinical models of Parkinson's disease (PD), melatonin has been shown to enhance the severity of this condition while its antagonism, using constant light or pinealectomy, facilitates recovery. To test this hypothesis further, the present study employed the melatonin analogues ML-23 and S-20928 in a post-6-OHDA injection regime to determine whether they may have a favorable effect on the symptoms of this more chronic model of PD. When ML-23 was injected I.P. in a dose of 3 mg/kg twice daily for 3.5 days after 6-OHDA, significant improvement in motor function and regulatory deficits was observed. Similarly, the injection of S-20928 in a 1 mg/kg dose (I.P.), in the same regimen, facilitated modest improvement in motor function and regulatory deficits while the larger dose enhanced the severity of behavioural deficits and produced severe side effects causing deterioration in condition during the course of drug administration. ML-23 administration totally abolished the 6-OHDA-induced mortality, which accompanies dopamine (DA) degeneration, while S-20928 had no effect on this parameter. These results suggest that some melatonin analogues can aid in recovery from DA depleting lesions after DA degeneration has commenced and the recovery is not attributable to the antioxidative properties of this hormone. While the exact mechanism by which ML-23 and S-20928 are exerting their therapeutic effect is unclear, it is possible that antagonism of melatonin receptors may play some role and this should be considered when assessing the potential of melatonin analogues for treatment of human neuropsychiatric disorders.

Keywords: Melatonin; Melatonin receptor antagonists; ML-23; S-20928; Parkinson's disease; Neuropsychiatric disease

1. Introduction

There is a growing interest in the role of the pineal gland and melatonin secretion in neuropsychiatric disorders and, in particular, Parkinson's disease (PD). Current theories have focused on the antioxidative properties of melatonin and many regard this characteristic as the most important in relation to its normal physiological function and in the compromised function of neurodegenerative disease. In this capacity, it is proposed to act as a natural deterrent to oxidative stress underlying neuropsychiatric disorders such as PD (Reiter et al., 1999; Mayo et al., 1998; Jin et al., 1998). The practical outcome of such a hypothesis, however, has major limitations in that dopamine (DA) degeneration has progressed to an advanced stage for several decades before the disease can be diagnosed (Horstink and Morrish, 1999). At this late stage, melatonin, as an antioxidant, would serve little to improve the condition. In fact, there is experimental

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evidence that administration of melatonin in some models of PD is capable of exacerbating some aspects of motor impairment (Willis and Armstrong, 1999; Burton et al., 1991).

In view of this controversy and of reports demonstrating that melatonin has little or no effect in the treatment of PD itself (Shaw et al., 1973; Papavasiliou et al., 1972), other theories as to the role of melatonin in neuropsychiatric disease are emerging. Advocates of melatonin for therapeutic application in PD and other neuropsychiatric disorders fail to address commonly reported findings such bradykinaesia and deterioration of motor performance after the central or peripheral administration of melatonin (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). The implementation of a drug, which causes bradykinaesia, in a disease where this symptom is a cardinal feature, would contraindicate its use in the therapeutic regimen. One possible explanation is that while melatonin may prevent oxidative stress in the early stages of the degenerative process, it may exacerbate various aspects of DA degeneration during the more chronic stages of the disease. This is supported by reports which demonstrate that melatonin reverses experimental PD when administered at the time of neurotoxic insult (Dabheni-Sala et al., 2001), while the antagonism of melatonin, using methods such as constant light and pinealectomy (Artemenko and Levin, 1996; Willis and Armstrong, 1999; Willis and McLennan, 2001b), alleviates the symptoms during the more advanced stages of disease in animals and man. To address this question, the present experiments were undertaken with the aim of validating this concept by determining whether pharmacological antagonism of melatonin, using the melatonin analogues ML-23 (Zisapel and Laudon, 1987) and S-20928 (Guardiola-Lemaître and Delagrange, 1995; Delagrange et al., 2003; Merle et al., 2000), to antagonize the melatonin receptor, could improve experimental PD in a manner similar to that reported after pinealectomy or constant light. While ML-23 was selected on the basis of its described function as a melatonin receptor antagonist in Syrian hamster brain synaptosomes (Anis and Zisapel, 1991; Zisapel and Laudon, 1987), we acknowledge that this has been subsequently challenged (Buzzell et al., 1990; Chong et al., 1993; Sugden, 1992) and that ML-23 may be a partial agonist (Iuvone and Gan, 1994). However, in this capacity, it may also function to antagonize the melatonin receptor (Nonno et al., 1999). Furthermore, given the recent suggestion that the search for 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 this purpose.

2. Method

Fifty-three outbred male, Sprague Dawley rats were obtained from the Bronowski Institute colony or from Monash University Animal Services. Rats were housed individually in wire mesh cages with standard food pellets made available ad libitum from the cage floor. Tap water was made available from bottles attached to the front of each cage. Animals ranged in weight from 250 to 350 g at the commencement of surgery. Room temperature was maintained at 22±2 °C with a 12-h light/dark cycle with lights on at 0700 h. The room was illuminated with two fluorescent tubes with the intensity of light within each cage averaging 250 lx during the lights on phase of the light/dark cycle. All experiments were performed under the auspices of the Animal Experimentation Ethics Committee of the Bronowski Institute of Behavioural Neuroscience implementing protocols conforming to the National Guidelines for the Care and Use of Animals for Scientific Purposes.

2.1. Surgery

After habituation into the colony for at least 7 days, rats were anaesthetized with 89 mg/kg of Alphaxalon and then placed in a stereotaxic instrument. The site of cannulation for intracranial (I.C.) injection for achieving experimental PD was the posterior lateral hypothalamus (Willis and Armstrong, 1998) just rostral to the midbrain diencephalon border in the bundle of nigro-striatal fibres. To achieve experimental Parkinsonism, rats were placed in a stereotaxic instrument whereby 23-gauge stainless steel cannulae were implanted just dorsal to the intended site of injection at the coordinates AP=−1.8 mm, L=±1.8 mm, D=−6.1 mm. The injection needle extended 2 mm beyond the cannula tip in a ventral direction to minimize damage to the injection site (Willis et al., 1976). All coordinates were relative to bregma and in the plane of Pelligrino et al. (1979). This position has been found to be effective in producing severe Parkinsonian-like effects in animals (Fink and Smith, 1979; Willis and Armstrong, 1998, 1999; Willis and Smith, 1985). Rats were allowed at least 10 days of recovery before commencing the formal of each study.

2.2. Solutions and injections

2.2.1. Intracerebral (I.C.) 6-OHDA injections

6-Hydroxydopamine hydrobromide (Sigma, St. Louis, MO, USA) was mixed in a concentration of 8 μg/μl and injected in a volume of 2 μl per site. Injections were made at a rate of 1 μl/min and the needle was left in situ for at least 30 s after each injection was complete to insure that the drug diffused from the end of the needle. 6-OHDA was dissolved in saline ascorbic solution to prevent rapid oxidation of the drug (Willis and Armstrong, 1999; Willis et al., 1976). New solutions of drug were prepared immediately prior to

injection with stock solutions kept refrigerated or on ice until used. All solutions were kept shielded from light and then discarded immediately at the end of each injection session.

2.2.2. Intraperitoneal (I.P.) ML-23, S-20928 or vehicle injections

ML-23 (M-(2,4,dinitrophenyl)-5-methoxytriptamine) and S-20928 were synthesized by AMRAD. ML-23 was weighed then dissolved in approximately 0.5 ml of 10% dimethylsulfoxide (DSMO) then brought to volume to achieve a 3 mg/ml solution with the final volume of injection being 1.5 ml/kg. S-20928 was weighed then dissolved in approximately 0.5 ml of 10% in DMSO then brought to volume to achieve 1.0 and 5.0 mg/ml solutions. The final solutions were injected in a volume of 1 ml/kg. Control injections were made with a 10% DMSO/water mixture. All injections were made via the I.P. route. The doses of ML-23 and S-20928 were determined on the basis of previous work with these compounds (Oaknin-Bendahan et al., 1995; Merle et al., 2000).

For the ML-23 study, there were three groups employed in a balanced paradigm with seven animals randomly assigned to each group post-surgery. The first group was injected with I.C. 6-OHDA followed by I.P. vehicle. The second group was injected with I.C.6-OHDA then ML-23 at 3 mg/kg. The third group served as a control group receiving intracerebral vehicle then I.P. ML-23 in a dose of 3 mg/kg. Implementation of the last group permitted the assessment of the effects of intracerebral injection and of the side effects of ML-23.

In the S-20928 study, four groups were employed in a balanced paradigm with eight animals randomly assigned to each group post-surgery. The first group was injected with I.C. 6-OHDA followed by I.P. vehicle. The second group was injected with I.C. 6-OHDA then S-20928 in a dose of 1 mg/kg. The third group was injected with I.C. 6-OHDA then S-20928 at 5 mg/kg. The fourth group served as a control group receiving I.C. vehicle then I.P. Implementation of the last group served as controls permitting the assessment of the effects of I.C. and I.P. injections.

Seven consecutive I.P. injections of ML-23 or S-20928 were made at 12 h intervals, with the first occurring approximately 12 h (=2100 h) after the intracerebral injection of 6-OHDA (=0900 h). The drug injection regime was selected on the basis of previous research demonstrating that strategic injection of DA receptor antagonists in the same regimen for 3 days post 6-OHDA enhances recovery from experimental PD (Glick and Greenstein, 1974). Since subsequent research has demonstrated that such recovery is not necessarily due to its effect on striatal DA receptors (Willis et al., 1983a), the use of this regime is particularly useful in the present application given that extra-striatal, circumventricular sites such as the pineal may be implicated (cf. Willis and Armstrong, 1998; Willis et al., 1983a; Carlisle and Reynolds, 1961). In addition, it has been demonstrated that melatonin exerts a protective effect on degenerating DA neurons when it is administered before or during neurotoxic treatment. Therefore, implementation of a post-treatment regime in the present study reduces the possibility that injection of ML-23 or S-20928 is interfering with the primary neurotoxic insult induced by 6-OHDA.

2.2.3. Behavioural, regulatory and histological assessment

Control measurements for all motor parameters were made at least 24 h prior to 6-OHDA injection during the light (1000–1500 h) and dark phase (2200–0300 h) of the light/dark cycle. Motor function was assessed during the light and dark phases at two crucial time periods after 6-OHDA injection. The first time of measurement was on or before day 7 post-6-OHDA and is referred to as the "acute phase" of testing. At this time, deficits become increasingly severe until death occurs or spontaneous recovery commences, that is, recovery without the aid of artificial feeding. This permits the assessment of the impact of brain lesions and the therapeutic potential of a treatment on motor function and mortality without confounding the procedure with artificial feeding. The object of testing at this time is to assess the impact of DA degeneration using the optimal number of animals prior to spontaneous death. The second time of measurement was between days 12 and 15 and was termed the "recovery phase" of testing. This period was also chosen on the basis of previous work (Willis and Armstrong, 1999; see Willis and Smith, 1985 for review) and is defined as the time during which recovery from the acute effects of the neurotoxic insult and homeostatic control has returned whereby animals are capable of regulating their nutritive intake on their own and brain lesions are no longer life threatening after this time.

