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Behavioral Phenotypes Associated with MPTP Induction of Partial Lesions in Common Marmosets (*Callithrix jacchus*)

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Abstract

Parkinson's disease is a chronic neurodegenerative disorder with the core motor features of resting tremor, bradykinesia, rigidity, and postural instability. Non-motor symptoms also occur, and include cognitive dysfunction, mood disorders, anosmia (loss of smell), and REM sleep disturbances. As the development of medications and other therapies for treatment of non-motor symptoms is ongoing, it is essential to have animal models that aid in understanding the neural changes underlying non-motor PD symptoms and serve as a testing ground for potential therapeutics. We investigated several non-motor symptoms in 10 adult male marmosets using the MPTP model, with both the full ($n = 5$) and partial ($n = 5$) MPTP dosing regimens. Baseline data in numerous domains were collected prior to dosing; assessments in these same domains occurred post-dosing for 12 weeks. Marmosets given the partial MPTP dose (designed to mimic the early stages of the disease) differed significantly from marmosets given the full MPTP dose in several ways, including behavior, olfactory discrimination, cognitive performance, and social responses. Importantly, while spontaneous recovery of PD motor symptoms has been previously reported in studies of MPTP monkeys and cats, we did not observe recovery of any non-motor symptoms. This suggests that the neurochemical mechanisms behind the non-motor symptoms of PD, which appear years before the onset of symptoms, are independent of the striatal dopaminergic

transmission. We demonstrate the value of assessing a broad range of behavioral change to detect non-motor impairment, anosmia, and differences in socially appropriate responses, in the marmoset MPTP model of early PD.

Keywords

Parkinson's disease; MPTP; non-motor symptoms; nonhuman primate model

1. Introduction

Non-motor symptoms commonly occur in patients with Parkinson's Disease (PD)[1, 2]. Such symptoms include cognitive dysfunction ranging from mild cognitive impairment to dementia, psychotic symptoms including hallucinations and delusions, and mood disorders of depression and anxiety[3, 4]. Many non-motor symptoms precede the onset of motor symptoms. Additionally, these non-motor symptoms, particularly depression and cognitive impairment, are key factors that contribute to poor quality of life[5]. Current management of these conditions frequently relies upon therapies designed for psychiatric conditions and are not specific to PD; these medications often result in intolerable side effects such as a worsening of the motor symptoms of PD[4]. As the development of medications specifically designed for the treatment of non-motor symptoms of PD is ongoing, there is a need for animal models that aid in understanding the neural changes underlying non-motor PD symptoms and can serve as a testing ground for potential therapeutics.

The nonhuman primate model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is widely regarded as the most appropriate model of PD[6, 7]. Key features of PD are replicated including oxidative stress, reactive oxygen species, energy failure, and inflammation[8]. However, this model does not appear to result in the formation of Lewy Bodies[9, 10]. The MPTP monkey model is primarily used to discern behavioral and symptomatic components of PD, rather than mechanisms of cell death, as this model exhibits behavioral and neuroanatomical similarities to the human condition showing a bilateral Parkinsonian syndrome. This is the best-established and validated model of motor dysfunction in PD and as such evaluation for new markers in the realm of cognition and affect can be performed with established expectations regarding motor impairment and its assessment.

Models of high dose MPTP-induced loss of dopaminergic neurons in monkeys have been well studied and characterized relative to motor function[11]. Typically a full MPTP model is used, which results in 85–90% loss of nigral tyrosine hydroxylase-positive neurons (TH+). Iravani *et al.*[12] reported on a partial MPTP model in marmosets that produced less severe lesions – 60% loss of nigral TH+ cells. In general the partial MPTP subjects displayed overall motor activity that was comparable to controls and motor disability scores that were significantly lower than that of the full MPTP model. However, Iravani *et al.*[12] did not report on non-motor behavioral assessments. Determination of non-motor impairments may be of particular importance in partial MPTP models that are designed to represent earlier phases of PD when gross motor impairments may be less evident.

