

Research report

Compromised circadian function in Parkinson's disease: Enucleation augments disease severity in the unilateral model

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ABSTRACT

Like enucleation, lateral hypothalamic (LH) lesions sever the connection between the retina and the pineal thereby simulating ambient exposure to constant darkness. While LH lesions have been employed to study either circadian function or Parkinson's disease (PD) independently, the application of such lesions to study circadian involvement specifically in this disease has never been attempted. In the present study, unilateral lesions of the LH, which compromise nigro-striatal dopamine (NSD) function, were combined with enucleation ipsilateral or contralateral to the hemisphere where 6-hydroxydopamine was applied. In addition to the observation that hemi-enucleation produced patterns of motor function that were grossly atypical compared to visually intact rats, hemi-enucleation ipsilateral to the side of NSD system denervation produced impairment of horizontal movement, limb retraction, ambulation and spontaneous or L-dopa induced turning. This impairment was more severe than that seen in rats with unilateral 6-OHDA lesions alone. Furthermore, hemi-enucleation contralateral to the side of unilateral NSD system denervation resulted in significantly improved performance on several parameters. While the rate of mortality in rats receiving unilateral 6-OHDA plus ipsilateral enucleation was similar to that occurring after bilateral lesions, it was not accompanied by severe weight loss and wasting that typically occurs in the acute stages of experimental PD. These results demonstrate the importance of the visual and circadian systems in PD and are consistent with reports that identify impaired circadian involvement as a major component in a wide range of neurological and neuropsychiatric conditions.

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1. Introduction

In 1967, Moore et al. [49] embarked upon a classic study to determine the anatomical connections between the retina and the pineal gland. In view of the integratory role that the lateral hypothalamus (LH) serves in homeostatic function [44,53,68,70] and of the large number of ascending and descending fibres that the LH carries [48,69], LH lesions were eventually found to encroach upon the retino-hypothalamic tract (RHT) and thereby compromised pineal function [47]. Like enucleation, LH lesions increased pineal production of the precursor enzyme hydroxyl-indole-*o*-methyl-transferase (HIOMT) and of its product melatonin [47,49]. In essence, for studying circadian function, lesioning the LH served the same purpose as enucleation, with various combinations of these two techniques producing a bilateral effect that was equivalent to exposing an organism to an ambient environment of complete darkness.

Perhaps coincidentally, the LH also plays an important role in various aspects of motor function and in the pathological consequences of neuropsychiatric disorders such as Parkinson's disease (PD) [6,35,44,48,73,74]. In the first instance, it carries the fibres of passage of the nigro-striatal dopamine (NSD) system extending from the cell bodies in the substantia nigra, through the LH and into the corpus striatum, putamen and globus pallidus [48]. Secondly, the LH and its sub-structures contain the highest incidence of Lewy body formation: the primary pathological feature of PD itself [32,35,40,41,73]. Given this important function in PD and that LH lesions plus enucleation compromise circadian function [49] the next logical step was to determine the effect of LH lesions plus enucleation on experimental PD. This approach is justified since PD patients commonly experience circadian-associated symptoms including insomnia, depression, akathisia and REM sleep-behaviour disorder [1,5,10,21,23,25,31,52,62] and intimates that the LH might well be the anatomical substrate that integrates and mediates NSD and circadian system involvement in this disorder.

One line of current research is based on the assumption that pineal function is severely compromised in PD and that melatonin

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tonin secretion is deficient [60,67]. Even though this antioxidative approach has limited theoretical grounding, this assumption provides that melatonin supplementation may serve to correct the melatonin deficiency and prevent the progressive degeneration characterizing this disorder [36–38]. This hypothesis is maintained even in the presence of unequivocal evidence that the melatonin levels are elevated in PD and its models [11,14] and that the ratio of melatonin to dopamine (DA) is elevated at least 6 to 1 in favor of melatonin [71]. Nevertheless, implementation of the experimental design employed by Moore et al. [49] permits the testing of this hypothesis in a systematic manner. While unilateral lesions of the LH produce a less severe form of experimental PD, the strategic combination of LH lesions with unilateral enucleation would produce functional blindness and increase the endogenous secretion of melatonin [4,42,49,64]. If the antioxidative hypothesis is correct, this in turn, should facilitate recovery from experimental PD [17,36,37]. Conversely, if circadian involvement after unilateral LH lesions combined with ipsilateral enucleation adversely affects NSD system function [74,75] then the severity of experimental PD would be hypothesized to be more severe than with unilateral lesions or with enucleation alone. Whichever the outcome, this study would add clarity as to the role of the circadian system in PD and other neuropsychiatric disorders and would help to assess the feasibility of implementing melatonin as a candidate for therapeutic intervention.

2. Materials and methods

Thirty five out bred male, Sprague–Dawley rats were obtained from the Bronowski Institute colony or from Monash University Animal Services. This species was selected on the basis of previous work which employed a S/D derivation to examine the effects of LH lesions on the anatomy of circadian function [49]. While it is acknowledged that albino rats do have compromised visual function [57] the extensive use of this strain in PD research [73] and for studying circadian function [71] justifies the use of this strain for the present purpose. Rats were housed individually in wire mesh cages with standard food pellets (Clarke King®/Barastoc®) made available *ad lib* from a feeding grid. Tap water was made available *ad lib* 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 $22 \pm 2^\circ\text{C}$ with a 12-h light:12-h dark cycle (LD) with lights on at 07:00 h. The room was illuminated with two fluorescent tubes with the intensity of light within each cage averaging 250 lux during the lights on phase of the LD 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.

3. Surgery

After habituation into the colony for at least 7 days, rats were pre-medicated with atropine sulphate (0.06 mg/kg s.c.) and then anaesthetized with a Ketamine® (55 mg/kg)/Xylazine® (10 mg/kg) mixture (i.m.). In 28 animals, the right or left eye was surgically removed using aseptic technique and the wound was then closed with 2 or 3 sutures. Immediately after this, each rat was then placed in a stereotaxic instrument. The site of cannulation for eventual intracranial (i.c.) injection for achieving experimental PD was the posterior lateral hypothalamus (PLH [73]) just rostral to the midbrain/diencephalon border in the bundle of NSD system fibres. A 23 gauge stainless steel cannulae was implanted on one side of the brain just dorsal to the intended site of injection at the coordinates AP = -1.8 mm; L = ± 1.8 mm; D = -6.1 mm. Half of the animals in each group were implanted unilaterally on the left side while the other half were implanted on the right. The injection needle extended 2 mm beyond the cannulae tip in a ventral direction to minimize damage to the injection site [77]. All coordinates were relative to bregma and in the plane of Pellegrino et al. [54]. This position has been found to be effective in produc-

ing severe Parkinsonian-like effects in animals [73–75,78] as the fibres of the NSD traverse this area [48]. An additional group of 7 animals were implanted with intracerebral cannulae but enucleation was not performed. At the completion of intracranial surgery rats were injected with 12 mg/kg Reversine® (s.c.), which was used as a reversal agent for the Xylazine®. All rats were injected with the analgesic Meloxicam® (10 mg/kg, i.m.) at the completion of surgery. Rats were kept warm after surgery and then allowed at least 10 days of recovery before commencing the formal part of the study.

4. Groupings, solutions and injections

4.1. Intracerebral 6-hydroxydopamine (6-OHDA) injections

6-OHDA hydrobromide (Sigma, St. Louis, MO, USA) was mixed in a concentration of $8 \mu\text{g}/\mu\text{l}$ and injected in a volume of $2 \mu\text{l}$ per site. Injections were made at a rate of $1 \mu\text{l}$ per 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 [74,77]. 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.