Locomotion and rearing were measured with the aid of a 50×30×20 cm PVC box fitted with infrared sensors. The total number of squares crossed and the number of rearings onto the hind feet during a 10-min test session were measured and recorded with the aid of a microprocessor. A series of three motor reflex tests were performed immediately at the conclusion of the open field test (Willis and Armstrong, 1999). The latency to retract the left and right front limbs when they were elevated 2.5 cm from the table surface, the latency to step up or down from a raised platform when the rear torso was elevated and the latency to ambulate outside of a 4×6 cm rectangle were tested during each test session directly after each animal was removed from the locomotion chamber as described and used previously (Balagura et al., 1969; Willis and Armstrong, 1999). Body weight was measured daily commencing at about 1000 h for at least 9 days prior to and 17 days after 6-OHDA injection. Food and water intake were measured for a 2-h period commencing at 1900 h on day 10 (ML-23 Study) or day 5 (S-20928 Study), post 6-OHDA treatment. Powdered food was made available from dishes designed to

minimize spillage (±1 gm/day) and water was made available from calibrated cylinders attached to the cage front (spillage=±2 ml/day).

Clinical examination of each animal was undertaken during the course of drug administration when animals were handled each day. Parameters including grooming, eye closure, kyphosis, penile erection, general muscle tone and haematuria were assessed on a regular basis in all groups. Each parameter was rated on a four-point scale with 0=not present, 1=slight, 2=moderate and 3=severe. Percent mortality was determined by comparing the total number of spontaneous deaths with the total number of animals for each group.

The performance of rats in the I.C. 6-OHDA plus I.P. vehicle injected groups was compared to that of the I.C. 6-OHDA plus I.P. drug injected groups in each study and subjected to statistical comparison between the means for each of the day and night testing sessions when each drug was tested during the acute and recovery phases of the experiments. In a balanced paradigm, a group of I.C. vehicle plus I.P. drug or vehicle were also run to determine if the therapeutic agent possessed any side effects and to control for the I.C. and I.P. injection procedures (controls). Additional analysis was undertaken at the end of the experiment by combining the locomotion and rearing data from the day sessions for the acute and recovery phases for each respective group in each study. Similarly, the locomotion and rearing data for the night sessions for the acute and recovery phase testing were combined to reveal any overall day/night effects. Statistical analysis employed was a one-way ANOVA with Tukey's HSD for multiple comparisons (SPSS 11.0 for Windows) unless otherwise indicated. The confidence levels were chosen pre hoc and set at 5% to depict a minimal significant effect while confidence levels ranging from .06 to .09 depicted a significant trend.

At the end of the study, all remaining animals were sacrificed with pentobarbitone sodium (325 mg/ml). Each animal was injected with 0.5 ml of the stock solution. The entire brain was removed and placed in 10% formalin. After fixing each brain, they were sectioned and the site of injection was examined bilaterally to permit the identification of the site of I.C. injection. The extent of damage and anatomical position for each injection site was defined in relation to anatomical landmarks and then transcribed onto mapped coronal sections as defined by Pelligrino et al. (1979).

3. Results

As shown in Fig. 1, the I.P. injection of ML-23 for 3 days post 6-OHDA caused significant, long-lasting improvement in body weight regulation commencing on the second day post 6-OHDA and lasting for the duration of the study. At the end of the observation period, rats treated with ML-23 showed a 35 g increase in body weight over those treated with 6-OHDA+vehicle. A one-way ANOVA revealed a significant treatment effect (df=3,540; F=21.08; p=.0001). Tukey's HSD for multiple comparisons revealed no significant difference between the control group and 6-OHDA+ML-23 injected group (p=.83). However, the 6-OHDA+vehicle injected group showed a significant decrease in body weight compared to both groups as revealed by this post hoc comparison (p=.0001 in both cases).

As illustrated in Fig. 2, animals injected with 1 or 5 mg/kg of S-20928 or vehicle showed a significant drop in body

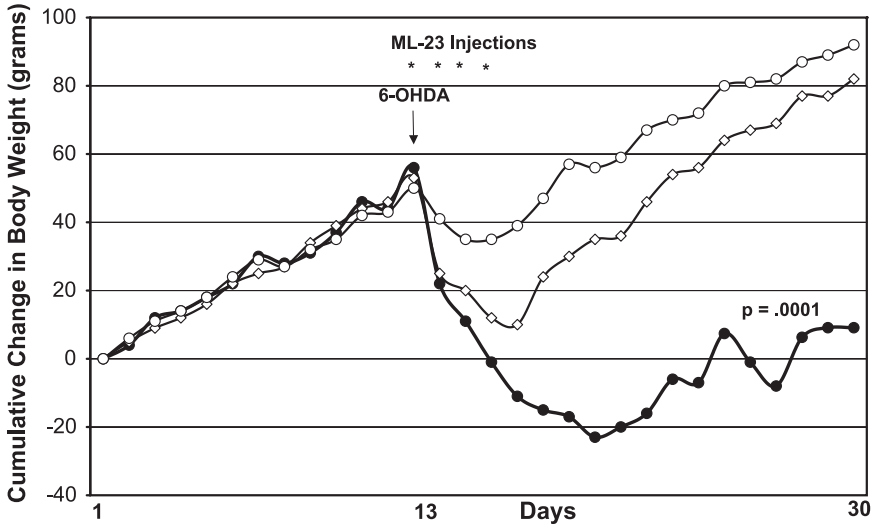

Fig. 1. The effect of 3 mg/kg of ML-23 on 6-Hydroxydopamine (6-OHDA) induced experimental PD: the mean cumulative change in body weight. The open circles represent the mean daily body weight of rats receiving I.C. injection of vehicle followed by I.P. injection of ML-23 (3 mg/kg). Open diamonds represent I.C. 6-OHDA injections followed by I.P. injection of ML-23, while the closed circles represent animals injected I.C. with 6-OHDA followed by I.P. vehicle. I.C. injections were made on the morning of day 13 and this is marked with an arrow. I.P. injections were made on the 4 days marked with an asterisk with the first in the P.M. 12 h after 6-OHDA injection on day 13. The p value shown depicts the level of significance for the comparison between the group injected with 6-OHDA plus ML-23 compared to rats injected with 6-OHDA plus vehicle.

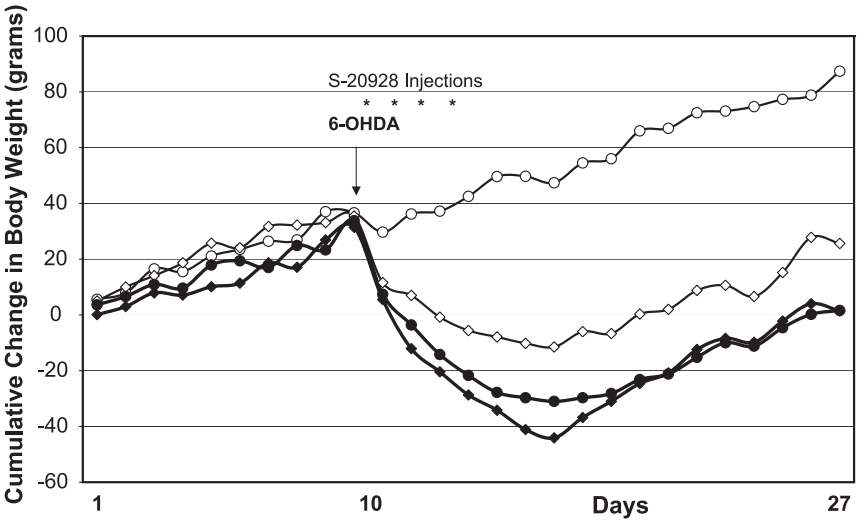

Fig. 2. The effect of 1 or 5 mg/kg of S-20928 on 6-Hydroxydopamine (6-OHDA) induced experimental PD: the mean cumulative change in body weight. The open circles represent the mean daily body weight of rats receiving I.C. injection of vehicle followed by I.P. injection of vehicle (1 ml/kg). Open diamonds represent I.C. 6-OHDA followed by I.P. S-20928 (1 mg/kg) while closed diamonds represent I.C. 6-OHDA followed by I.P. S-20928 (5 mg/kg). Closed circles represent animals injected I.C. with 6-OHDA followed by I.P. injection of vehicle and these served as a control group. I.C. injections were made on the morning of day 10 and this is marked with an arrow. I.P. injections were made on the 4 days marked with an asterisk with the first occurring in the P.M. 12 h after 6-OHDA on day 10.

weight compared to controls after 6-OHDA injection and continuing for the duration of the study (ANOVA: df=3, 540; F=21.08, p=.0001). Further analysis with Tukey's HSD test revealed that the drop in body weight was significant for all three groups compared to controls (p=.0001 in all cases). When analysis was performed for the critical 6 days post 6-OHDA, on a day-to-day basis, the 6-OHDA+vehicle group showed a significant drop in body weight. Post hoc testing using Tukey's HSD revealed the difference on day 1 (ANOVA with df=3,28 for all comparisons; F=99.8; p=.0001), day 2 (F=2.78; p=.04), day 3 (F=5.3; p=.04) and day 4 (F=5.1; p=.006), compared to controls. This group also showed a significant trend toward enhanced body weight loss on day 5 relative to controls (F=2.53; p=.09). Rats injected with 1 mg/kg of S-20928 only showed significant loss of body weight compared to controls on day 1(F=9.8; p=.002) and day 3(F=5.3; p=.04), with a significant trend toward enhanced body weight loss on day 4 (F=5.1; p=.07). Rats injected with 5 mg/kg of S-20928 showed significant loss of body weight compared to controls on day 1(F=9.8; p=.001), day 3 (F=5.3; p=.03) and day 4 (=5.1; p=.009).

Fig. 3 illustrates the effect of ML-23 or S-20928 on the locomotor performance (horizontal movement) for rats treated with 6-OHDA during the acute and recovery test sessions. When the performance of 6-OHDA plus ML-23 treated group was compared to that of vehicle treated controls, no difference was observed (left traces; ANOVA, df=2,39; F=3.73; p=.91). However, Tukey's post hoc revealed that the number of squares crossed during the 10-min test session for rats treated with 6-OHDA plus vehicle, during the acute phase of testing, was significantly impaired when compared to that seen for controls (p=.03). When tested during the recovery phase, several days later, the horizontal movement for all three groups was similar and not significantly different. Note, however, that three animals in each of the 6-OHDA+vehicle or 6-OHDA plus S-20928 groups died during the acute phase of testing and this contributed to the lack of observable effect and spontaneous recovery contributed to the improved condition.

The grouped traces on the right of Fig. 3 illustrate the differences in horizontal movement between the three test groups employed in the S-20928 study during the acute and recovery phases of testing. ANOVA revealed a significant treatment effect (df=3,60; F=3.37; p=.02). Post hoc multiple comparison revealed that while the group injected with 5 mg/kg were similar to controls (p=.12) during the acute phase of testing, rats injected with 1 mg/kg or vehicle after 6-OHDA were significantly different from controls (p=.05 and .03, respectively). When tested during the recovery phase, ANOVA again revealed a significant treatment effect (df=3,63; F=11.2; p=.0001). Post hoc analysis with Tukey's multiple comparisons revealed that the 5 mg/kg group showed the most severe decrement in horizontal movement and this was highly significant (p=.001) when compared to control performance. Similarly, although not as severely, animals treated with vehicle after 6-OHDA were significantly impaired at this time when compared to controls (p=.01). There was a significant trend for those animals treated with the 1 mg/kg dose to also display a slight decrement when compared to controls (p=.07). Multiple comparison also revealed a significant increase in the severity of impairment in the rats treated with 5 mg/kg of S-20898 compared to those treated with 6-OHDA plus vehicle (p=.03).