As the partial MPTP model may more closely replicate human pathology[12], particularly in the early phases of the disease, characterizing the non-motor symptoms is essential. Here we describe several non-motor phenotypes in the partial MPTP marmoset model. In order to make the best functional use of the partial MPTP regimen – in particular to effects of interventions or therapeutics – it is desirable to have reliable behavioral phenotypes that are associated with this early PD model. We examined sensory tasks, social tests and sensorimotor tasks, in addition to established assessment of gross motor impairment, to determine whether reliable phenotypic change could be identified in the putative model of early PD.

2. Methods

2.1 Subjects

We tested 10 research naïve, vasectomized male marmosets [*M* age = 2.9 years; range 2.0 – 5.0 years] housed at the Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX. Each male was socially housed with a female throughout the study except during MPTP dosing and selected testing procedures (described below). Room temperature ranged between 76° F and 84° F (set point of 80° F), with a 12h light-dark cycle with lights off at 19:00. Fresh food was available *ad libitum*; the base diet consisted of a purified diet (Harlan Teklad TD130059 PWD) and Mazuri diet (AVP Callitrichid 5LK6). Animals also received small amounts of fresh fruits, seeds or dairy products daily. This research was approved by the Institutional Animal Care and Use Committee at Texas Biomedical Research Institute and abided by all applicable U.S. Federal laws governing research with nonhuman primates.

Subjects were randomly assigned to either the full ($n = 5$) or partial ($n = 5$) MPTP model condition. Baseline behavioral assessments occurred prior to MPTP induction over a period of 12 weeks. Multiple sessions of each task were conducted during this time period. Subjects were then injected subcutaneously with the neurotoxin MPTP dissolved in 0.9% sterile saline. Two MPTP dosing schedules were used: (1) 2 mg/kg (full MPTP model) for three consecutive days and (2) 1 mg/kg (partial MPTP model) for three consecutive days. Treated animals were maintained in quarantine for five days following the last injection. During this time their food and water intake were closely monitored. No animals required hand feeding intervention following dosing. After this initial recovery period, behavioral assessments were conducted for 12 weeks; multiple sessions of each task were again conducted. The development of clinically evident PD progression was determined via daily observations in the home cage[13] (see Table 1).

2.2 Histology

At 13 weeks following MPTP treatment, subjects were deeply anesthetized with 20mg Ketamine and 50mg Nembutal and perfused transcardially with 120 ml phosphate buffered saline (PBS, pH 7.4) followed by an equal volume of ice-cold 4% paraformaldehyde solution in PBS. The brains were removed to quantify PD pathology via assessment of TH+ neurons in the nigral section. Brains were post-fixed overnight in 4% paraformaldehyde solution in PBS at 4°C. The tissues were cryoprotected in 30% sucrose solution, embedded

in Tissue-Tek OCT compound, and processed for cryosectioning. Anatomical landmarks were determined according to Paxinos *et al.*[14]. Nigral sections were cut in the coronal plane using a Leica CM 3050 S cryostat at 40 μm thickness and mounted onto electrostatically charged slides. Every fourth section was stained and counted.

We processed for tyrosine-hydroxylase (TH) IHC using a standard avidin–biotin complex (ABC) method. Briefly, sections were treated with 1% bovine serum albumin in phosphate-buffered saline containing 0.3% Triton X-100 for 1 hour and then incubated with rabbit anti-TH (Millipore AB152) at 1:1,000 dilution for 48 hours at 4 °C. Sections were rinsed in phosphate-buffered saline and incubated with 0.5% biotinylated goat anti-rabbit secondary antibody for 1 hour, followed by avidin–biotin peroxidase complex (ABC Elite Kit; Vector Laboratories, Burlingame, CA) at room temperature for 1 hour. The chromogen used was 3,3′-diaminobenzidine tetrahydrochloride (DAB Kit; Vector SK-4100). Sections were cover-slipped with Cytoseal (Electron Microscopy Services).