4.2. Intraperitoneal (i.p.) injections

To induce rotation L-dopa was obtained from a commercial source (Sigma–Aldrich, U.S.A.) and was mixed in distilled, deionized water and then dissolved by adding a few drops of HCl and brought to a final concentration of 50 mg/ml. The peripheral decarboxylase inhibitor Benserazide (R-044602) was also obtained from a commercial source (Sigma–Aldrich, U.S.A.) and mixed in a concentration of 50 mg/ml.

4.3. Treatment groups

There were 5 groups of 7 animals per group employed in the present study.

Group 1 ($N=7$) was not enucleated. This was a standard, unilateral Parkinsonian model preparation with the condition induced by i.c. injection of 6-OHDA on one side of the brain. Damage from neurotoxic administration in the brain was achieved on either the left or right side and this was systematically varied within the group to achieve a balanced design.

Group 2 ($N=7$) was enucleated on the left or right side and this was again systematically varied within the group to achieve a balanced design. Sometime later each animal was rendered Parkinsonian and received an i.c. injection of 6-OHDA via the implanted cannula in the hemisphere ipsilateral to the side of enucleation.

Group 3 ($N=7$) were also enucleated on the left or right side and this was systematically varied within the group. Sometime later each animal was rendered Parkinsonian with an i.c. injection of 6-OHDA via the implanted cannula in the hemisphere contralateral to the side of enucleation.

Group 4 ($N=7$) served as a control group and underwent unilateral enucleation on the left or right side and this was systematically varied as with previous groups to achieve balance. Sometime later, each animal received an i.c. injection of vehicle via the implanted cannula in the hemisphere ipsilateral to the side of enucleation.

Group 5 ($N=7$) served as a second control group and were enucleated on the left or right side and this parameter was systematically varied. Sometime later, each animal received an i.c.

injection of vehicle via the implanted cannula in the hemisphere contralateral to the side of enucleation.

5. Procedure

5.1. Behavioural assessment

Control measurements for all motor parameters were made at least 24 h prior to i.c. injection in all groups. Independent variables were measured during the light and the dark phase of the LD cycle between 10:00–15:00 h and again at 20:00–01:00 h, respectively, with at least 36 h allowed between consecutive measurements. Assessment was first undertaken during the “acute phase” of experimental PD which occurs up to and including day 9 post i.c. injection. The earliest time of testing was undertaken on the fifth day post 6-OHDA. This corresponds to a critical time when animals with bilateral lesions of the NSD become severely affected by this acute form of PD whereby death occurs or spontaneous recovery commences (that is, recovery without the aid of artificial feeding). While animals with unilateral lesions do not experience this life threatening form of the disease, this is still the time when the most severe deficits are exhibited. The second time of measurement was between days 12–15 after the induction of PD and is termed the “recovery phase” of testing. This period is defined as the time during which recovery from the acute effects of the neurotoxic insult and homeostatic control has returned (after day 9 post i.c. injection) whereby animals are capable of regulating nutritive intake on their own and brain lesions are no longer life threatening. These two phases of testing have been defined previously [73,78].

Locomotion and rearing were measured with the aid of a 900 mm(length) × 500 mm(width) × 300 mm(height) PVC box fitted with machine vision with motion detection capabilities. The total number of movements within the horizontal plane and the number of rearing associated movements in the vertical plane during each 10 min test session were measured and recorded with the aid of specialized software. A series of three motor reflex tests were performed immediately at the conclusion of the open field test [74]. These tests included the latency to retract the left and right front limbs when they were elevated 25 mm from the table surface, the latency to step up or down from a raised platform when the rear torso was elevated 25 mm and the latency to ambulate outside of a 90 mm × 170 mm rectangle. These tests are derivations of those described previously [6,74]. The test chamber and all surfaces and apparatus were thoroughly washed between the testing of each animal to avoid contamination which may cause distraction during testing. Body weight was measured on intermittent days commencing at about 10:00 h for at least 3 days prior to and 29 days after 6-OHDA injection.

Commencing on the third week after i.c. injection each rat was placed in a rotometer for a 10 min period and the number of clockwise and counterclockwise rotations was measured. Spontaneous turning was measured during the light phase and the dark phase of the LD cycle and no drugs were administered during these sessions to induce turning. This light phase and dark phase testing of spontaneous turning was repeated during the fourth week post i.c. injection. On the fifth and sixth week post-injection the number of clockwise and counterclockwise rotations during the light and dark phase was measured 45 min after the i.p. injection of L-dopa (50 mg/kg), which was preceded 30 min earlier by an i.p. injection of the peripheral decarboxylase inhibitor Benserazide (50 mg/kg). The number of turns was determined in relation to the side of the brain receiving i.c. injection and this was translated to ipsi- and contralateral forms of rotation.

The statistical analyses employed were either one-way ANOVA with Tukey's HSD for post hoc multiple comparisons or Chi-Square

Test (linear by linear association) (SPSS 14.0 for Windows) Levine's test for homogeneity of variance was performed and if significant, or if the data were badly skewed or if *n* was dramatically reduced as a result of natural attrition due to the acute effects of the experimental form of the disease, non-parametric analyses were employed. There were several crucial comparisons utilized in the present study. The first was between the hemi-PD group (group 1-normal vision) and the hemi-PD group with ipsilateral enucleation (group 2). The second was between the hemi-PD group (group 1-normal vision) PD and the hemi-PD group with contralateral enucleation (group 3). The third and fourth order comparisons were made between both hemi-PD (groups 3 and 4) and their respective controls receiving i.c. vehicle and either ipsi- or contralateral enucleation (groups 4 and 5). In some instances data transformation was employed when the distribution for a given set of scores was not normal. Analysis was performed on ratio scores obtained by dividing the test score by the control score. As the hypothesis permitted prediction of the direction of the expected outcome, a one sided test was employed with exact significance. Alpha was set *a priori* at $p < 0.05$ and alpha values ranging from 0.06 to 0.09 indicated significant trends.

5.2. Histological assessment

At the end of the study all remaining animals were sacrificed with pentobarbitone sodium (325 mg/ml) with each animal being injected with 0.5 ml of the stock solution. The entire brain was removed and placed in 10% formalin. After fixing for at least 2 weeks, brains were sectioned and examined 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 and published previously [54]. Given that the present experiment was an anatomical study and injection placement was essential to demonstrate anatomical consistency between this and previous work involving the LH [49] DA analysis was not performed. However, given our previous experience with these lesions (for reviews see [74,78], and the experience of others [6,48,49,73]), it is reasonable to assume that such lesions not only affect NSD function but that they induce a reliable form of experimental PD. Furthermore, because of the number of unexpected, spontaneous deaths in the enucleation plus ipsilateral lesion group, a meaningful DA analysis for comparison between groups could not be performed.