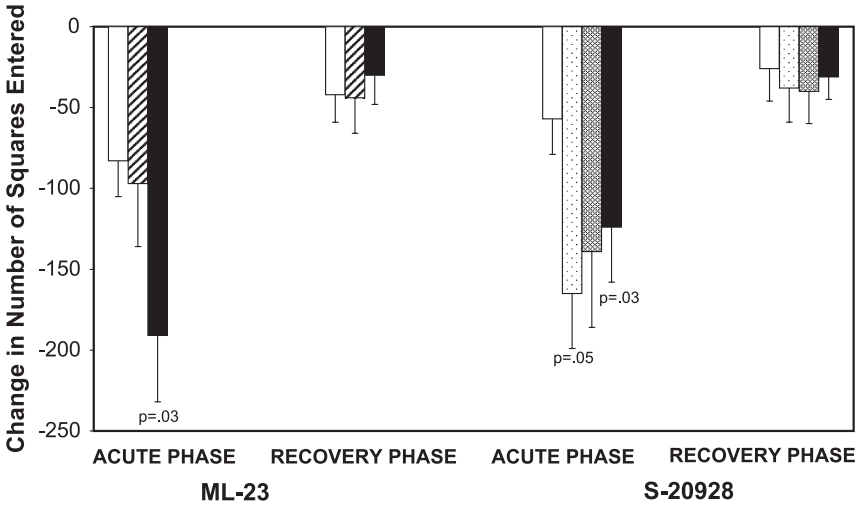

Fig. 3. The effect of ML-23 or S-20928 treatment on locomotion (horizontal movement) in experimental PD. The change in number of squares entered for each group was calculated on the basis of control performance prior to I.C. 6-OHDA injection and was compared to test performance post-6-OHDA injection. The open bars represent the mean change in number of squares crossed for control animals injected I.C. with vehicle then I.P. with ML-23 (left traces) or with I.C. vehicle then I.P. with vehicle at 1 ml/kg (right traces). The diagonal bars represent the mean of animals injected I.C. with 6-OHDA plus I.P. ML-23, while the filled bars represent those animals receiving I.C. 6-OHDA plus I.P. vehicle. The white bars with light stippling represent the mean of animals injected with 6-OHDA plus 1 mg/kg of S-20928 while the dark cross-hatched bars represent those injected with 5 mg/kg of this drug. The acute phase test measurement occurred during days 5–7 while the recovery phase test measurements occurred during days 15–17. The level of significance is expressed above the bars where statistical significance occurred. The *T*-bars represent the standard error of the mean.

As illustrated in Fig. 4, a significant treatment effect was revealed between the three groups tested for vertical movement (rearing)(ANOVA: df=2,39, F=6.1, p=.005). Post hoc analysis revealed that animals treated with ML-23 and tested during the acute phase showed a similar performance to that of controls (p=.851) and this was significantly better than the performance of those animals receiving 6-OHDA+vehicle (p=.006). Similarly, rats treated with 6-OHDA+vehicle were significantly impaired when compared to controls (p=.02). ANOVA again revealed a significant treatment effect on the number of rearings between groups (df=2,39; F=7.01; p=.003) during the recovery phase. Tukey's multiple comparisons revealed a significant difference between control animals and those

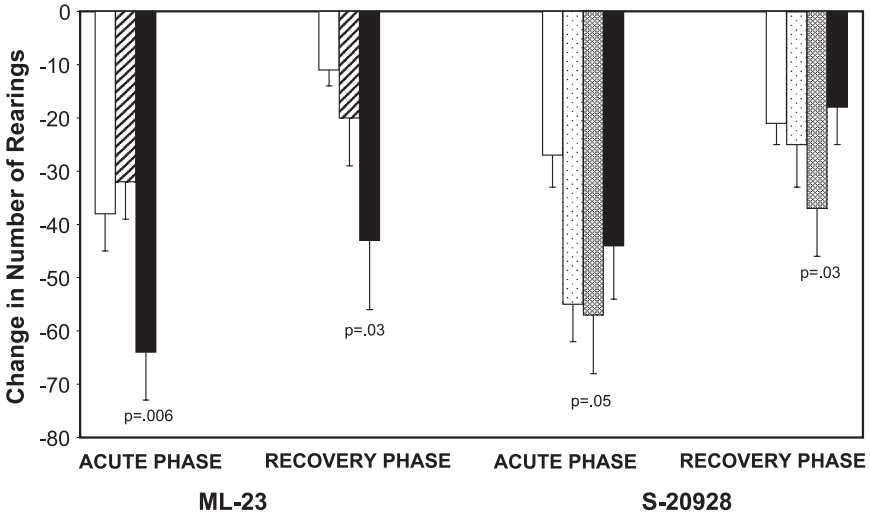

Fig. 4. The effect of ML-23 or S-20928 treatment on rearing (vertical movement) in experimental PD. The change in number of rearings for each group was calculated on the basis of control performance prior to I.C. 6-OHDA injection and was compared to test performance post-6-OHDA injection. The open bars represent the mean change in number of squares crossed for control animals injected I.C. with vehicle then I.P. with ML-23 (left traces) or with I.C. vehicle then I.P. with vehicle at 1 ml/kg (right traces). The diagonal bars represent the mean of animals injected I.C. with 6-OHDA plus I.P. ML-23, while the filled bars represent those animals receiving I.C. 6-OHDA plus I.P. vehicle. The white bars with light stippling represent the mean of animals injected with 6-OHDA plus 1 mg/kg of S-20928 while the dark cross-hatched bars represent those injected with 5 mg/kg of this drug. The acute phase test measurement occurred during days 5–7 while the recovery phase test measurements occurred during days 15–17. The level of significance is expressed above the bars where statistical significance occurred. The *T*-bars represent the standard error of the mean.

injected with 6-OHDA plus vehicle (p=.002). Rats injected with 6-OHDA+vehicle were also significantly impaired in their ability to rear onto the hind feet when compared to those injected with 6-OHDA plus ML-23 (p=.03).

Fig. 5 illustrates the effects of ML-23 and S-20928 on the ability to perform the first of three motor tests assessed at the end of each open field session. ANOVA revealed a significant treatment effect for the latency to retract a limb during the acute phase of testing (df=2,81; F=8.87; p=.0001). Post hoc analysis revealed that the latency to retract a limb after 6-OHDA+ML-23 was not significantly different from that of controls (p=.650) while the group treated with 6-OHDA plus vehicle were significantly impaired (p=.0001). A significant difference between the 6-OHDA+ML-23 and the 6-OHDA and vehicle treated rats was also detected (p=.007). During the recovery phase, a significant treatment effect was observed (ANOVA: df=2,81; F=4.97; p=.009) in respect to the latency to retract a limb. Multiple comparisons performed on that data revealed that impairment in the 6-OHDA+ML-23 (p=.01) or 6-OHDA+vehicle (p=.03) were similar for the two groups, when compared to control performance. Similarly, the injection of 6-OHDA produced a significant treatment effect in respect to the latency to retract during acute phase testing for animals treated post-6-OHDA with vehicle or either dose of S-20928 after 6-OHDA (ANOVA: df=3,124; F=5.05; p=.002). Tukey's multiple comparisons revealed a significant difference only between control animals and those injected with 6-OHDA+1 mg/kg of S-20928 (p=.001). During the recovery phase of testing, the latency to retract was significantly impaired as a result of treatment (ANOVA: df=3,124; F=4.15; p=.008). Multiple comparisons again revealed the significant difference to lie in the impaired performance of the 5 mg/kg treated group, when they were compared to controls (p=.005).

ML-23 administration significantly improved impaired performance on the latency to step from a raised platform during the acute phase of testing as this group was not significantly different to controls (ANOVA: df=2,39; F=4.46; p=.473). Post hoc testing revealed that the significant difference detected occurred between the 6-OHDA+vehicle treated and the control group (p=.01). During recovery phase testing, the latency to step was significantly impaired only in the 6-OHDA+ML-23 group compared to controls (ANOVA with Tukey's HSD: 2,39; F=4.7; p=.01) (Fig. 6).

The latency to step in animals treated with vehicle or S-20928 was not significantly affected during the acute phase of testing as they did not vary significantly from controls and ANOVA revealed no main effect (df=3,60; F=1.56; p=.208). Similarly, during the recovery phase of testing a significant main effect was detected (ANOVA: df=3,60; F=2.76; p=.05). However, Tukey's multiple comparisons revealed that only the S-20928 injected animals showed a significant trend toward impaired latency to step (p=.07). Otherwise, all other treatment groups were similar to controls on this parameter.

The latency to ambulate during the acute test phase was significantly impaired in the 6-OHDA+vehicle treated animals compared to control animals injected with I.C. vehicle+I.P. ML-23 (Fig. 7). ANOVA with Tukey's multiple comparisons revealed this to be statistically significant (df=2,39; F=8.04; p=.001) while animals injected with 6-OHDA+ML-23 did not differ significantly from controls

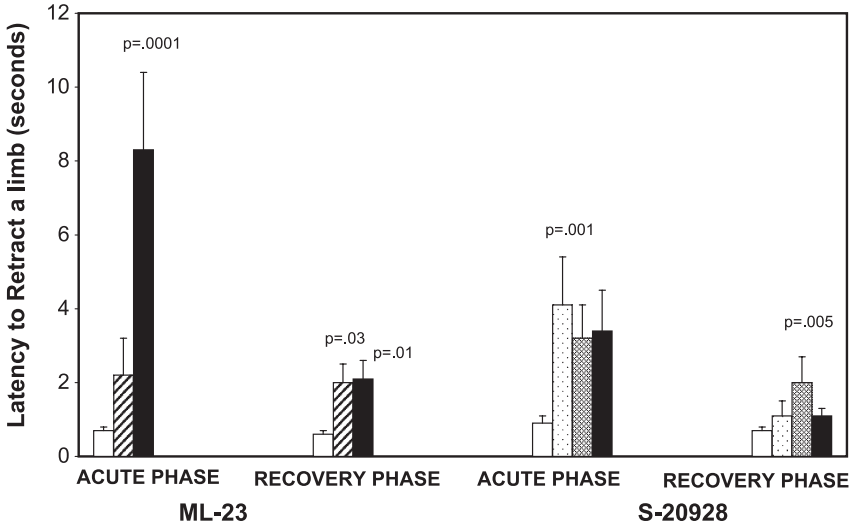

Fig. 5. The effect of ML-23 or S-20928 treatment on latency to retract a limb in experimental PD. The open bars represent the mean latency to retract for control animals injected I.C. with vehicle then I.P. with ML-23 (left traces) or with I.C. vehicle then I.P. with vehicle at 1 ml/kg (right traces). The diagonal bars represent the mean of animals injected I.C. with 6-OHDA plus I.P. ML-23, while the filled bars represent those animals receiving I.C. 6-OHDA plus I.P. vehicle. The white bars with light stippling represent the mean of animals injected I.C. with 6-OHDA plus 1 mg/kg of S-20928 while the dark cross-hatched bars represent those animals injected with I.C. 6-OHDA plus 5 mg/kg of this drug. The acute phase test measurement occurred during days 5–7 while the recovery phase test measurements occurred during days 15–17. The level of significance is expressed above the bars where statistical significance occurred. The T-bars represent the standard error of the mean.