2.3 Cell counting

The total number of TH+ neurons in the substantia nigra pars compacta (SNpc) was estimated using the optical fractionator method in combination with unbiased counting rules, an approach that is not affected by either the volume of SNpc or the size of the neurons. The SNpc in each 40 μm thick section was outlined at 2.5× magnification using Stereo Investigator workstation (MicroBrightField, Williston, VT) attached to an Axioplan 2 imaging microscope (Carl Zeiss), fitted with a DEI-750 CE video camera (Optronics, Goleta, CA) and a LEP MAC5000 motorized stage controller (Ludl Electronic Products, Hawthorne, NY). Anatomical landmarks were determined according to Paxinos *et al.*[14]. Then at random start, TH+ neurons were counted from every fourth serial section throughout the entire extent of the SNpc using a 63× oil immersion objective (numerical aperture 1.4). The counter (AI) was blinded to condition. Cells were counted only when their nuclei were optimally visualized, which occurred only in one focal plane.

2.4 Behavioral assessments

2.4.1 Gross motor impairment—To assess gross motor impairment we recorded 20-minute videos of the males in their home cage twice weekly. Videos were then scored by a trained observer who was blind to the experiment. In order to compare more traditional qualitative PD impairment scores with quantitative evaluations of changes to marmoset species-specific behavior, the videos were scored two ways. First, the scorer assessed the video using a traditional impairment scoring regime in which they watched a 5-min. segment from the middle of the video and gave an impairment score based on their overall impression of the animal movement (PD score). An animal was scored for range of movement, bradykinesia, postural abnormality, and checking behavior using a 0 – 4 ranking scheme (see Table 1; [13]). Next the scorer assessed the same video segment using Observer 5.0 (Noldus Information Technology, Leesburg, VA) to record all occurrences of shifts in the animal's ranking for each behavior assessed in the PD scoring scheme. At the end of the time frame Observer tallied the ranking for each behavior (Quantitative PD Score). Both the PD score and the Quantitative PD score were then weighted using the standard Fox scoring system

(Johnston & Fox 2015). Impairment scores from the videos were averaged together for each individual animal as either pre- or post-dose for comparison.

2.4.2 Hourglass task—Verhave et al.[15] validated a task to assess impairment in the marmoset's natural righting reflex in the MPTP model. We employed this task in the present study. Marmosets were placed in a cylinder (approximately 15.24cm diameter, 17.78 cm height) and turned 180° to an upside down position. The animal was turned every 30s for a total of five turns of the hourglass. Subjects were tested twice prior to dosing and then tested monthly post-dosing. Sessions were video-recorded and scored (time to reorient to the upright position) using Observer 5.0.

2.4.3 Activity level—In order to evaluate daily activity patterns an Actical actimeter (Philips Respironics, Bend, Oregon) was placed in a pouch (Lomir Biomedical Inc., Quebec, Canada) on a modified ferret harness. Subjects were fitted for a harness and then habituated slowly over the course of six sessions of increasing length, starting at 15 min. and ending in overnight sessions. The actimeter was programed with the individual's ID and weight and was set to record 15s epochs for the duration of the trial. Trials lasted 48 hrs during which subjects wore the harness containing the actimeter and were separated by a mesh divider from their pairmate (to prevent accidental removal of the harness or actimeter). All other husbandry activities remained the same. At the end of the 48 hrs the harness was removed and data were downloaded from the actimeter. Data analysis included removal of the first 10 minutes and the last 10 minutes of recordings as this was animal handling time and not daily activity recordings. Average hourly activity counts for day and night hours were then calculated.

In order to evaluate activity patterns the daily data were coded to estimate the time the animal went to sleep at night, the time they awoke in the morning, the number of times they were at rest during the day, and the number of times they were aroused at night. The following criteria were set to score each event within the actimeter output. To score the initiation of each rest period the output was scanned for five minute periods (20, 15 second epochs) with zero activity counts. The time the animal went to sleep at night was estimated as the time at which there was at least a 5-minute period with zero counts near the time of lights out (1900h). The initiation of arousal periods during the dark phase were designated as at least three consecutive epochs with a greater than zero score, or a single epoch with greater than 50 counts, arousal periods were separated by at least five minutes of zero counts. The time they awoke in the morning was determined by the same criteria as arousal occurring in proximity to the lights on phase (0700). For each 48-hour collection time daily averages were determined for the variables sleep, wake, rest, and arousal. Furthermore, the percent of time with zero activity was determined for each 24-hour period. Repeated measures ANOVA was used to examine differences between dose concentrations and time for each variable of interest.