6. Results

Prior to assessment of the dependant variables resulting from combining enucleation with PD, the effects of enucleation alone on horizontal and vertical movement were assessed on the basis of control, pre-Parkinsonian performance (Fig. 1). While the mean number of horizontal movements was higher during the day than at night for all enucleated animals combined, this difference was not significant indicating that typical day/night differences in horizontal movement were not seen (day = 321 ± 23.4 vs. night 244 ± 39 ; $F = 17.206$, d.f. = 3,108, $p = 0.392$). Conversely, vertical movement scores during the day in enucleated animals was lower than that exhibited during the night suggesting normal circadian rhythmicity for this parameter (day = 441 ± 37.1 vs. night = 572 ± 36.4 ; $F = 17.206$, d.f. = 3,108, $p = 0.042$). However, when the total amount of vertical movement in enucleated animals during the day was compared to the amount of horizontal movement they engaged in a disproportionately higher amount of vertical movement and this trend was significant (day vertical vs. day horizontal, 441 ± 37.1

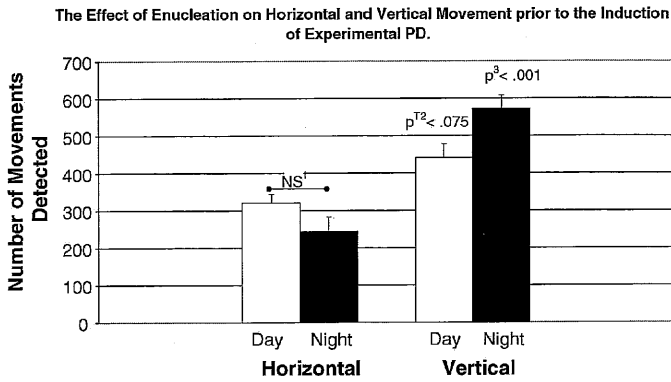


Fig. 1. Prior to the induction of experimental PD the effects of enucleation on horizontal and vertical movement were assessed in all enucleated rats. While the mean number of horizontal movements during the day was greater than during the dark phase of the light/dark cycle (left traces) this difference was not significant. From a historical perspective, this is the complete opposite of the usual expectation since rats are nocturnal animals and their daytime activity is routinely reported to be significantly lower than that occurring at night [61]. The right traces demonstrate the changes in vertical movement during the day and night in hemi-enucleated rats. While the vertical movement during the day in enucleated animals was significantly lower than that expressed during the night ($p=0.042$), the number of vertical movement during the day and night was significantly greater than the number of horizontal movements exhibited during those time periods (p^2 and $p^3 < 0.001$ in both cases). From previous research [73,75] the number of horizontal movement would be expected to outnumber the vertical movements in normal animals and the discrepancy is thereby attributed to enucleation.

vs. 321.3 ± 23.5 , respectively, $F=17.206$, d.f. = 3,108, $p=0.075$ -trend). Similarly, the amount of vertical movement at night was also disproportionately high in relation to horizontal movement and this difference was also significant (572 ± 36.4 vs. 321.3 ± 27 ; $F=17.206$, d.f. = 3,108, $p < 0.001$).

Fig. 2 depicts the effects of enucleation on horizontal movement in hemi-Parkinsonism. When tested in the day, during the

acute phase of the disease, no significant changes in horizontal movement were exhibited. All groups of rats decreased their horizontal movement score, with their performance ranging from 100 to 250 counts below their control day performance. This is often observed shortly after i.c. injection and may be due to the injection procedure or to the handling process itself and is observed shortly after the injection, if at all. However, during the test night the mean number of counts increased to a level that was above that of their control performance, with the exception of those rats that had been enucleated on the side ipsilateral to the side where the NSD systems was denervated by an injection of 6-OHDA. While this decrease represents a significant trend toward more severe impairment of horizontal movement when compared to rats with PD alone ($\chi^2=2.136$, $n=14$, d.f. = 1, $p=0.089$), those rats with contralateral enucleation and experimental PD were significantly better than Parkinsonian rats with ipsilateral enucleation ($\chi^2=3.689$, $n=14$, d.f. = 1, $p=0.023$). While Parkinsonian rats with ipsilateral enucleation were not significantly better than their non-PD controls at this time, their horizontal activity was significantly reduced when compared to rats with contralateral enucleation plus PD ($\chi^2=3.925$, $n=14$, d.f. = 1, $p=0.021$). During the recovery phase of experimental PD, during the day, there was no difference between animals with PD, PD plus ipsilateral enucleation, PD plus contralateral enucleation or control animals with contralateral enucleation ($F=0.962$, d.f. = 4,30, $p=0.462$). However rats with PD plus ipsilateral enucleation showed significantly impaired horizontal movement when compared to their ipsilateral controls and the difference was highly significant ($\chi^2=5.743$, $n=14$, d.f. = 1, $p=0.004$). There were no significant differences observed between any of the 5 groups during testing at night during the recovery phase ($F=1.844$, d.f. = 4,30, $p=0.146$).

The effects of ipsi- or contralateral enucleation on the ability to retract a limb in experimental hemi-parkinsonism are expressed in Fig. 3. During the day of the acute phase of testing, PD and Parkinsonian rats with ipsilateral enucleation were not significantly

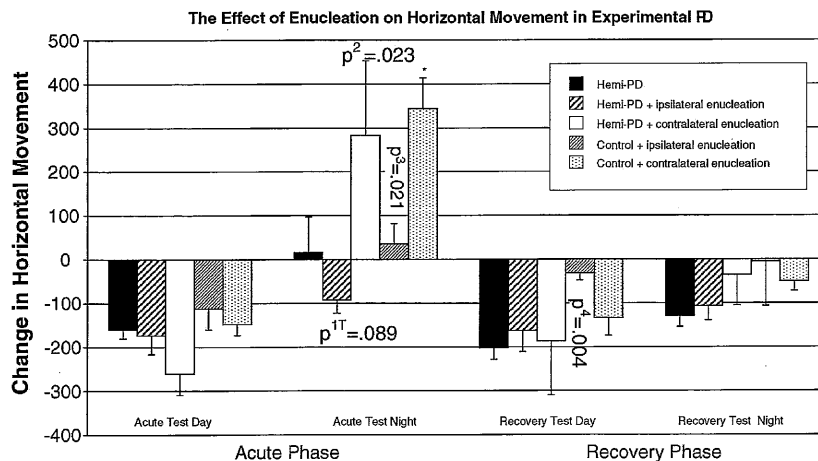


Fig. 2. The effect of enucleation on horizontal movement during the acute and recovery phase of experimental PD. Measurements during the acute phase occurred prior to the ninth day after induction of PD with intracerebral 6-OHDA, during which time spontaneous death can occur in some animals. Testing during the recovery phase occurred between days 12–15, post 6-OHDA and during which spontaneous recovery is seen. Animals were maintained in a 12-h light:12-h dark cycle with testing during the day occurring between the hours of 10:00 and 14:00h and testing during the night between 22:00 and 02:00h. When acute phase performance was compared to pre-6-OHDA control performance, only the rats with unilateral PD plus ipsilateral enucleation showed a decrement. This trend was significant suggesting impairment of horizontal movement in rats with PD and ipsilateral enucleation (large diagonal stripes; $p^{1T}=0.89$) when compared to those with PD only (solid black). Rats with enucleation contralateral to the side where unilateral PD was induced (open bar) showed an 8-fold increase in horizontal movement when compared to rats with PD plus ipsilateral enucleation and this was significant (solid black; $p^2=0.023$). In an additional comparison between PD rats with ipsilateral enucleation and vehicle injected control rats with contralateral enucleation showed a 6-fold difference in horizontal movement and this difference was also significant ($p^3=0.021$). Control rats with ipsilateral enucleation (mottled) were significantly better than PD rats with ipsilateral enucleation (asterisk, $p < 0.05$). During the night of the acute phase only the control rats with ipsilateral enucleation were significantly different to their experimental counterpart ($p^4=0.004$). No significant difference between the groups was observed during the night of the recovery phase. The mean values represent the change in horizontal movement comparing pre and post 6-OHDA performance. The T-bars represent the standard error of the mean.