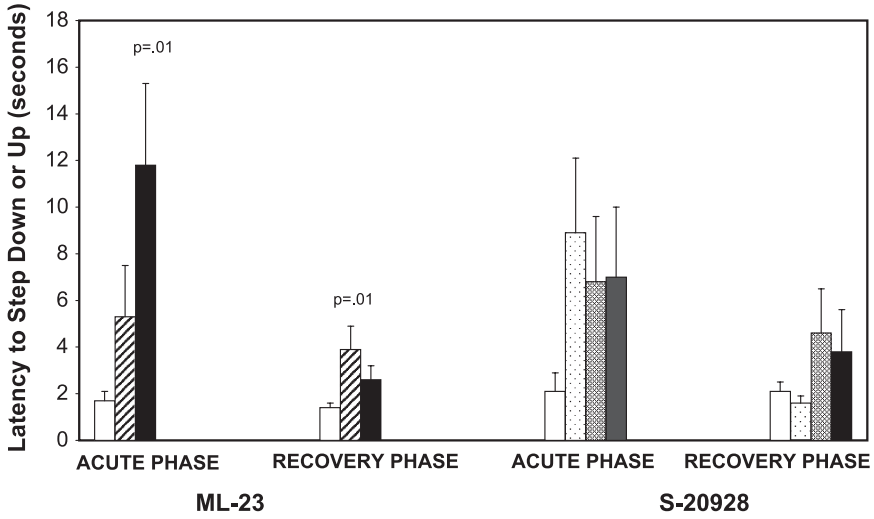

Fig. 6. The effect of ML-23 or S-20928 treatment on latency to step from a raised platform in experimental PD. The open bars represent the mean latency to step for control animals injected I.C. with vehicle then I.P. with ML-23 (left traces) or with I.C. vehicle then I.P. with vehicle at 1 ml/kg (right traces). The diagonal bars represent the mean of animals injected I.C. with 6-OHDA plus I.P. ML-23, while the filled bars represent those animals receiving I.C. 6-OHDA plus I.P. vehicle. The white bars with light stippling represent the mean of animals injected I.C. with 6-OHDA plus 1 mg/kg of S-20928 while the dark cross-hatched bars represent those animals injected with I.C. 6-OHDA plus 5 mg/kg of this drug. The acute phase test measurement occurred during days 5–7 while the recovery phase test measurements occurred during days 15–17. The level of significance is expressed above the bars where statistical significance occurred. The *T*-bars represent the standard error of the mean.

(p=.176). Other than a significant trend for ML-23 treated, 6-OHDA injected rats that showed slight impairment for latency to ambulate during the recovery phase of testing (ANOVA: df=2,39; F=2.74; p=.06), no other differences in performance on this parameter were observed at this time. The latency to ambulate for S-20928 or vehicle injected animals was not significantly altered during the acute phase of testing as no main effect was seen (ANOVA: df=3,63, F=2.05; p=.116). However, during the recovery phase, the 6-OHDA treated rats injected with 5 mg/kg of S-20928 were significantly impaired on this parameter compared to controls (ANOVA: df=3,63; F=7.67; p=.0001), to those receiving 6-OHDA+1 mg/kg of S-20928 (p=.002) or to rats injected with 6-OHDA+vehicle (p=.03).

When the number of squares crossed during the day test sessions for the acute and recovery period for rats treated with ML-23 was, there were no significant differences between any of the treatment groups for this parameter (ANOVA: df=2,39; F=1.49; p=.238. $\bar{x}$ difference scores: 6-OHDA+vehicle, $\bar{x}$=−116.4 S.E.=±39.8: 6-OHDA+ML-23,

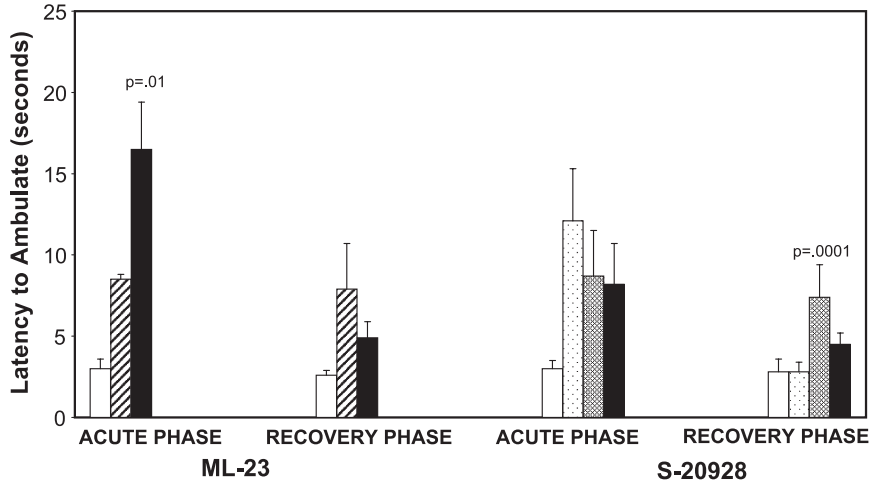

Fig. 7. The effect of ML-23 or S-20928 treatment on latency to ambulate in experimental PD. The open bars represent the mean latency to ambulate for control animals injected I.C. with vehicle then I.P. with ML-23 (left traces) or with I.C. vehicle then I.P. with vehicle at 1 ml/kg (right traces). The diagonal bars represent the mean of animals injected I.C. with 6-OHDA plus I.P. ML-23, while the filled bars represent those animals receiving I.C. 6-OHDA plus I.P. vehicle. The white bars with light stippling represent the mean of animals injected I.C. with 6-OHDA plus 1 mg/kg of S-20928 while the dark cross-hatched bars represent those animals injected with I.C. 6-OHDA plus 5 mg/kg of this drug. The acute phase test measurement occurred during days 5–7 while the recovery phase test measurements occurred during days 15–17. The level of significance is expressed above the bars where statistical significance occurred. The T-bars represent the standard error of the mean.

$\bar{x}$=−109.4 S.E.=±32.7: vehicle+ML-23 (control), $\bar{x}$=−91.1±23.5). Similarly, combined night test sessions for this parameter were unaffected by the various ML-23 or vehicle treatments (ANOVA: *df*=2,39; *F*=.660, *p*=.522. $\bar{x}$ difference scores: 6-OHDA+vehicle, $\bar{x}$=−90.8 S.E.=±35.8: 6-OHDA+ML-23, $\bar{x}$=−31.6 S.E.=± 28.8: vehicle+ML-23 (control), $\bar{x}$=−33.4±13.2).

There was only a slight trend to rear less during the combined day test sessions for animals injected with 6-OHDA+vehicle, compared to controls (ANOVA with Tukey's HSD: *df*=2,39, *F*=2.91; *p*=.07. $\bar{x}$ difference scores: 6-OHDA+vehicle, $\bar{x}$=−50.7 S.E.=±6.5: 6-OHDA+ML-23, $\bar{x}$=−33.6 S.E.=±7.5: vehicle+ML-23, $\bar{x}$=−29.4±5.7). Otherwise, there were no other differences detected. During the combined night sessions, there was no significant difference in rearing for rats treated with 6-OHDA+ML-23 compared to controls as revealed by ANOVA with post hoc comparisons. Those rats treated with 6-OHDA plus vehicle did show significant impairment in rearing behaviour during the same combined test periods in the dark (ANOVA: *df*=2,39; *F*=8.44; *p*=.999 and *p*=.003, respectively; $\bar{x}$ difference scores: 6-OHDA+vehicle, $\bar{x}$=−57.5, S.E.=± 8.6: 6-OHDA+ML-23, $\bar{x}$=−18.3 S.E.=±7.6: vehicle+ML-23, $\bar{x}$=−18.6±6.9).

The injection of either dose of S-20928 has no effect on the number of squares crossed during the day, after 6-OHDA treatment. (ANOVA: *df*=3,60; *F*=2.05, *p*=.116: 6-OHDA+vehicle, $\bar{x}$=−118.8, S.E.=±24.4: 6-OHDA+1 mg/kg, $\bar{x}$=−147.3, S.E.=±31.3: 6-OHDA+5 mg/kg, $\bar{x}$=−140.2, S.E.=±32.4: vehicle+vehicle (control), $\bar{x}$=−57.4, S.E.=±25.2). During the night there was a significant trend for the number of squares to decrease after 6-OHDA+vehicle treatment when compared to control performance while the number of squares crossed by the 1 mg/kg (*p*=.476) or 5 mg/kg dose groups (*p*=.254) were not significantly different from controls. (ANOVA plus Tukey's HSD: *df*=3,60; *F*=2.13, *p*=.06 for the 6-OHDA+vehicle group, $\bar{x}$=−133.0, S.E.=±30.0: 6-OHDA+1 mg/kg, $\bar{x}$=−87.0, S.E.=±28.8: 6-OHDA+5 mg/kg, $\bar{x}$=−104.6, S.E.=±39.3: vehicle+vehicle (control), $\bar{x}$=−26.3, S.E.=±15.8).

Rearing onto the hind limbs during test sessions in the light were not affected significantly by drug treatment in any of the groups tested after vehicle or S-20928 injection. (ANOVA: *df*=3,60; *F*=1.245, *p*=.302: 6-OHDA+vehicle, $\bar{x}$=−31.5, S.E.=±7.6: 6-OHDA+1 mg/kg, $\bar{x}$=−47.2, S.E.=±5.9: 6-OHDA+5 mg/kg, $\bar{x}$=−38.1, S.E.=±8.7: vehicle+vehicle, $\bar{x}$=−29.9, S.E.=±5.5). During the dark phase of the light/dark cycle rats injected with 5 mg/kg of S-20898 showed a significant decrease in rearing performance for the acute and recovery sessions combined and compared to controls (ANOVA with Tukey's HSD: *df*=3,60; *F*=5.15, *p*=.003: cf. 6-OHDA+vehicle, $\bar{x}$=−30.5, S.E.=±9.1: 6-OHDA+1 mg/kg, $\bar{x}$=−31.9, S.E.=±6.8: 6-OHDA+5 mg/kg, $\bar{x}$=−55.8, S.E.=±7.2: vehicle+vehicle (control), $\bar{x}$=−17.9, S.E.=±3.7). Analysis using post hoc multiple comparisons revealed that rats injected with 1 mg/kg of S-20928 or vehicle were not significantly different from controls at this time.

Results from the food and water intake tests are expressed in Fig. 8. During the recovery phase of testing, at day 10 after 6-OHDA injection, there was a significant difference in the quantity of food eaten by 6-OHDA+ML-23 injected animals compared to those injected with 6-OHDA+vehicle (ANOVA: *df*=2,15; *F*=5.95; *p*=.01), albeit

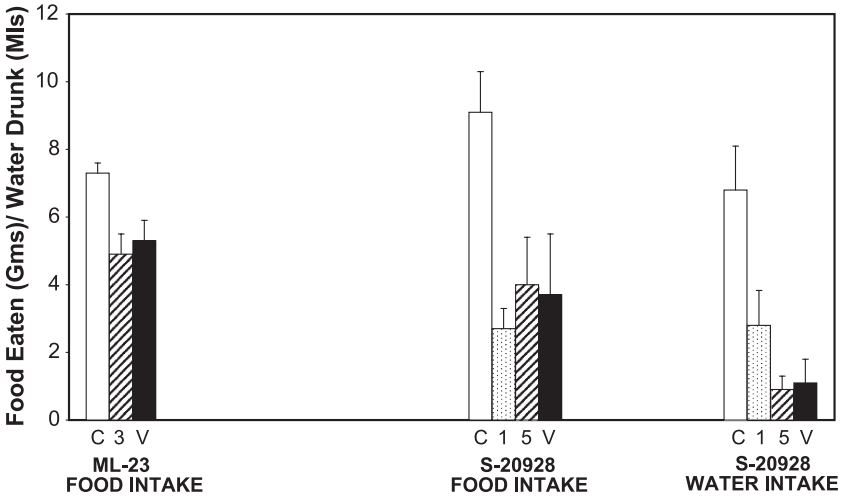

Fig. 8. The effect of ML-23 or S-20928 on food and water intake in experimental Parkinson's disease. Rats treated with ML-23 (left trace) were tested at 1900–2100 h on day 10 after 6-OHDA injection while S-20928 injected rats were tested on day 5 post-6-OHDA (right traces). For the ML-23 and food intake study, the open bars represent the mean food intake of rats injected with I.C. vehicle and I.P. ML-23 or I.C. vehicle plus I.P. vehicle. These animals served as controls. The diagonally banded bars represent rats injected I.C. with 6-OHDA then I.P. with ML-23. In the S-20928 study, the filled bars represent food and water intake for rats injected I.C. with 6-OHDA then I.P. with vehicle. The light stippled bars represent animals injected I.C. with 6-OHDA then I.P. with 1 mg/kg S-20928. The diagonal bars represent the mean response of rats injected I.C. with 6-OHDA plus 5 mg/kg S-20928. Statistical comparisons revealed no significant treatment effects on these parameters. The *T*-bars represent the standard error of the mean.