2.4.4 Olfactory test—We took advantage of a well-characterized response of male marmosets to a natural olfactory cue - the scent-mark of females. Male marmosets display increased tongue flicks, mounts and ejaculations when exposed to female odors. Ziegler et al.[16] demonstrated predictable differences in male behavior can be elicited by a disk

impregnated with female scent mark versus a disk impregnated with vehicle, with no female scent present. Scent was collected from 15 females that were 10 to 14 days postpartum by placing a small glass stopper in the cage for them to scent mark on. The stopper was collected and the urine on the stopper was pipetted into a falcon tube, followed by washing the stopper with 300μl 1:1 ethanol:water mix with the supernatant being added to the falcon tube. Samples were frozen at −20°C until all were collected. Then the scent samples were thawed and mixed into a single mixture of all females. The mix was aliquotted into 100 μl test samples and frozen at −80°C until used in a trial. A vehicle consisting of a 1:1 ethanol:water mix was also aliquotted and frozen. For the scent trial subjects were randomly assigned to receive either the vehicle disk or the scent disk on day one, and received the other disk on day two. Presentation of the disk consisted of removing the female from the home cage and placing the disk in the home cage with the male. Behavior was recorded for 15 min, the disk was removed and the female was then returned to the homecage. Behavior was recorded for an additional 5 minutes. Behaviors recorded included interactions with the disk (contact, licking, sniffing), presence of an erection, and interactions with the female. On the second day the same procedure was followed with presentation of the alternate disk. All trials were also recorded for further video review. Subjects were tested once prior to dosing and four times post-dosing.

2.4.5 Social intruder test—Exposure to an intruder animal has been studied historically in marmosets as a measure of their social bonding to a partner and reactiveness to an unfamiliar social situation. This type of trial offers a unique opportunity to assess an impaired animal's ability to react to a social situation. For these trials the subject and his pair mate were moved in their home cage to an empty test room. They were given 5 minutes to acclimate to the new room. A 10-min baseline pre-trial behavioral assessment was conducted. Then a cage holding an unfamiliar intruder male was rolled into the testing room and placed within 2 cm of the home cage; behaviors were recorded for 20-min. The cage containing the intruder was removed from the test room and a 10-min post-trial behavioral assessment was conducted. Behaviors recorded during the trials included affiliative social interactions, aggressive interactions, location of the focal male and attentiveness (see Table 2). Trials were conducted with different randomly assigned unfamiliar intruders each time. Social intruder trials were conducted once for each focal male prior to MPTP dosing and post-dosing each focal male was tested every other week, resulting in five post-dose trials.

2.5.6 Staircase tasks—The staircase reaching task assesses independent limb function and visuospatial integration[17, 18]. Subjects were tested in their home cage; a temporary divider was inserted to separate the subject from the cage mate during testing. In this task, subjects were required to reach through vertical slots that are placed in the middle of a Plexiglas apparatus (hill task) or placed at the left and right sides (valley task), which was attached to the front of their home cage. The hill staircase or valley staircase, each having 4 steps per side, was secured to the Plexiglass. A piece of marshmallow was placed on the tread of each step. The subject had to orient himself so that he could extend one arm through the slot to retrieve the marshmallow.

Subjects were allowed a maximum of 3 min to retrieve the marshmallows. Only successful retrievals (food item grasped and securely taken through the Plexiglas slot) were scored. The score for each marshmallow was determined by its distance from the relevant slot – score of 1 for the nearest step, to a score of 4 for the farthest step. The total score was summed to result in a maximum score of 10 per side.

Each subject received both the Hill and Valley Staircase tasks, in a random order, during each test session. Subjects were tested weekly for two months prior to dosing and then tested weekly for 12 weeks post-dosing.