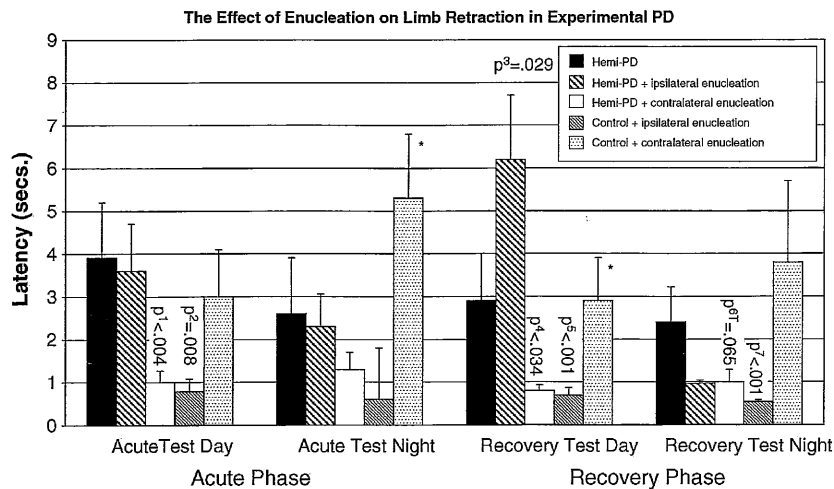


Fig. 3. The effect of enucleation on latency to retract a limb during the acute and recovery phases of experimental PD. Measurements during the acute phase occurred prior to the ninth day after induction of PD with i.c. 6-OHDA, during which time spontaneous death can occur. Testing during the recovery phase occurred after day nine usually between days 12 and 15 post 6-OHDA and during which spontaneous recovery is seen. Animals were maintained in a 12-h light/12-h dark cycle with testing during the day occurring between the hours of 10:00 and 14:00 h and testing during the night between 22:00 and 02:00 h. During the light phase of the light/dark cycle (test day) PD rats bearing contralateral enucleation retracted more than 3 times faster than those with PD only and this was significant (open bars, $p^1 = 0.004$) as did vehicle injected control rats with ipsilateral enucleation (small diagonal stripes) showed a similar difference compared to intact rats with PD and this was highly significant ($p^2 = 0.008$). During the dark phase of the light/dark cycle during the acute phase testing only the control rats with contralateral enucleation were significantly different to their control counterparts with ipsilateral enucleation (mottled bar; asterisk $p < 0.05$). During the recovery day test PD rats with ipsilateral enucleation were almost twice as slow on latency to retract than were those with PD alone, (Diagonal stripes versus solid black, $p^3 = 0.029$). Conversely, rats with PD plus contralateral enucleation were significantly better than those with PD alone ($p^4 = 0.034$) while vehicle injected control rats with ipsilateral enucleation were nearly 3 times faster and significantly better than their contralateral counterparts with experimental PD (small diagonal stripes; $p^5 < 0.001$). Vehicle injected control rats with contralateral enucleation were significantly impaired compared to their Parkinsonian counterparts (mottled bar; asterisk $p < 0.05$). During the night test of the recovery phase there was a significant trend depicting a tendency for PD rats with contralateral enucleation to perform better than PD rats ($p^{6T} = 0.065$) while vehicle injected control rats with ipsilateral enucleation were significantly faster to than their PD counterparts ($p^7 < 0.001$). The mean values are represented with the T-bars representing the standard error of the mean.

different in their performance. However, rats with contralateral enucleation plus PD were significantly faster (1.0 ± 0.27) than rats with PD alone (3.9 ± 1.3 ; $\chi^2 = 5.743$, $n = 14$, d.f. = 1, $p = 0.004$). Control rats receiving i.c. vehicle plus ipsilateral enucleation (mean = 0.79 ± 0.3) were significantly better than their Parkinsonian counterparts with ipsilateral enucleation (3.6 ± 1.1 ; $\chi^2 = 4.936$, $n = 14$, d.f. = 1, $p = 0.008$). During the night of the acute phase testing, rats in all groups were similar in latency to retract with the exception of control animals with contralateral enucleation and they were significantly impaired compared to ipsilaterally enucleated control rats (respectively, 5.3 ± 1.5 vs. 2.6 ± 1.3 ; $F = 3.309$, d.f. = 4,65, $p = 0.011$).

During the recovery phase of testing, during the day, rats with ipsilateral enucleation plus PD were significantly more impaired at limb retraction (6.2 ± 1.5) compared to rats with PD only (2.9 ± 1.1 ; $\chi^2 = 3.498$, $n = 28$, d.f. = 1, $p = 0.029$). In addition PD rats with contralateral enucleation (0.8 ± 0.13) were significantly faster at retracting a limb than were those with PD alone (2.9 ± 1.1 ; $\chi^2 = 3.414$, $n = 28$, d.f. = 1, $p = 0.034$). Parkinsonian rats with ipsilateral enucleation were significantly impaired (mean = 6.2 ± 1.5) compared to their control counterparts with i.c. vehicle plus ipsilateral enucleation and this was highly significant, (0.69 ± 0.18 , $\chi^2 = 9.064$, $n = 28$, d.f. = 1, $p < 0.001$). Parkinsonian rats with contralateral enucleation (0.8 ± 0.13) were also significantly faster than their control counterparts receiving i.c. vehicle plus contralateral enucleation at this time (3.0 ± 1.1 , $\chi^2 = 3.717$, $n = 28$, d.f. = 1, $p = 0.012$).

At night testing during the recovery phase there was a significant trend for rats with PD alone to take longer to retract a limb (2.4 ± 0.82) than did PD rats with contralateral enucleation (1.3 ± 0.4 ; $\chi^2 = 2.347$, $n = 28$, d.f. = 1, $p = 0.065$). Control rats receiving intracerebral vehicle plus ipsilateral enucleation (0.97 ± 0.07) were significantly better than their Parkinsonian counterparts with ipsilateral enucleation (1.0 ± 0.29) and the differ-

ence between the two groups was highly significant ($\chi^2 = 14.908$, $n = 28$, d.f. = 1, $p < 0.001$).

The latency to step down from an elevated platform when the rear torso was elevated was not significantly changed by enucleation in any of the groups tested (results not shown). The average time required to perform this task ranged from 3 to 7 s with the longest times exhibited by the PD only group, however none of the differences were significant.

The effects of enucleation on the ability to ambulate are expressed in Fig. 4. During day testing in the acute phase of experimental PD, Parkinsonian rats with ipsilateral enucleation were significantly more impaired (17.7 ± 4.2) compared to rats with PD alone (5.8 ± 2.3) and this difference was highly significant ($\chi^2 = 4.370$, $n = 14$, d.f. = 1, $p = 0.017$). Parkinsonian rats with contralateral enucleation (6.2 ± 1.5) were not significantly different on this parameter from PD rats (5.8 ± 2.3) but they were significantly quicker than PD rats with ipsilateral enucleation (17.7 ± 4.2 , $F = 4.131$, d.f. = 4,30, $p = 0.055$). Parkinsonian rats with ipsilateral enucleation were significantly impaired in ambulation (mean = 17.7 ± 4.2) compared to their control counterparts with i.c. vehicle plus ipsilateral enucleation (3.3 ± 1.0) and this difference was highly significant, ($F = 4.131$, d.f. = 4,30, $p = 0.01$). Ambulation at night during the acute phase or during the day or night during the recovery phase of the disease does not yield any significant differences between any groups.