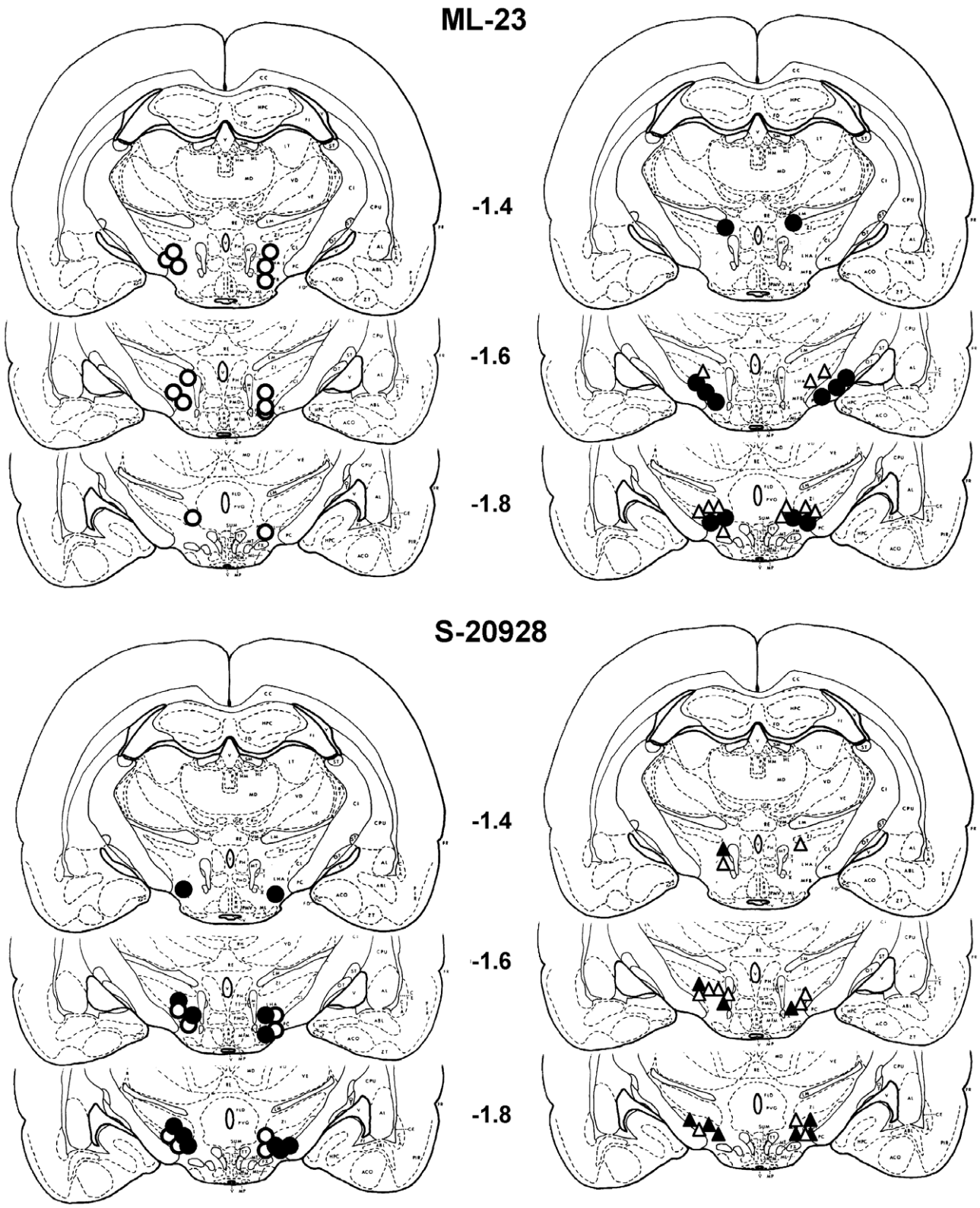

Fig. 9. Anatomical maps from Pelligrino et al. (1979), depicting injection sites after the I.C. injection of 6-OHDA with the centre of the lesion depicted for each injection site. The upper two map groups represent the locus of injection for the ML-23 study. The open circles represent sites of injection for rats receiving I.C. vehicle plus I.P. ML-23. The filled circles represent the injection sited for rats injected I.C. with 6-OHDA then I.P. vehicle. The open triangles represent injection sites in rats injected I.C. with 6-OHDA then I.P. with ML-23. The lower two map groups represent the injection loci for the S-20928 study. The open circles represent sites of injection for rats receiving I.C. vehicle plus I.P. ML-23. The filled circles represent the injection sites for rats injected I.C. with 6-OHDA then I.P. vehicle. The open triangles represent injection sites in rats injected I.C. with 6-OHDA then I.P. with 1 mg/kg of S-20928 while the filled triangles represent the injection sites in rats injected I.C. with 6-OHDA then I.P. with 5 mg/kg of S-20928. The placement of the lesions generally ranged in an anterior–posterior position extending from 1.4 to 1.8 mm posterior to bregma as indicated.

a change of minor magnitude. The occurrence of a significant trend to reduce food intake in the presence of 6-OHDA+vehicle administration when compared to control intake (p=.07), while 6-OHDA+ML-23 injected rats did not, suggests that the significant difference between ML-23 and vehicle administration noted above is probably due to a type I error.

Similarly, neither the 1 or 5 mg/kg dose of S-20928, nor the injection of vehicle caused significantly improvement in the depressed food intake at day 5 after 6-OHDA injection, as all were significantly reduced when compared to controls (ANOVA with multiple comparisons: df=3,28;F=5.107; p=.007; p=.04; and p=.02, respectively). None of these groups differed from each other at this time. In addition, water intake was significantly depressed in all three groups, compared to control intake, with Tukey's multiple comparisons revealing these differences to be highly significant (ANOVA: df=3,28;F=20.9; p=.0001 in all cases). Neither of the groups treated with 6-OHDA+S-20928 or 6-OHDA+ vehicle differed from each other at this time.

Assessment of all rats on clinical parameters during the time of testing revealed that only those treated with 5 mg/kg of 20898 showed more severe impairment of grooming, increased eye closure, and kyphosis, around the time of I.P. injection than did the animals injected with 1 mg/kg of S-20928 or vehicle. In addition, penile erection, loss of general muscle tone and haematuria were seen more frequently in these animals than in any other group. Rats in the 1 mg/kg of S-20928 group showed these signs less frequently, and with less severity, than the 5 mg/kg dose group. These symptoms disappeared 1–2 days after I.P. injections ceased while they were not observed in animals injected I.P. with vehicle or ML-23.

The mortality of rats treated with 6-OHDA was prevented by ML-23 administration as none of this group became moribund and died during the course of the experiment. Three of the rats treated with 6-OHDA+ vehicle died between the 7th and 18th day of observation (47%). In the S-20928 study, three out of eight rats died in the 6-OHDA plus 1 mg/kg group, the 6-OHDA plus 5 mg/kg group and the 6-OHDA plus vehicle group. In both experiments, all animals that died as a result of the acute severity of DA depleting lesions could not be assessed during the recovery phase of the experiment. Therefore, the impact or longer term physiological and behavioural parameters was limited to those animals experiencing spontaneous recovery.

Histological examination of the brain tissue post-mortem (Fig. 9) revealed that the lesions resulting from the injection of 6-OHDA or vehicle into the PLH caused necrotic tissue damage of a similar volume of tissue and in similar anatomical placement in all three groups tested. Necrosis depicting the site of injection was located just rostral to the substantia nigra, extending from the lateral hypothalamus, through the PLH and into the anterior part of the substantia nigra.

4. Discussion

These results demonstrate that the administration of some melatonin analogues can reverse the PD like deficits seen in more chronic models of PD and can facilitate recovery of motor and homeostatic control. Traditionally, one of the most difficult parameters to recover in the bilateral, 6-OHDA model for this disorder is reduced food intake and loss of the ability to regulate body weight. Consequently, animals ether die within a few days after 6-OHDA injection or they have to be maintained with artificial feeding for several weeks until spontaneous recovery occurs. Even when DA replacement therapy is employed (Dunnett and Björklund, 1984; Ljungberg and Ungerstedt, 1976), the reinstatement of such regulatory functions is not readily achieved. However, when the melatonin analogue, ML-23, was employed in a paradigm shown previously to induce recovery from DA depleting lesions using other drugs (Glick and Greenstein, 1974), a permanent, complete recovery was observed.

In the present study, we have employed a paradigm which has been used frequently to ascertain the role of DA receptor supersensitivity in recovery from DA degenerating lesions of the brain. Traditionally, DA receptor antagonists or tyrosine hydroxylase (TH) enzyme inhibitors have been administered 3 days prior to or 3 days after DA depletion. This produces a reduction of DA receptor occupancy in the striatum thereby inducing a state of paucity of transmitter and hypersensitivity of the postsynaptic receptor ensues. In previous studies, we have challenged this model by employing a DA receptor antagonist which fails to cross the blood–brain barrier and yet produces a robust recovery of motor control and regulatory function (Willis et al., 1983a). This suggested that extra-striatal mechanisms were involved in the aetiology and recovery from DA depleting lesions. In the present study, we choose this regime of drug administration for two additional reasons. In the first instance, the time of first injection in this regime occurs at least 12 h after the administration of 6-OHDA and interference of the drug with the acute neurotoxicity of 6-OHDA is unlikely (Antolin et al., 2002; Kim et al., 1998; Mayo et al., 1999; Reiter, 1998). Since the cascade of degenerative events would already be well passed by the time the first ML-23 or S-20928 injection occurred, it is unlikely that an antioxidative effect is responsible for the observed behavioural recovery. Contemporary work with melatonin, suggesting that it has prophylactic utility in PD as an antioxidant, is linked with a mechanism of action dependent on administration around the time of neurotoxic insult. This is based on reports demonstrating that melatonin loses its efficacy in this capacity about 1 h after neurotoxin administration (i.e. Iacovitti et al., 1997). With the profile of recovery depicted by body weight loss after 6-OHDA illustrated in Fig. 1 showing a pattern or recovery that strengthens over several days post-6-OHDA, such an effect is not consistent with a simple antioxidative

effect. Secondly, a similar injection regime has been employed with the TH inhibitor alpha-methyl-*para*-tyrosine (AMPT) to induce DA receptor hyperactivity prior to induction of DA degeneration thereby reducing the severity of experimental PD (Glick et al., 1972). It so happens that AMPT injection also causes dramatic reduction of nocturnal pineal melatonin (Zimmerman et al., 1994), and like the drugs employed in the present study, such treatment would reduce melatonin bioavailability providing an alternative explanation for the observed recovery in DA-deficient rats. This phenomenon may well involve an extra-striatal mechanism. (Carlisle and Reynolds, 1961; cf. Glick and Greenstein, 1974; Willis et al., 1983a). Further to this, relief of bradykinaesia, rigidity, insomnia and depression have been reported in studies employing bright light therapy to induce recovery from PD in animals (Willis and Armstrong, 1999) and man (Artemenko and Levin, 1996; Willis and McLennan, 2001b) and such treatment has also been linked to reduced melatonin bioavailability (Rosenthal et al., 1986). Consistent with this interpretation is the suggestion that melatonin may have a more acute (non-antioxidative) role in facilitating DA degeneration and that prevention of melatonin signaling may be beneficial in mediating recover from PD. We suspect that the pineal plays a major role in this regard, and in relation to the psychiatric side effects which typify long-term use of DA replacement therapy (Willis and McLennan, 2001a).