2.5.7 Conveyor task—A Remo conveyor was used to assess executive function in subjects treated with the lower dose MPTP. Subjects were trained to retrieve rewards (dehydrated marshmallows) from a moving conveyor belt attached to their home cage. Subjects were trained to criterion of 80% successful retrieval of the reward presented in an alternating manner. In order to examine executive function subjects were presented with two treats at the same time, an unpreferred treat (apple) and a preferred treat (marshmallow) following it. Subjects had to control impulsivity to allow the unpreferred treat to pass and retrieve the preferred reward; due to the speed of the conveyor they could not retrieve both rewards. In the 30 trial session animals were presented with dual treats six times, separated by presentations of a single treat.

Subjects were tested prior to MPTP dosing and then tested weekly following release from quarantine. Videos of the sessions were scored to assess whether the animal successfully retrieved the treat from the belt or not. If the attempt failed, the failure was scored as a grab attempt, knocking the treat from belt, or an aborted attempt. If no attempt was made attentiveness was assessed and scored as 1) the animal was watching the belt attentively, 2) the animal was at the belt but not attending to the treat, or 3) the animal was out of view. During the dual treat trials the animal was assessed for the following behaviors: attempted retrieval of treat or decoy, successful retrieval, ate retrieved item, dropped retrieved item, second attempt to grab multiple items, hesitation, or attempt failed. In addition, we examined whether retrieval of the decoy or treat varied within a session from the first trial of the session, middle and last presentation. An animal learning the task should show an increase in retrieval of the treat and decrease in decoy attempts within a session, while animals unable to control impulsivity or learn the task would show no change in the retrieval of the decoy.

2.5.8 Data analysis—We first determined the extent of dopaminergic loss in the SNpc for each subject in the full and partial MPTP dose condition. The total number of TH+ positive neurons was expressed as a percentage of the number of TH+ cells found in untreated healthy controls. To evaluate non-motor symptoms, repeated measures ANOVA were conducted for each behavioral assessment. We were interested in determining if any observed non-motor impairment would recover over time, as spontaneous recovery from PD motor symptoms, associated with a compensatory increase in striatal DA release, has been reported [19, 20]. If the analysis found no significant differences on the behavioral measures post-dosing, then the post-dosing data points were averaged into a single data point for each individual for further analysis to improve power.

3. Results

3.1 Histology

Subjects in the low dose condition had, on average, 45.7% of TH+ neurons as compared to healthy controls; subjects in the high dose condition had, on average, 19.07% of TH+ cells found in healthy controls (Figure 1).

3.2 Gross motor impairment

The full MPTP subjects had a mean increase in PD score of 18.93 ($t(4) = 5.157$, $p = 0.007$) and a mean increase in Quantified PD score of 18.0 ($t(4) = 5.315$, $p = 0.006$). The partial MPTP subjects had a much more modest change in PD score 3.03 ($t(4) = 2.11$, $p = 0.102$) and Quantified PD score 1.44 ($t(4) = 3.71$, $p = 0.021$). The mean gross impairment scores, pre- and post-dosing, for the full and partial MPTP subjects are illustrated in Figures 2a (PD score) and 2b (Quantified PD score).

3.3 Hourglass task

On average, the marmosets took 6s to right themselves following a flip (full dose pre: 6.21s ± 0.8s, post: 5.85s ± 0.7s; partial dose pre: 6.13s ± 0.9s, post: 6.04s ± 0.6s). There were no significant differences pre- or post-dosing, or due to dose regimen for the hourglass duration to flip.