The effect of enucleation on spontaneous rotation is expressed in Fig. 5. When testing occurred during the day, there was no significant difference between Parkinsonian rats with ipsilateral enucleation and PD alone, however, the amount of ipsilateral turning in rats with hemiparkinsonism plus contralateral enucleation (12.6 ± 1.3) was significantly greater than that seen in animals with pure hemiparkinsonism (6.4 ± 1.1 ; $F = 3.772$, d.f. = 4,65, $p = 0.045$). When a similar comparison is made between the PD group

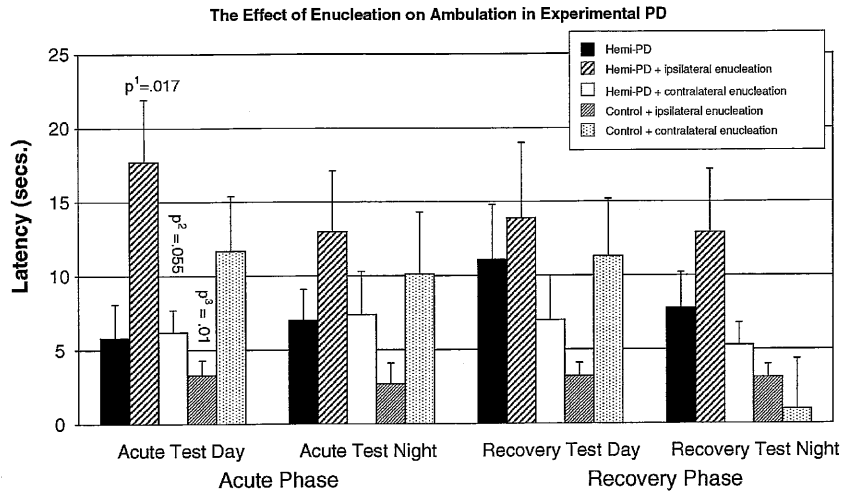


Fig. 4. The effect of enucleation on latency to ambulate from a prescribed area during the acute and recovery phase of experimental PD. Measurements during the acute phase occurred prior to the ninth day after induction of PD with i.c. 6-OHDA, during which time spontaneous death can occur in some animals. Testing during the recovery phase occurred after day nine usually between days 12 and 15 post 6-OHDA and during which spontaneous recovery is seen. Animals were maintained in a 12-h light:12-h dark cycle with testing during the day occurring between the hours of 10:00 and 14:00 h and testing during the night between 22:00 and 02:00 h. During the acute test day PD rats bearing ipsilateral enucleation were more than three times slower on this task than were those with PD and intact vision (diagonal striped bars, $p^1 = 0.017$). PD rats with contralateral enucleation were significantly faster than PD rats with ipsilateral enucleation at this time (open bars; $p^2 = 0.055$), while vehicle injected control rats with ipsilateral enucleation were significantly faster than their Parkinsonian counterparts with intact vision (small diagonal stripes, $p^3 < 0.01$). At all other times of testing during the acute and recovery phases of experimental PD no significant differences in latency to ambulate were observed. The mean values are shown with the T-bars representing the standard error of the mean.

(6.4 ± 1.1) and vehicle injected controls with ipsilateral enucleation (12.6 ± 1.3) an increase in the ipsilateral turning ratio is also seen ($F = 3.772$, $d.f. = 4,65$, $p = 0.021$). It was also noted that ipsilateral turning decreased significantly in rats with PD plus ipsilateral enucleation compared to vehicle injected control rats with ipsilateral enucleation (7.6 ± 1.9 vs. 12.6 ± 1.3 ; $\chi^2 = 3.457$, $n = 28$, $d.f. = 1$, $p = 0.030$).

During night testing, contralateral turning in PD rats with ipsilateral enucleation was significantly higher (16.2 ± 1.7) when compared to their vehicle injected controls with ipsilateral enu-

cleation (9.6 ± 0.77 ; $\chi^2 = 7.771$, $n = 28$, $d.f. = 1$, $p = 0.002$). Note that this was the converse of the ipsilateral/contralateral ratio seen during the day, with Parkinsonian rats bearing ipsilateral enucleation. The ratio of contralateral to ipsilateral rotations exhibited by pure Parkinsonian rats (15.9 ± 1.8) was also greater than that of control rats with ipsilateral enucleation (9.6 ± 0.77) and this was also significant ($\chi^2 = 6.709$, $n = 28$, $d.f. = 1$, $p = 0.002$).

L-Dopa induced turning was also affected by enucleation and this is depicted in Fig. 6. With the cut off score set at 20 rotations per hour (3.3 per 10 min test) the number of rats that met

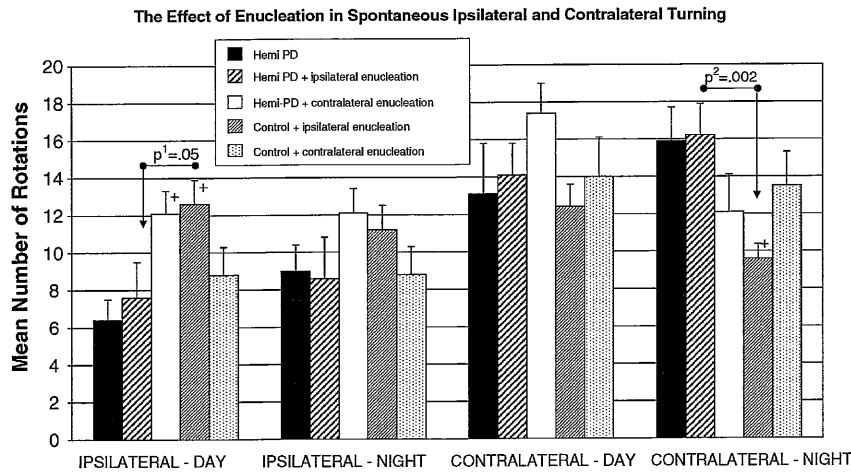


Fig. 5. The effect of enucleation on spontaneous ipsilateral and contralateral turning during the day and night in experimental PD. On the third week after intracerebral 6-OHDA injection testing was commenced at 01:00 h of the light/dark cycle while night testing commenced at 22:00 h. All animals were tested twice during the day and twice during the night over a two-week period. During day testing, the amount of ipsilateral turning was significantly increased in PD rats bearing contralateral enucleation (open bars) when compared to PD rats with normal vision (closed bars; comparison marked with a (+) indicates $p < 0.05$). Vehicle injected control rats with ipsilateral enucleation (small diagonal bars) also showed more ipsilateral turning than PD rats without enucleation (closed bars; comparison marked with a (+) indicates $p < 0.05$). Enucleated rats with ipsilateral Parkinsonism (diagonal bar) also showed decreased turning when compared to enucleated, vehicle injected controls (small diagonal bar) when tested on this parameter during the day (diagonal bars: black arrow depicts comparison; $p^1 < 0.05$). The only other significant difference observed at any other time of testing was an increase in contralateral turning during the night. A significant increase in contralateral turning was observed between PD rats with ipsilateral enucleation (diagonal bar) and their vehicle injected controls bearing ipsilateral enucleation (small diagonal bar; black arrow depicts comparison; $p^2 = 0.002$). Note that the relationship between the ipsi- and contralateral turning ratio for these two groups is the completely reversed during the day versus the night tests. Otherwise, no other changes were seen in any of the other groups. The mean values are represented with the T-bars representing the standard error of the mean.