One of the most interesting findings in the present study is the differential effects that drug administration had on motor responses in the light versus the dark phase of the light/dark cycle. Vertical movement seemed to be particularly sensitive to the reparative effect of ML-23 at night. The night-time performance of ML-23 injected rats with experimental PD was the same as controls for the duration of the experiment, an effect that was not observed in 6-OHDA treated rats injected with vehicle. Given that rats are nocturnal: increased activity would be predicted in the dark for all groups. The ML-23 injected group showed the most remarkable improvement in rearing during testing in the dark (+47%). Control rats improved slightly less (+37%) while 6-OHDA+vehicle injected rats deteriorated (-7%). Similarly, in the S-20928 study, a significant trend toward enhanced impairment of horizontal movement was observed at night in 6-OHDA+vehicle treated controls, but not in Parkinsonian rats treated with either dose of drug. The night-time rearing performance of control rats improved by 54%, as did the performance of rats treated with 1 mg/kg (+40%) or 5 mg/kg (+26%) of the active drug while rats injected with 6-OHDA+vehicle deteriorated when tested in the dark (−12%). These findings add further support to the contention that melatonin is directly involved in aetiology of experimental PD and that the observed treatment effects are mediated by pineal melatonin. This theme is currently under intensive investigation.

The use of melatonin as an adjunct therapy for PD to either halt progressive degeneration or for the symptomatic treatment of PD is problematic. In the first instance, when a PD patient presents, DA degeneration is well in progress and at such advanced stages melatonin would not be expected to be particularly effective. It is generally accepted that clinical PD is characterized by degeneration of at least 80% of the ascending DA system. Ideally, to overcome this, melatonin therapy would have to commence 10–30 years prior to diagnosis. In this respect, the early diagnosis of PD this far in advance is unrealistic not to mention the potential for unwanted side effects with prolonged use (Guardiola-Lemaître, 1997; Weaver, 1997). So the time, dose and duration of melatonin administration in PD is riddled with many practical limitations. The most promising application for melatonin in PD is to retard or halt the progressive degeneration characterizing this disease. However, there have been many attempts to do so using other compounds with antioxidative properties but this has met with limited success (Baronti et al., 1992; Fahn, 1996; Gerlach et al., 1994a; 1994b; Stocchi and Olanow, 2003). Given that there are other antioxidants, more potent and effective than melatonin (Martinez-Cruz et al., 2002) and that most antioxidants (including melatonin) also possess pro-oxidative qualities (Kienzl et al., 1995; Medina-Navarro et al., 1999; Oyanagui, 1997; Tang et al., 1998; Waddington and Crow, 1979; Youdim et al., 1989), further work with other antioxidative candidates is in order before a consensus is reached about the suitability of melatonin in this capacity.

On balance, the reports suggesting that melatonin is of therapeutic benefit in PD do not present an overwhelmingly convincing story. In the original clinical studies where melatonin was administered to PD patients (Antón-Tay, 1974; Antón-Tay et al., 1971), there were difficulties with experimental design and interpretation. Firstly, the definition of each patient's condition prior to treatment and their response after treatment commenced and during drug withdrawal was not detailed or standardized. Secondly, the doses of melatonin employed were large (1.2 g/day), making a meaningful interpretation as to the therapeutic mechanism difficult to decipher (Guardiola-Lemaître, 1997). Thirdly, the melatonin was mixed with 2% alcohol solution, a combination which will enhance the sedative effect of melatonin (Willis and McLennan, 2001a,b). Furthermore, subsequent attempts to replicate the work showed that melatonin therapy was ineffective in treating PD (Papavasiliou et al., 1972; Shaw, 1977; Shaw et al., 1973). This, combined with conflicting reports describing inhibitory effects of melatonin upon DA synthesis and release (Alexiuk and Vriend, 1993; Nowak et al., 1992; Tenn and Niles, 1997; Zisapel et al., 1982; 1985), makes the DA replacement theory using melatonin unlikely and does not encourage the use of melatonin in the treatment of PD. It is entirely possible that symptomatic relief, such as reduction in tremor and anxiety, may be related to a sedative effect similar to that observed with the benzodia-

zepines (Garfinkel et al., 1999; cf. Antón-Tay, 1974; Dowling and Aminoff, 1999; Patterson and Vickers, 1984; Shaw, 1977). The introduction of melatonin for treating secondary symptoms of PD presents additional problems such as increased falling (Culebras, 1992), reduced vigilance (Wirz-Justice and Armstrong, 1996), and decreased motor performance (Araghi-Nikham et al., 1999; Arushanyan and Ovanesov, 1989; Bradbury et al., 1985; Burton et al., 1991; Chuang and Lin, 1994; Minneman et al., 1976; Reis et al., 1963; Rodriguez et al., 1984; Willis and Armstrong, 1999). Each of these factors should be carefully and thoroughly examined before melatonin is considered as an adjunct therapy in PD (Mayo et al., 1998; Zisapel, 2001; Bruguerolle and Simon, 2002).

Like the putative melatonin receptor antagonist, ML-23, the antagonist S-20928 also possesses anti-Parkinsonian properties that enhance recovery in a chronic model of PD. However, unlike ML-23, the observed therapeutic effects of S-20928 are not as robust with dose-dependent properties and toxicity limiting its utility. While some anti-Parkinsonian effects were seen with the 1 mg/kg dose, the 5 mg/kg caused deleterious side effects. Haematuria, penile erection, exaggerated kyphosis and abdominal pain during I.P. injection were much more severe in the 5 mg/kg group than in the 1 mg/kg or vehicle treated groups. It is likely that these side effects interfered with the recovery from the 6-OHDA-induced PD. Whether this is an issue specifically related to S-20928 or whether it is a problem associated with some classes of melatonin receptor antagonists is unclear. Body weight was improved significantly by the lower dose of S-20928, suggesting that motor versus regulatory deficits may be differentially sensitive to the effects of different antagonists. Previous work using non-pharmacological methods of melatonin antagonism, namely pinealectomy and light (Willis and Armstrong, 1999), is devoid of side effects suggesting that melatonin antagonism in itself is not responsible for the observed complications. These deleterious versus the therapeutic effects may be specific to the dose of S-20928 employed and further work with doses less than 1 mg/kg may elucidate the answer.

The dose-related effects of S-20928 might be similar to those observed with DA replacement. In the case of L-DOPA, the dose–response curve is bell-shaped with moderate doses facilitating recovery while higher doses cause motor impairment (Fredriksson et al., 1990; Bell et al., 1971; Fink and Smith, 1979; Willis et al., 1983b; Asami et al., 1986; Messiha, 1989). In fact, this phenomenon could account for the high incidence of psychiatric side effects and motor abnormalities seen in the clinic after prolonged use of these drugs (Banerjee et al., 1989; Cardoso and Jankovic, 1997; Olanow and Houser, 1990). Further dose–response studies with other melatonin receptor antagonists in more chronic models (Gerlach and Riederer, 1996) should also shed light on the nature of the inhibitory versus facilitatory effects observed with some antagonists.

Of particular significance is the finding that ML-23 was effective in totally eliminating the high morbidity associated with this acute model of PD, while S-20928 had no effect on this parameter. Typically, the mortality rate after bilateral, intracerebral 6-OHDA is 30–50% in most studies undertaken over the past 20 years (see Willis and Smith, 1985; Willis and Armstrong, 1998). Reduced mortality is similar to that observed in studies which specifically employed melatonin and the analogue ML-23, to increase the longevity of survival in normal rats (Oaknin-Bendahan et al., 1995). In that study, it was assumed that the increased survival of melatonin treated rats would be reversed by the administration of the antagonist ML-23; however, both compounds increased life span and no explanation as to the basis for this phenomenon was forthcoming. The current study confirms the life enhancing characteristics of ML-23 and, for the first time, demonstrates that such an effect can be observed in models of human disease. While we are yet to elucidate the precise mechanism by which this decreased mortality occurs, our choice of this drug as a potential candidate for clinical development was made on the basis of this feature. Such a feature is now recognized as an important criterion for selecting potential drug candidates for diseases of advancing age (Reiter, 1998).

That the antagonism of melatonin might be the mechanism underlying the observed effect is supported by inference from numerous studies. For example, melatonin secretion is associated with increased occurrence of seborrhea (Maietta et al., 1991). Interestingly, seborrhea is a common secondary symptom associated with PD (Burton et al., 1970, 1973). Both seborrhea and the negative symptoms of PD can be corrected with light therapy in man (Artemenko and Levin, 1996; Willis and McLennan, 2001b) and similar effects on motor function have been achieved in animal models of the disease (Willis and Armstrong, 1999). Consistent with this, L-DOPA therapy has been reported to reduce the occurrence of seborrhea in parallel with its therapeutic efficacy on PD (Burton et al., 1970, 1973) and may do so by reducing melatonin secretion (Rosenthal et al., 1986). Given the relationship between (1) light therapy and remission from depression (Rosenthal et al., 1986) or PD (Willis and McLennan, 2001a,b; Artemenko and Levin, 1996), (2) exposure to daylight, reduced melatonin secretion and its effects on Seborrheic dermatitis (Maietta et al., 1991), (3) the occurrence of Seborrheic dermatitis in PD and its remission with L-DOPA therapy (Burton et al., 1970, 1973), (4) the antidepressive properties of the melatonin receptor antagonist Luzindole (Dubocovich et al., 1990), and (5) the common occurrence of depression prior to and during the course of PD (Deniker et al., 1975; Fonda, 1985; Girotti et al., 1986; Okun and Watts, 2002), an intriguing picture emerges which suggests that excess melatonin and the pineal play a major role in PD, DA replacement and related neuropsychiatric disorders (Willis and Armstrong, 1998; Willis and McLennan, 2001a).