3.4 Activity level

The full MPTP subjects displayed significantly lower activity counts post-dosing ($t(4) = 6.655$, $p = 0.003$) while the activity counts of partial MPTP subjects did not change ($t(4) = 0.145$, $p = 0.891$; Figure 3). There was a significant dose by time interaction for the number of rest periods during the day ($F(1, 39) = 15.782$, $p = 0.000$), with full dose animals exhibiting significant increases in the number of rest periods during the day after dosing (full pre = 12.3, post = 25.6; partial pre = 14.1, post =14.6). Additionally full dose animals exhibited a significantly higher number of arousals post dose ($F(1, 39) = 4.181$, $p = 0.048$; pre = 2.3, post = 6.3). The percent of overall time during a 24-hour period with zero activity was also significantly increased for full dose animals ($F(1, 39) = 13.68$, $p = 0.001$; pre = 72.7%, post= 88.8%). We found no shift in the circadian rhythm of the animals associated with MPTP dosing.

3.5 Olfactory Test

An insufficient number of the full MPTP subjects displayed any behaviors indicating preference for the female-scented disk. We suspect this was due to the testing situation, wherein the female mate was in the room during testing. The protocol was therefore refined for testing of the partial MPTP subjects by not having the female mate present in the room during testing. With this change, four out of five of the partial MPTP subjects displayed a preference for the female-scented disk, with the most robust behavioral difference between disks being licking frequency per trial.

Figure 4 provides the mean ratio of female-disk to control-disk licks per trial. While there was variation between males in their responsiveness to the female disk prior to dosing, four

of the five animals demonstrated a preference for the female disk (ratio greater than 1). The ratio of female-disk to control-disk licks declined post-dosing in all subjects (Wilcoxon signed rank test, $Z = 1.826$, $p = 0.068$), centering around 1.0 post-dosing, indicating no preference for either disk. Importantly, the overall rate of licking disks, in general, did not change after dosing.

3.6 Social intruder task

Repeated measures analysis of pre- and post- dose exposure to an intruder was assessed using multivariate analysis of variance 2 doses × 2 trials (pre, post) × 3 times (pre, during, post trials) and *post hoc* analysis with Bonferroni correction. There were no significant differences found between the five weeks post dosing for any variable. Therefore, all of the post-dose data were collapsed into a single data point for each individual. Subjects were found to exhibit significant differences for a number of social and agonistic behaviors during the intruder trials. Males attended to females significantly more following an intruder trial than pre- intruder exposure (p=0.022) and during the intruder trial (p=0.031) (F(2,16) = 8.83, p=0.003) (Figure 5a). Additionally, there was a trial by dose interaction (F(1,8)=22.51, p=0.001) with full MPTP dose males being more attentive to females prior to dosing than after dosing. Male twitter calls were significantly associated with a time by dose interaction (F(2,16)=19.219, p=0.000) and trial by time interaction (F(2,16)=4.592, p=0.027). There were significant differences in twitter call behavior associated with the dose regime, with full dose animals calling more often than partial dose animals (even prior to dosing) (F(1,8) = 21.262, p = 0.002) (Figure 5b). While there was a significant trial by time interaction (F(2,16) = 6.259, p=0.01) for the presence of erection, with males more likely to have an erection prior to dosing during the intruder trial, there were no significant effects of dosage for this behavior.

Agonistic and aggressive behaviors typically described during marmoset intruder paradigms include increased rates of scent marking, arch walking, genital displays and attack behaviors in the presence of an intruder male, in addition to increased chuck vocalizations (short staccato calls). Males dosed with MPTP (full and partial model) showed very low rates of arch walking and attack behavior during any of the intruder exposures. Males, regardless of dosing regimen, were found to chuck significantly more in the pre-dose intruder trial than in the post-dose trials (F(1,8) = 9.658, p = 0.014), and significantly more during the intruder exposure rather than pre- or post- exposure (F(1,8) = 10.23, p =0.001) (pre = 0.001, during = 0.006, post < 0.001, occurrences per minute). Male genital display activity was significantly associated with the time point in the trial (F(2,16) = 43.884, p < 0.001; pre 0.003, during = 0.027, post = 0.004) and an interaction of trial * dose (F(1,8) = 7.151, p = 0.028) (Figure 5c). Male scent marking activity was a significant interaction of trial * time (F(2,16) = 4.207, p = 0.034) with males scent marking more during exposure to an intruder and doing so less after the exposure, and after dosing with the high dose, the scent marking post intruder trial dropped to almost zero (Figure 5d).