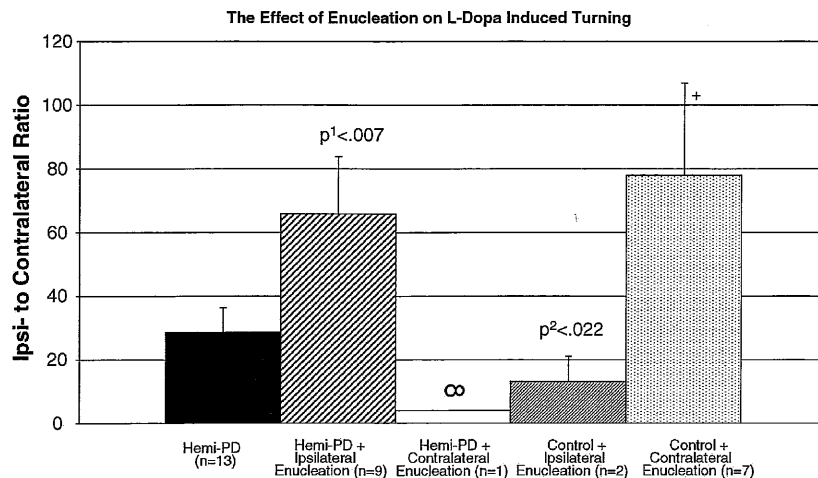


Fig. 6. The effect of enucleation on L-dopa induced ipsilateral turning during the recovery phase of experimental PD. On the fifth and sixth week post 6-OHDA injection the number of clockwise and counterclockwise rotations during the light and dark phases was measured 45 min after the i.p. injection of L-dopa (50 mg/kg), which was preceded 30 min earlier by an i.p. injection of the peripheral decarboxylase inhibitor Benserazide (50 mg/kg). The number of turns was determined in relation to the side of the brain receiving intracerebral injection and was expressed as ipsi- or contralateral forms of rotation. The bar graphs shown depict the mean ratio of ipsilateral to contralateral turns in rats that met the criterion of turning at least 20 turns per hour (≥ 3.3 turns per 10 min test). Rats with PD plus ipsilateral enucleation (Striped bar) showed more than a two-fold predominance of ipsilateral turns compared to those with PD alone (solid bar; $p^1 = 0.007$) while the vehicle injected controls with ipsilateral enucleation showed significantly less turning rats with ipsilateral enucleation plus PD (small diagonal stripes; $p^2 = 0.022$). Vehicle injected control rats with contralateral enucleation engaged in a significantly higher ratio of ipsi to contralateral turning than did rats with PD alone and this was significant ($p < 0.05$ marked with +). The number of rats reaching the set criterion of ≥ 3.3 turns per 10 min test is indicated in the parentheses. Infinity indicates that the number of rats in the PD plus contralateral enucleation group meeting this criteria was so low ($n=1$) that meaningful statistical analysis was not possible. The T-bars representing the standard error of the mean.

this criterion were as follows for the ipsi- to contralateral ratio: PD Group, $n=13$; PD with ipsilateral enucleation, $n=9$; PD with contralateral enucleation, $n=1$; Controls plus ipsilateral enucleation, $n=2$; Controls plus contralateral enucleation, $n=7$. The ratio of ipsi- to contralateral turning was significantly increased at all time periods combined in Parkinsonian rats with ipsilateral enucleation (65.8 ± 18) compared to rats with PD alone (26.6 ± 7.7) and this difference was highly significant ($\chi^2 = 5.908$, $n=22$, $d.f. = 1$, $p = 0.007$). When ipsilaterally enucleated PD rats were compared to their non-PD controls with ipsilateral enucleation (13.0 ± 8) this difference was also significant ($\chi^2 = 3.046$, $n=10$, $d.f. = 1$, $p = 0.022$). Control rats without PD but with contralateral enucleation (77.9 ± 29.4) were also significantly impaired when compared to the group with only PD ($\chi^2 = 3.717$, $n=20$, $d.f. = 1$, $p = 0.036$). Only one animal in the PD plus contralateral enucleation group reached the criterion of 3.3 rotations per 10 min test so statistical comparisons between this and other groups could not be made. Calculation of the contra- to ipsilateral ratio in all five groups produced values that were so low that the criterion set above was reached on only 3 out of 70 occasions and statistical testing was not possible.

Fig. 7 depicts the changes in body weight for the duration of the study in all 5 groups tested. For the 6 days period following 6-OHDA injection the change in body weight was not significantly different between the 5 groups tested ($F = 0.639$, $d.f. = 4, 100$, $p = 0.636$). This suggests that the severity of PD induced by 6-OHDA was similar between groups. When a comparison was made between all groups for the last two days of measurement no significant difference was found ($F = 0.327$, $d.f. = 4, 65$, $p = 0.859$).

At the completion of the study between the sixth week and seventh week post 6-OHDA, spontaneous mortality was observed in the PD plus ipsilateral enucleation group. During the two week period between completion of the study and preparation of tissue for histological examination a total of 4 of the 7 animals were found dead in their cage (57%). No other incidences of spontaneous death were observed in any of the other groups. Body weight was maintained at a similar level in all groups so the observed deaths were not predictable in this regard. Fig. 8 depicts the mortality rate

observed in the present study in relation to several other studies utilizing the bilateral model of PD.

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 in all five groups. The anatomical placement of injections extended caudally from -1.8 mm posterior to bregma, just in front of the nigra and extended rostrally just beyond a position -1.4 mm from bregma. The most dorsal position of necrosis extended to the level of the ventral aspect of the medial lemniscus and the zona incerta while injection sites extended ventrally to the level of the arcuate nucleus at the tip of the internal capsule. The injection sites

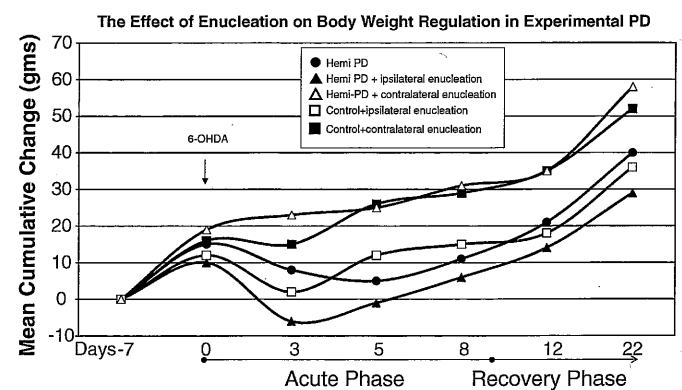


Fig. 7. The effect of enucleation on body weight regulation in experimental Parkinson's disease for six days prior to and 22 days after the induction of PD using intrahypothalamic injections of 6-OHDA. The mean cumulative change in body weight for the days of observation is indicated. Measurement commenced on the seventh day prior to intracerebral injection and then again on day 0 indicated by the black vertical arrow. Measurements were again taken on days 3, 5, 8, 12 and 22 post 6-OHDA. The acute phase of the disease is represented by the black line extending from day 5 to 9 while the recovery phase is represented by the line extending from day 9 to the end of the study. Statistical analysis of the change in body weight during the 3 days of measurement on days 3, 5 and 8 (acute phase) post 6-OHDA and for days 12 and 22 (recovery phase) post 6-OHDA indicated that no significant difference in body weight occurred.

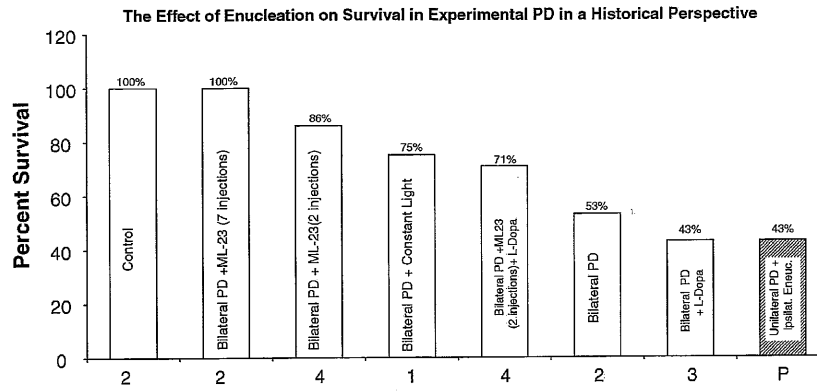


Fig. 8. The effect of enucleation on mortality expressed within a historical context. The mortality of rats receiving various treatments that alter melatonin function in experimental PD is represented. The results are obtained from 4 studies published between the years of 1998 and 2005 and include results from the present study. Publication 1 utilizes various methods for antagonizing melatonin and examines the effect upon the bilateral model of experimental PD. Publication 2 utilized the melatonin antagonist ML-23 in 7 consecutive doses to recover experimental PD in the bilateral model during the acute phase of experimental PD. Publication 3 utilizes the bilateral model and examines the effect of traditional L-dopa treatment on pineal function in the bilateral model of PD. Publication 4 examines the dose dependant effects of ML-23 in the bilateral and acute models of PD and the present study (P) examines the effect of enucleation on the expression of symptoms in the unilateral model. The citation from which the date was taken is expressed in parentheses beneath each bar graph). Mortality is expressed in percent survival and the value for each graph is represented above each trace. (ipsilat. = ipsilateral; enuc. = enucleation).