The mechanism involved in the reparative effects observed with ML-23 may be mediated by its ability to antagonize melatonin and to counteract melatonin's effect on the cytoskeleton and impaired axoplasmic transport in dying neurones. In this regard, there are similarities between the effects that melatonin has on cellular structure and function with those occurring in neuropsychiatric conditions, including PD (Appel, 1981; Gajdusek, 1985; Goldman and Yen, 1986; Schmid, 1993). Earlier work with melatonin has shown that, like other mitosis inhibitors and neurotoxins, it inhibits axoplasmic transport and interferes with cytoskeletile organization (Cardinalli and Friere, 1975; Huerto-Delgadillo et al., 1994; Matsui and Machado-Santelli, 1997). Pharmacological antagonism of the melatonin receptor provides the opportunity to test this hypothesis as MT1 receptor subtypes provide direct access to the subcellular compartment and subcellular organization (Bordt et al., 2001). We have not, however, ruled out the possibility that ML-23 may be acting directly on the cytoskeletile structure as it passes readily through cellular membranes and accesses intracellular compartments. (Finocchiaro and Glikin, 1998; Melchiorri et al., 1995; Reiter, 1998).

The present work suggests that circadian influences work via the lateral hypothalamus and related parts of the diencephalon and that such areas may be involved in the etiology of PD. Previous work has explored the anatomical relationship of this area in the circuitry from the retina to the pineal gland in mammalian brain (Rowland, 1976; Axelrod et al., 1966). By virtue of this anatomical relationship, we suspect that the pineal is involved to a much larger degree in the aetiology of PD than has been suggested previously. Recent work concerning the role of the pineal gland in DA replacement therapy suggests that it acts via its influence on the pineal gland rather than by replacing deficient DA per se (Willis and McLennan, 2001a; Willis et al., 1983a; Ghaemi et al., 2001). This position is confirmed by the present work as compounds such as ML-23 have little or no direct effect on central DA.

In conclusion, the object of the present study was to test the hypothesis that pharmacological antagonism of the melatonin receptor would be as effective as other methods of melatonin antagonism in recovering rats from experimental PD. The present work, taken together 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 conditions with such neuropathological aetiologies. From the broader perspective and taking into account (1) the ubiquitous nature of melatonin, (2) its dwindling functional importance in the ageing organism (Reyes, 1982), and (3) the high incidence of neuropsychiatric conditions such as PD and dementia which are marked by cytoskeletile and cellular transport pathology (Appel, 1981; Gajdusek, 1985; Goldman and Yen, 1986; Price et al., 1987; cf. Cardinalli and Friere, 1975) suggests further that this hormone may play a role in these conditions during some stage of the degenerative process (Cardinalli and Friere, 1975; Matsui and Machado-Santelli, 1997; Meléndez et al., 1996; Willis and McLennan, 2001a; see review; Willis and Armstrong, 1998). From the present work, it may be concluded that a complex role for melatonin in the aetiology of PD is likely and intimates that blocking of melatonin function or decreasing its bioavailability may offer a novel approach to the treatment of this debilitating degenerative disease.

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References

Alexiuk NAM, Vriend JP. Melatonin reduces dopamine content of the neurointermediate lobe of male Syrian hamsters. Brain Res Bull 1993;32:433–6.

Anis Y, Zisapel N. Affinity labeling of melatonin binding sites in the hamster brain. Biochem Biophys Res Commun 1991;178 1147–52.

Antolin I, Mayo JC, Sainz RM, Del Brio Mde L, Herrera F, Martin V, et al. Protective effect of melatonin in a chronic experimental model of Parkinson's disease. Brain Res 2002;243:163–73.

Antón-Tay F. Melatonin; effects on brain function. Adv Biochem Pharmacol 1974;11:315–24.

Antón-Tay F, Diaz JL, Fernandez-Guardiola A. On the effect of melatonin upon human brain. Its possible therapeutic implications. Life Sci 1971;10:841–50 [Part 1].

Appel SH. A unifying hypothesis for the cause of amyotrophic lateral sclerosis Parkinsonism and Alzheimer disease. Ann Neurol 1981;10: 499–905.

Araghi-Nikham M, Lane M, Watson RR. Modification of physical movement in old C57BL/6 mice by DHEA and melatonin supplementation (44404). PSEMB 1999;221:193–7.

Artemenko AP, Levin YJ. Light therapy in patients with Parkinsonism. Ž Nevropatol Psihiatr SS Korsakova 1996;96(3):63–6.

Arushanyan EB, Ovanesov KB. Peculiarities of psychotropic effect of melatonin depending on dose and time of day. Farmakol Toksikol 1989;52:33–7.

Asami T, Kuribara H, Todocoro S. Effects of repeated administration of Bromocriptine on ambulatory activity in mice, and changes in methylamphetamine sensitivity in Bromocriptine-experienced mice. Yakubutsu Seishin, Kudo 1986:309–17.

Axelrod J, Snyder SH, Heller A, Moore RY. Light-induced changes in pineal hydroxyindole-*O*-methyl transferase: abolition by lateral hypothalamic lesions. Science 1966;154:898–9.

Balagura SA, Wilcox RH, Cochina DV. The effects of diencephalic lesions on food intake and motor activity. Physiol Behav 1969;4: 629–633.

Banerjee AK, Falkai PG, Savidge M. Visual hallucinations in the elderly associated with the use of levodopa. Postgrad Med J 1989;65:358–61.

Baronti F, Davis TL, Bouldry RC, Mouradin MM, Chase TN. Deprenyl effects on levodopa, pharmacodynamics, mood and free radical scavenging. Neurology 1992;42:541–4.

Bell LJ, Iversen LL, Uretsky NJ. Time course of the effects of 6-Hydroxydopamine on catecholamine-containing neurones in rat hypothalamus and striatum. Br J Pharmacol 1971;42:88–99.

Bordt SL, McKeon RM, Li PK, Witt-Enderby PA, Melan MA. N1E-115 neuroblastoma cells express MT-1melatonin receptors and produce neurites in response to melatonin. Biochim Biophys Acta 2001;1499: 257–64.

Bradbury AJ, Kelly ME, Smith JA. Melatonin action in the midbrain can regulate forebrain dopamine function both behaviorally and biochemically. In: Brown GM, Wainwright SD, editors. The pineal gland: endocrine aspects. Oxford: Pergamon Press; 1985. p. 327–32.

Bruguerolle B, Simon N. Biologic rhythms and Parkinson's disease: a pharmacologic approach to considering fluctuation in function. Clin Neuropharmacol 2002;25(4):194–201.

Burton JI, Cartlidge M, Schuster S. Effect of L-DOPA on the seborrhea of Parkinsonism. Lancet 1970, July;4:19–20.

Burton JI, Cartlidge M, Schuster S. Effect of L-DOPA on the seborrhea of Parkinsonism. Br J Dermatol 1973;88:475–9.

Burton S, Daya S, Potgeiter B. Melatonin modulates apomorphine-induced rotational behaviour. Experientia 1991;47:466–9.

Buzzell GR, Menendez-Palaez A, Troiani ME, Meneill ME, Reiter R. Effects of short-day photoperiods and of N-(2,4-dinitrophenyl)-5-methoxytryptamine, a putative melatonin synthesis in the harderian gland of the Syrian hamster, mesucricetus auratus. J Pineal Res 1990;8(3):229–35.

Cardinalli DP, Friere F. Melatonin effects on brain: interaction with microtubule proteins, inhibition of fast axonal flow and induction of crystalloid and tubular formation in the hypothalamus. Mol Cell Endocrinol 1975;2:317–30.

Cardoso K, Jankovic I. Dystonia and dyskinaesia. Psychiatr Clin North Am 1997;20:821–38.

Carlisle HJ, Reynolds RW. Effect of amphetamine on food intake in rats with brain stem lesions. Am J Physiol 1961;201:965–7.

Chong NW, Evans JE, Sugden D. N-bromoacetyl-5-methoxytryptamine: an irreversible melatonin ligand? Biochem Biophys Res Commun 1993;193:1355–61.

Chuang JI, Lin MT. Pharmacological effects of melatonin treatment on both locomotor activity and brain serotonin release in rats. J Pineal Res 1994;17:11–6.

Culebras A. Update on disorders of sleep and the sleep–wake cycle. Psychiatr Clin North Am 1992;15:467–89.

Dabherni-Sala F, DiSanto S, Franceschini D, Skaper SO, Givsti P. Melatonin protects against 6-OHOA-induced neurotoxicity in a role for mitrochrondial complex I activity. FASEBJ 2001;15(1):164–70.

Delagrange P, Atkinson J, Boutin JA, Casteilla L, Lesieur D, Misslin R, et al. Therapeutic perspectives for melatonin agonists and antagonists. J Neuroendocrinol 2003;15(4):442–8.

Deniker P, Ginistet D, Loo H. Psychotropic drugs and mechanisms of mood regulation. Encephale 1975;1:159–62.

Dowling GA, Aminoff MJ. Melatonin for the treatment of sleep disturbance in Parkinson's disease. Epilepsy Int; Gen Clin Neurol: Interesting cases SP-1, p. sp1.003–4.

Dubocovich ML, Mogilnicka E, Areso PM. Antidepressant-like activity of the melatonin receptor antagonist, luzindole (N-0774), in the mouse behavioural despair test. Eur J Pharmacol 1990;182(2):313–25.

Dunnett SB, Björklund A. Aphagia and adipsia after serial bilateral lesions. Appetite 1984;5:263–5.

Fahn S. Controversies n the therapy of Parkinson's disease. Adv Neurol 1996;60:477–86.

Fink JS, Smith GP. L-DOPA repairs deficits in locomotor and investigatory exploration produced by denervation of catecholamine terminal fields in the forebrain of rats. J Comp Physiol Psychol 1979;93:66–73.

Fonda D. Parkinson's disease in the elderly: psychiatric manifestations. Geriatrics 1985;40:109–12.

Fredriksson A, Plaznik A, Sandström E, Jonsson G, Archer T. MPTP induced hypoactivity in mice: reversal by L-DOPA. Pharmacol Toxicol 1990;67:295–301.

Finocchiaro L.M, Glikin GC. Intracellular melatonin distribution in cultured cell lines. J Pineal Res 1998;24:22–34.

Gajdusek GC. Hypothesis: interference with axonal transport of neurofilament as a common pathogenic mechanism in certain diseases of the central nervous system. N Engl J Med 1985;312:714–9.

Garfinkel D, Zisapel N, Wainstein J, Laudon M. Facilitation of benzodiazepine discontinuation by melatonin. A new clinical approach. Arch Int Med 1999;159:2456–60.

Gerlach M, Riederer P. Animal models of Parkinson's disease: an empirical comparison with the phenomenology of the disease in man. J Neural Transm, Suppl 1996;103:987–1041.

Gerlach M, Ben-Shachar D, Riederer P, Youdim MBH. Altered brain metabolism of iron as a cause of neurodegenerative disease. J Neurochem 1994;63:793–807.

Gerlach M, Youdim MBH, Riederer P. Is selegiline neuroprotective in Parkinson's disease? J Neural Transm 1994;41(Suppl.): 177–188.

Ghaemi M, Rudolf J, Hilker R, Herholz K, Heiss WA. Increased pineal F-Dopa uptake in related to severity of Parkinson's disease—a PET study. J Pineal Res 2001;30(4):213–9.

Girotti F, Carella F, Grassi MP, Solveri P, Marano R, Caraceni T. Motor and cognitive performance of Parkinson's patients in the on and off phases of the disease. J Neurol Neurosurg Psychiatry 1986;48:657–60.

Glick SD, Greenstein S. Facilitation of lateral hypothalamic recovery by post-operative administration of α-methyl-*p*-tyrosine. Brain Res 1974;73:180–3.

Glick SD, Greenstein S, Zimmerberg B. Facilitation of recovery by α-methyl-*p*-tyrosine after lateral hypothalamic damage. Science 1972;177:534–5.

Goldman JE, Yen S-H. Cytoskelatal protein abnormalities in neurodegenerative diseases. Ann Neurol 1986;19:209–23.