3.7 Staircase task

A two-way mixed ANOVA was conducted on the retrieval scores for full and partial MPTP model (between-subjects factor) and time of test (pre- and post- treatment; within-subjects factor). Scores from two types of tasks (hill and valley) were evaluated separately.

For the hill task, the results of the two-way mixed ANOVA showed a significant main effect of time of test, $F(1,8) = 8.612$, $p = 0.019$. Performance on the hill task declined for subjects in both conditions post-treatment (M pre = 8.24, M post = 5.77). There was no significant interaction between condition and time of test, $F(1,8) = 2.206$, $p = 0.176$, such that the time of test did not depend on the MPTP dosage (Figure 6a).

For the valley task, there was no significant main effect of time of test, $F(1,8) = 0.241$, $p = 0.636$. (M pre = 5.86, M post = 6.38). Additionally, there was no significant interaction between condition and time of test, $F(1,8) = 1.463$, $p = 0.261$, such that the effect of the time of test did not depend on the level of the condition (Figure 6b).

3.8 Conveyor task

The successful retrieval of an alternating presentation of a single treat was significantly affected by the side of presentation and the time after dosing ($F(4,16) = 6.621$, $p = 0.002$). While successful retrieval rate was lower immediately following dosing, the success rate recovered 4 weeks post-dosing (Figure 7a). Animals displayed a higher rate of attentive failures on week 1 post dosing than during any other session ($F(4,16)=3.778$, $p=0.024$). For the dual treat presentation we found no significant change in percent of successful treat retrieval attempts across the post-dose sessions (Figure 7b). There was no significant increase in overall failure or in the retrieval of the decoy over the preferred treat. Examining trials within a session across the five sessions, there was a significant decrease in the retrieval of the decoy in the last trial of each session ($F(1,4)=10.286$, $p=0.033$) (Figure 7c).

A summary of the observed behavioral changes for each test for the full and partial MPTP dosed animals can be found in Table 3.

4. Discussion

Many non-motor symptoms associated with PD, such as sleep abnormalities, impaired cognition, and impaired sense of smell, appear years before the onset of motor symptoms[21, 22]. While the underlying pathophysiology of the non-motor symptoms remains unclear and may involve widespread neuronal loss in systems other than the nigrostriatal pathway[23], it is important to characterize and quantify non-motor symptoms displayed in animal models of PD. The research presented here demonstrated several of these non-motor symptoms in the MPTP marmoset model of early stage PD, including social, cognitive, and olfactory changes. Importantly, while spontaneous recovery of PD motor symptoms has been reported in studies of MPTP monkeys and cats [20, 24, 25], we did not observe recovery of any non-motor symptoms. This suggests that the neurochemical mechanisms behind the non-motor symptoms of PD, which appear years before the onset of symptoms, are independent of the striatal dopaminergic transmission. Combined with previous studies showing impairment in sleep[26], agitation and psychosis-like behaviors

including hallucinations, stereotypies, and hyperkinesia[27], a more thorough documentation of the behavioral phenotypes of the MPTP marmoset model has emerged. Specifically, we demonstrated the value of assessing a broad range of behavioral change to detect non-motor impairment, anosmia, and differences in socially appropriate responses, in the marmoset MPTP model of early PD.

PD scoring of impairment in humans and many model species has relied on qualitative post hoc assessments of a few key behaviors including bradykinesia and range of movement [28]. While these values are very important for comparing impairment across model species and experimental trials we were interested in assessing this impairment score in a more quantitative way. We also wished to compare this scoring to changes in species-appropriate behaviors following MPTP dosing. We found that animals receiving the full MPTP dose had quite similar PD and Quantitative PD scores. However, subjects receiving the partial MPTP dose has substantially lower Quantitative PD scores. This suggests impressions of impairment may be higher than true impairment at this stage in disease progression. While a PD score provides a measure of impairment, assessing a broad range of behavioral changes strengthens our ability to detect non-motor associated impairment in this model.