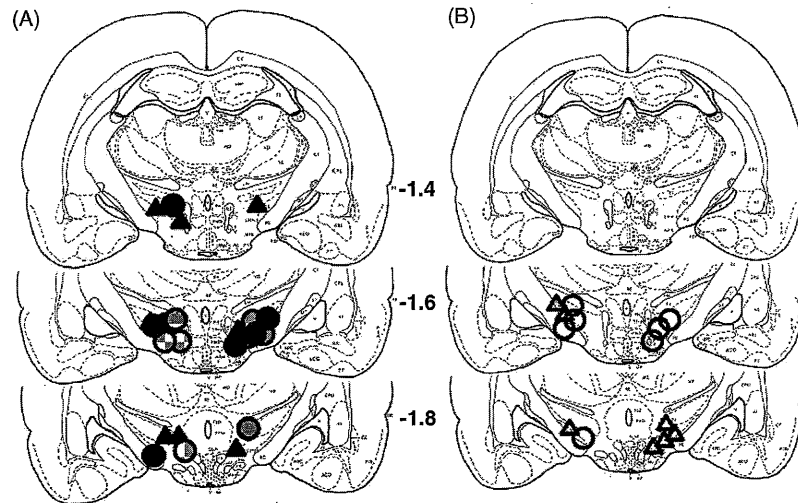


Fig. 9. Histological plates depicting the sites of injection of 2 μ l of an 8 μ g/ μ l solution of 6-hydroxydopamine or of 2 μ l vehicle in cannulated rats that underwent enucleation. In Plate A, 6-OHDA injected rats without enucleation are depicted by the solid black circles, and those receiving 6-OHDA plus ipsilateral enucleation are depicted by circles with diagonal bans, while those receiving intracerebral vehicle plus ipsilateral enucleation are depicted by the solid black triangles. In plate B, the open circles represent rats receiving intracerebral 6-OHDA plus contralateral enucleation while the open triangles depict rats injected with vehicle and contralateral enucleation. The centres of lesions were located 1.4–1.8 mm posterior to bregma in the plane of the de Groot coordinate system as depicted in Pellegrino et al. [54]. The sites of injection were similarly positioned for each group to predict a similar pattern of diffusion of 6-OHDA to axons of the adjacent NSD system and a homogenous effect of this neurotoxin may be assumed [77]. This would produce a spread of damage that is similar to other studies employing 6-OHDA to induce depletion of nigro-striatal DA [44,77].

were similarly positioned for each group so as to permit a similar pattern of diffusion of 6-OHDA to the adjacent NSD system and a homogenous effect of this neurotoxin might be assumed [76].

7. Discussion

These results demonstrate that the visual system plays an important role in the aetiology and progression of PD. Reports that visual function is impaired in PD are numerous but these deficits are attributed to the disease process and they are regarded as consequential to the primary loss of NSD function [3,45,65]. Conversely, the current findings suggest that loss of visual function may not only enhance the severity of motor deficits in PD, but that compromised visual function may even initiate and contribute to the ongoing process of NSD system degeneration. For example, in early anatomical studies examining the connections of the visual pathways, animals were enucleated with the intent of producing

traceable degenerative fibres that extended into the diencephalon and midbrain [26,30,48]. In those early studies the connections of the accessory optic system and the RHT were examined and defined. While these techniques were gross they did demonstrate that loss of retinal function could produce degenerative changes that extended deep into the brain. It is interesting to note that many of the brain sites specifically affected by experimental enucleation [26,43,47,81] also happen to be important in various aspects of PD [23,32,40,41,51,72]. Other studies suggest that retinal degeneration can produce “down stream” effects including degenerative effects in the midbrain and hypothalamus [26,56,63], transport of melatonin down the optic tract and into the brain [28] or enhanced secretion of melatonin [15,34] and it is such a change that may contribute to the degenerative events characterizing PD and other neuropsychiatric disorders [71]. Similarly, the retina may provide a port-of-call for neurotoxins such as 6-OHDA or Paraquat [9,16] that do not ordinarily cross the blood-brain barrier, and may be

the place where they may exert their initial neurotoxic effect. The degenerative process may then progress to critical diencephalic and mesencephalic structures and produce degenerative changes similar to those occurring in PD and its models [63,73]. Classic examples of this are found in studies such as those where retinal trauma induced by air pressure produced optic tract degeneration which progressed to the midbrain-diencephalon border [56]: an area critical in the development of neuropathological changes that characterize PD [73].

While the focus of the current study was on the interaction of enucleation with LH lesions, an interesting question that arises is the effect of hemi-enucleation alone on motor performance. While the design of the present experiment precludes a direct comparison of enucleated vs. normal vision, the performance of hemi-enucleated rats on motor function could be compared to the outcome of published studies on parameters of normal day/night activity in visually intact rats. For example given that rats are predominantly nocturnal and are horizontal movers, it is commonly observed that their horizontal activity is greater in the dark than in the light phase [61]. However, when hemi-enucleated rats were tested prior to the induction of PD, the amount of horizontal movement exhibited in the dark was equal to or less than that occurring during the day (Fig. 1). Furthermore, the expression of vertical movement is substantially less than that of horizontal movement in these species when their visual system is intact [73,74], but after hemi-enucleation the expression of horizontal movement is significantly reduced below that of vertical movement. It is interesting how similar movement altering effects of enucleation were also seen after unilateral i.c. injections of vehicle (see Figs. 3, 4 and 6) and compared to other groups such as PD plus ipsilateral enucleation the results were quite variable. For example, in enucleated, vehicle injected control animals limb retraction was slowed during both the acute and recovery phases during day and the night testing. Similarly, these animals showed impaired ambulation during the day and night of the acute phase but only during the day of the recovery phase. The amount of spontaneous ipsilateral turning was decreased during the day while ratio of ipsi to contralateral turning was enhanced. No such effect was observed with the i.c. vehicle plus ipsilateral control group illustrating the robust, but conflicting effects that enucleation itself can have on motor function making it difficult to fully appreciate the effects of 6-OHDA. Perhaps the effects of 6-OHDA and enucleation employed in the current study have a synergistic effect on melatonin secretion [49]. Blinding has been shown to produce DA deficiency and to increase striatal 5HT, both of which might enhance melatonin secretion [50]. Such effects in the presence of circadian modulation of DA function [39] may account for the variability observed in the present results and in the clinical syndrome, see ref. [71]. This illustrates the complexity of effects induced by such DA depleting lesions in the presence of compromised visual function and how their synergistic effect is virtually unexplored.

Handedness deserves some consideration particularly when the unilateral destruction of various brain systems is employed as the independent variable [27]. While the purpose and design of the present study somewhat precludes the importance of this factor in the endocrine parameters studied, an attempt to control for the effects on laterality was taken into account by systematically varying ipsilateral and contralateral enucleation in respect to the unilateral location of brain lesions within each group tested. This design would distribute any effects due to lateralization equally within each group. Attempts to analyze handedness within such a design would render the groups so small that meaningful analysis of results could not be performed. Nevertheless, cursory examination of the data for each group employed indicates that laterality probably had little, if any effect on the obtained results. Indeed, if

laterality did have an effect on any of the parameters studied then they could have only been minor as the difference between the groups for variables such as ambulation, latency to retract and rotation were of a large magnitude and these were highly significant. While laterality is an important variable to consider as it extends across species [55], future studies should be designed to specifically address this in relation to the involvement of the visual system in motor function.