Guardiola-Lemaître B. Toxicology of melatonin. J Biol Rhythms 1997;12:697–706.

Guardiola-Lemaître B, Delagrange P. Melatonin analogues: from pharmacology to clinical application. Eur J Med Chem 1995;30(Suppl.): 643s–51s.

Horstink MWIM, Morrish PK. Preclinical diagnosis of Parkinson's disease. Adv Neurol (Parkinson's disease) 1999;80(42):327–33.

Huerto-Delgadillo L, Antón-Tay F, Benetez-King G. Effects of melatonin on microtubule assembly depend on hormone concentration: role of melatonin as a calmodulin antagonist. J Pineal Res 1994;17:55–62.

Iacovitti L, Stull ND, Johnston K. Melatonin rescues neurones from cell death in tissue culture models of oxidative stress. Brain Res 1997;768: 317–26.

Iuvone PM, Gan J. Melatonin receptor-mediated inhibition of cyclic AMP accumulation in chick retinal cell cultures. J Neurochem 1994;63: 124–88.

Kienzl E, Puchinger L, Jellinger K, Linert W, Stachelberger H, Jameson RF. The role of transitional metals in the pathogenesis of Parkinson's disease. J Neurol Sci 1995;134(Suppl.):69–78.

Kim YS, Woo WS, Jin BK, Cho YH, Baik HH, Park CW. Melatonin protects 6-OHDA induced neuronal death of nigro striatal dopamine system. Neuroreport 1998;9:2387–90.

Jin BK, Shin DY, Jeong MY, Gwag MR, Baik HW, Yoon KS, et al. Melatonin protects nigral dopaminergic neurones from 1-methyl-4-phenylpyridinium (MPP+) neurotoxicity in rats. Neurosci Lett 1998;345:61–4.

Ljungberg T, Ungerstedt U. Reinstatement of eating by dopamine agonists in aphagic dopamine denervated rats. Physiol Behav 1976;13:277–83.

Maietta G, Rongioletti F, Rebora A. Seborrheic dermatitis and daylight. Acta Derm Venereol (Stockh) 1991;71:538–93.

Martinez-Cruz F, Pozo D, Osuna C, Espinar A, Marchante C, Guerrero JM. Oxidative stress induced by phenylketonuria in the rat: prevention by melatonin in, vitamin E and vitamin C. Neurosci Res 2002;69: 550–8.

Matsui DH, Machado-Santelli GM. Alterations in F-actin distribution in cells treated with melatonin. J Pineal Res 1997;23:169–75.

Mayo JC, Sainz RM, Antolin I, Rodriguez C. Ultrastructural confirmation of neuronal protection by melatonin against the neurotoxic 6-Hydroxydopamine cell damage. Brain Res 1999;818:271–7.

Mayo JC, Sainz RM, Uria H, Antolin I, Esteban MM, Rodriguez C. Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. J Pineal Res 1998;24:179–92.

Medina-Navarro R, Duran-Reyes G, Hicks JJ. Pro-oxidative properties of melatonin in the in-vitro interaction with the singlet oxygen. Endocr Res 1999;25:263–80.

Melchiorri D, Reiter RJ, Attia PM, Hara M, Burgos A, Nistico G. Potent protective effect of melatonin on in vivo paraquat-induced oxidative damage in rats. Life Sci 1995;56:83–9.

Meléndez J, Meldonado V, Ortega A. Effect of melatonin on β-tubulin and MAO-2 expression in N1E-115 cells. Neurochem Res 1996;21: 653–8.

Merle A, Faucheron JL, Delagrange P, Renard P, Roche M, Pellisier S. Nycthermeral variations of cholecystokinin action on intestinal motility in rats: effects of melatonin and S20928, a melatonin receptor antagonist. Neuropeptides 2000;34(6):385–91.

Messiha FS. Mouse strain dependant effect of amantadine on motility and brain biogenic amines. Arch Int Pharmacodyn Ther 1989;302:74–85.

Minneman KP, Lynck H, Wurtman RJ. Relationship between environmental light intensity and retinal-mediated suppression of rat pineal serotonin-*N*-acetyltransferase. Life Sci 1976;15:1791–6.

Nonno R, Fraschini F, Stankov BM. Methods for the evaluation of drug action at the human melatonin receptor subtypes. Biol Signals Recept 1999;8:32–40.

Nowak JZ, Kazula G, Golembiowski K. Melatonin increases *N*-acetyltransferase activity and decreases dopamine synthesis in light exposed chick retina: in vivo evidence supporting melatonin–dopamine interaction in retina. Neurochemistry 1992;59:1499–505.

Oaknin-Bendahan S, Anis Y, Nir I, Zisapel N. Effects of long term administration of melatonin and a putative antagonist on the aging rat. Neuroreport 1995;6:785–8.

Okun MS, Watts RL. Depression associated with Parkinson's disease Clinical factors and treatment. Neurology 2002;58(Suppl. 1):S63–70.

Olanow CW, Houser RA. The treatment of early Parkinson's disease. In: Marsden CD, Fahn S, editors. The assessment and therapy of Parkinson's disease. U.K.: Parthenin Publishing; 1990. p. 77–88.

Oyanagui Y. Natural antioxidants enhance and prolong the oxyradical/NO-related suppression of dexamethasone of ischemic and histamine paw edema in mice. Inflammation 1997;21:643–54.

Papavasiliou PS, Cotzias GC, Steck AJ, Bell M, Lawrence WH. Melatonin and parkinsonism. JAMA 1972, July 3;221:88–9.

Patterson AT, Vickers C. Sex and strain related effects of melatonin and 5-methoxytryptophol on open field behaviour in paired mice. Behav Brain Res 1984;13:107–13.

Pelligrino LL, Pelligrino AJ, Cushman AJ. Stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.

Price DL, Cork LC, Strubble RG, Kitt CA, Walker LC, Powers RE, et al. Dysfunction and death of neurones in human degenerative neurological diseases and in animal models. Ciba Found Symp 1987;126:30–48.

Reis M, Davis RH, Sideman MB, Plichta ES. Pineal gland and spontaneous activity in rats. J Endocrinol 1963;28:127–8.

Reiter RJ. Oxidative damage in the central nervous system: protection by melatonin. Prog Neurobiol 1998;56:359–84.

Reiter RJ, Tan DX, Cabrera J, D'Arpa D, Sainz RN, Mayo JC, et al. The oxidant/antioxidant network: role of melatonin. Biol Signals Recept 1999;8(1–2):56–63.

Reyes PF. Age related histological changes in the human pineal gland, (The Pineal and Its Hormones). Prog Clin Biol Res 1982;92:253–61.

Rodriguez M, Sosa J, Hernandez G, Mas M. Pineal indoles and testosterone affect exploratory activity of male rats. Experientia 1984;40:397–8.

Rosenthal NE, Sack DA, Jacobsen FM, James SP, Parry BL, Arendt J, et al. Melatonin in seasonal affective disorder and phototherapy. J Neural Transm 1986;21(Suppl.):257–67.

Rowland N. Endogenous circadian rhythms in rats recovered from lateral hypothalamic lesions. Physiol Behav 1976;16:257–66.

Shaw KM. Hypothalamo–pituitary–adrenal function in Parkinson's patients treated with melatonin. Curr Med Res Opin 1977;4(10):743–6.

Shaw KM, Stern GM, Sandler M. Melatonin and parkinsonism. Lancet 1973;1:271.

Schmid HA. Decreased melatonin biosynthesis, calcium efflux, pineal gland calcification and aging: a hypothetical framework. Gerontology 1993;39:189–99.

Stocchi F, Olanow CW. Neuroprotection in Parkinson's disease clinical trials. Ann Neurol 2003;53(Suppl.):S87–97.

Sugden D. Effect of putative melatonin receptor antagonists on melatonin-induced pigment aggregation in isolated xenopus leavis melanophores. Europ J Pharmacol 1992;213:405–8.

Tang YP, Ma YL, Chao CC, Chen KY, Lee HY. Enhanced glial cell line-revived neurotrophic factor mRNA expression upon (−)-deprenyl and melatonin treatments. J Neurosci Res 1998;53:593–604.

Tenn CC, Niles LD. Mechanisms underlying the anti-dopaminergic effect of clanazepam and melatonin in striatum. Neuropharmacology 1997;36: 1659–63.

Waddington JL, Crow TJ. Drug induced rotational behaviour following unilateral intracerebral injection of saline–ascorbic solution: neurotoxicity of ascorbic acid and monoamine independent circling. Brain Res 1979;161:371–6.

Weaver PR. Reproductive safety of melatonin: a "wonder drug" to wonder about. J Biol Rhythms 1997;12:682–9.

Willis GL, Armstrong SM. Orphan neurones and amine access: the functional neuropathology of Parkinsonism and neuropsychiatric disease. Brain Res Rev 1998;27:177–242.

Willis GL, Armstrong SM. A therapeutic role for melatonin antagonism in experimental models of Parkinson's disease. Physiol Behav 1999;66: 785–95.

Willis GL, McLennan CA. Pinealectomy and dopamine replacement therapy in models of Parkinson's disease. Melatonin and Biol Rhythms Sympos Sat Meet XXXIV Cong Physiol Sci Christchurch, New Zealand; 2001a. p. 10.

Willis GL, McLennan CA. Therapeutic effects of bright light therapy in Parkinson's disease: a series of case reports. Melatonin and Biol Rhythms Sympos Sat Meet XXXIV Cong Physiol Sci Christchurch, New Zealand; 2001b. p. O15.

Willis GL, Smith GC. Amine accumulation in behavioural pathology. Brain Res Rev 1985;9:109–32.

Willis GL, Singer G, Evans BK. Intracranial injections of 6-OHDA; comparison of the catecholamine depleting effects of different volumes and concentrations. Pharmacol Biochem Behav 1976;76: 207–13.

Willis GL, Smith GC, Kinchington PC. Peripheral DA receptor blockade facilitates behavioural recovery from nigrostriatal damage. Brain Res Bull 1983a;11:15–9.

Willis GL, Smith GC, McGrath BP. Deficits in locomotor behaviour and motor performance after central 6-hydroxydopamine or peripheral L-DOPA. Brain Res 1983b;266:279–86.

Wirz-Justice A, Armstrong SM. Melatonin: natures' soporific? J Sleep Res 1996;5:137–41.

Youdim MBH, Ben-Schachar D, Riederer P. Is Parkinson's disease a progressive siderosis of substantia nigra resulting in iron and melanin induced degeneration? Acta Neurol Scand 1989;126: 47–54.

Zimmerman RC, Krahn L, Klee G, Delgado P, Ory SJ, Lin SC. Inhibition of presynaptic catecholamine synthesis with alpha-methyl-*para*-tyrosine attenuates nocturnal melatonin secretion in humans. J Clin Endocrinol Metab 1994;79:1110–4.

Zisapel N. Melatonin–dopamine interactions: from basic neurochemistry to a clinical setting. Cell Mol Biol 2001;21:605–16.

Zisapel N, Laudon M. A novel melatonin antagonist effects melatonin-mediated processes in vitro and in vivo. Eur J Pharmacol 1987;136: 259–60.

Zisapel N, Egozi Y, Laudon M. Inhibition of dopamine release by melatonin: regional distribution in the rat brain. Brain Res 1982;246: 161–3.

Zisapel N, Egozi Y, Laudon M. Circadian variations in the inhibition of dopamine release from adult and newborn rat hypothalamus by melatonin. Neuroendocrinology 1985;40:102–8.