The unilateral model of PD has been used for more than two decades to screen anti-Parkinsonian drugs for potential clinical development [69]. This model was adopted as a standard tool because the bilateral model produced severe impairment of motor function to the point of death. Unilateral lesions to the NSD system avoided this mortality with rigorous contra- or ipsilateral turning in response to DA activating drugs and this was attributed to the development of denervation supersensitivity of DA receptors. In the present study, more than a two-fold difference was observed on 6-OHDA induced rotation between animals bearing typical unilateral 6-OHDA lesions of the NSD system and those bearing the same lesion plus ipsilateral enucleation. Conversely, when lesions were placed contralateral to the side of enucleation the resulting deficits were significantly less severe. In 13 out of 14 rotation tests performed on the PD group without enucleation, the criterion of at least 20 ipsilateral turns per hour was achieved, while only 1 in 14 cases occurred in the PD plus contralateral enucleation group. This is similar to the pattern observed when LH lesions were combined with contralateral enucleation being less effective than ipsilateral enucleation in facilitating melatonin release [49]. It was hypothesized that this was mediated by a crossover of retinal fibres at the optic chiasm on their way through the LH to the pineal. Ipsilateral enucleation destroyed fibres that projected to the opposite side of the brain to where LH lesions were made thereby producing the equivalent of bilateral enucleation. Conversely, LH lesions plus contralateral enucleation destroyed the same fibres at the level of the retina, and again at level of the LH, on the same side of the brain, impacting minimally on pineal melatonin secretion. Such an effect observed with contralateral enucleation in the present study is reminiscent of the process of denervation supersensitivity [69,71,82]. In the same way that contralateral enucleation facilitates recovery from 6-OHDA lesions in the present study, lesions or drug treatments that denervate DA receptors around the time DA depleting lesions are placed, also reduce the severity of subsequent lesions [33,79]. Such an effect may be mediated by the cross-over of degenerating fibres from the enucleated eye to the lesioned side, producing a pre-hypersensitivity before LH lesions and reducing the severity of motor deficits. In short, the traditional core phenomena used to describe NSD system function, that is DA deficiency and receptor hypersensitivity, can be achieved by involving the visual system. This suggests that more than three decades of research on NSD system function has been undertaken without factoring in the involvement of the visual and associated circadian systems. Given the preponderance of visual and circadian deficits in PD [71] a reappraisal of NSD function with regard to the visual system and anti-Parkinsonian chemotherapy is essential. This move might well lead to more effective pharmacological approaches involving new mechanisms that currently remain unexplored.

The mechanism of action responsible for the observed difference might well involve melatonin in a capacity that is remarkably different to the process of antioxidation [73,80]. While melatonin can induce a minor degree of recovery in the rotating rat when administered strategically around the time that degeneration is induced [12], melatonin might produce or enhance PD symptoms at other times [74]. The present study demonstrates further that strategic enucleation, combined with 6-OHDA induced degeneration, can produce a spectrum of symptoms consistent with the

dynamic state of DA degeneration observed in experimental models of the disease. For example, enucleation ipsilateral to the side of the brain where DA degeneration occurs increases the severity of experimental PD. This is consistent with the interpretation that the circadian system is involved in experimental PD and that it may even originate in the retina. When circadian elements are destroyed bilaterally either at the level of the retina, or in the brain at the level of the LH, a more severe form of the syndrome is precipitated. This is exactly what was found with retinal participation in pineal function as LH lesions plus ipsilateral enucleation enhance HIOMT activity and melatonin secretion [47,49] thereby producing the equivalent of bilateral enucleation. Although melatonin was not examined in the present study, the observed parallel between reported increases in melatonin secretion in previous work [49] and the enhanced PD observed in the present study strongly suggest the involvement of the circadian system, and more specifically, the retina in the enhanced behavioural impairment. We do, however, exert some caution in this interpretation since DA concentrations in the NSD system were not measured in the present study. While the methods employed have been shown to have a robust effect on the NSD system and produced a well defined form of experimental PD [44,68,69,72–75,77] verification of the interrelated processes operating between the visual, circadian and NSD systems need to be explored in detail. This may be best achieved using analytical techniques that simultaneously examine the DA/melatonin relationship in the retina, the mesencephalon and the pineal and this will undoubtedly prove to be a challenge for future research.

One of the most surprising findings of the present work is the high rate of mortality associated with the 6-OHDA plus ipsilateral enucleation group (Fig. 8). The mortality associated with acute, bilateral models of this disease are typically associated with aphagia, adipisia and gradual wasting until death during the acute period from day 6 to 9 post 6-OHDA. While the body weight was slightly suppressed toward the end of the study, this was only minor and all animals were still capable of maintaining their body weight at a level which was not consistent with the wasting typically associated with DA deficiency [44,82]. This suggests that there are aspects to the body weight loss, reduced appetite and mortality associated with LH lesions and DA deficiency that lie outside the realm of our current understanding of NSD function and we are speculating that this is likely to involve the circadian system.

It is interesting to note that previous studies which reduce the long term endogenous, bioavailability of melatonin by pinealectomy observe decreased neuronal survival in the senescent brain [19]. This has been linked to the occurrence of demerive type symptoms. Similarly, studies that increase the bioavailability of melatonin in preclinical models of PD claim that the administration of exogenous melatonin can reduce the severity of Parkinsonian deficits if it is administered around the time of 6-OHDA administration [36–38]. According to a large volume of literature spanning nearly four decades [7,8,11,47,48,71] lesions of the LH, whether they be induced by i.c. 6-OHDA or by enucleation, would also be expected to increase melatonin secretion. Such lesions would produce functional blindness with an expected outcome of increasing HIOMT activity and a net increase in melatonin secretion: particularly around the time of lesion placement. Such lesions would therefore be expected to provide some degree of protection from the debilitating effect of LH lesions and DA deficiency. Clearly, this is not the case and such lesions produce a severe form of PD in the presence of elevated melatonin levels and the converse appears to be true. There are numerous studies that report antagonism of melatonin by exposure to light can, in fact, enhance recovery from lesions that cause NSD system degeneration in animals and in man [12,18,20,29,58] and a similar effect can be achieved using pharmacological means to block melatonin [74–76]. If nothing else, these

conflicting results expose the complexity of circadian physiology as it relates to the aetiology and progression of neuropsychiatric disease.

In summary, the present results demonstrate the importance of visual and circadian system input into the aetiology and progression of PD. While there is a substantial literature demonstrating compromise of sensory function in PD and its experimental forms [2,44] this is the first demonstration of direct involvement of any sensory system in the aetiology and progression of this disorder with sensory deficits typically regarded as a consequence of DA degeneration [3,34,45,65]. In consideration of various accounts for the genesis of PD including genetic [24], viral [46], environmental [13] and compromised oxidative inputs [59–60,66] these have had limited impact on treating the disease or advancing our understanding as to the early detection, prevention or slowing of the degenerative process. In fact, one of the weakest links in these approaches is that they fail to explain the unilateral nature of PD as it commonly develops on one side of the brain (and body) and then gradually progresses to the other side as the disease progresses. On the other hand, the involvement of input from a bilateral sensory modality, such as vision, lends itself to a gradual, mature age onset course that closely resembles the onset and course experienced in PD [15,22]. The present findings and recent developments present an important case for circadian involvement in this regard and reveal a great deficiency in our understanding about the events that underlie such disorders. A new approach depicting how the DA and circadian systems cofunction is likely to yield more promising results that will alleviate the high morbidity associated with this disease and other neuropsychiatric conditions.

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