We did not detect differences in the righting response (tested via the Hourglass task) in marmosets given either the full or partial MPTP dose. These results are in contrast to a previous report which indicated marmosets displayed significant impairment in this task, with impairment remaining for three weeks after MPTP dosing [15]. Our inability to detect an effect of either the full or partial MPTP dose is likely due to a small difference in test administration. The diameter of the cylinder jar used by Verhave et al. [15] was approximately 13cm, whereas we used a cylinder jar approximately 15cm in diameter. This wider jar allowed all animals including those with severe impairment to right themselves.

Mild cognitive impairment is common in individuals with PD, and is recognized as a stage between no dementia and dementia [29, 30]. While impairment may be seen across several cognitive domains, the most frequent impairments are seen in executive function, attention, and memory [30]. In patients with PD with dementia, psychotic symptoms can also develop including visual hallucinations. Although such symptoms are often triggered by the dopaminergic medications used in treating PD, psychosis is now recognized as part of the disease process *per se* and continues even with reduction of the prescribed medication. Mood disorders such as depression and anxiety can also be a problem in early PD and often predate the motor symptoms by many years, but are common throughout the course of the disease. In our behavioral assessments we found no indication of changes in behavior that might be described as increased anxiety, depression or psychosis. The marmosets did not display higher rates of scratching, calling or attentiveness to nonsocial cues that were reported by Fox [28]. While it is possible that our animals did not have the same neural damage and behavioral outcome as previously reported, it is also possible that interpretations of species-specific behaviors differed between experimental groups, or that socially housing the animals during treatment prevented the development of such behavior.

The staircase task was developed to assess cognitive deficits in a marmoset model of stroke [18]. This task tests for disruptions in higher order cognitive processing, specifically

visuospatial integration. Only the hill component resulted in significant change in performance, with both full and partial MPTP dose marmosets showing a significant decrease in performance. The staircase task did not detect performance differences between marmosets receiving the full and partial MPTP dose. This may indicate that visuospatial performance is not affected in early PD.

Regarding the conveyor task, we had some concern that this form of assessment would not work in a Parkinsonian model due to animals' motor impairment. However, subjects with the partial MPTP dose displayed a decline in retrieval success immediately following dosing, they were still engaged with the apparatus and demonstrated the ability to choose a preferred treat. Thus it appears unlikely that there are significant cognitive declines in this task with the partial MPTP dose.

Olfactory impairment appears to be associated with the early stages of PD. This relationship is robust enough to generate interest in tests of olfactory acuity as an initial screening tool used to decide the relative merit of more expensive or invasive testing [31]. Miwa et al. [32] provided descriptive results of possible impaired olfactory ability in marmosets receiving a high, short-term MPTP regimen (4mg/kg, twice per day for two days). These marmosets had significantly impaired olfactory function and could not find a favored food (banana) placed in a hidden location within their cage. Additionally, they were described as eating banana that was treated with aversive odorants, something not done by animals prior to MPTP administration. However, no quantitative details of either of these tests are provided. In our study, we took advantage of a well-characterized response of male marmosets to a natural olfactory cue - the scent-mark of females. Male marmosets display increased tongue flicks, mounts and ejaculations when exposed to female odors. Ziegler et al. [16] demonstrated predictable differences in male behavior could be elicited by a disk impregnated with female scent mark from one impregnated with vehicle (no female scent present). In the present study we demonstrated this ability prior to dosing which was then lost in males given the partial MPTP dose. Thus, we were able to detect anosmia in the marmoset MPTP model of early stage PD.

REM sleep behavior disorder precedes PD in about one-third of patients. Mild dosing of MPTP marmosets produced significant changes in REM sleep [26]. While our actimeter data revealed no differences in night time activity it is unclear whether these type of data are a good proxy for interpreting sleep behaviors in the marmoset model. The actimeter can only be used to assess movement during timeframes and does not indicate whether the animal is at rest or asleep. The animals did not appear to actively locomote during the dark hours following dosing; however, we do not know if they were asleep or experiencing the same REM patterns as they did prior to dosing.

Our social intruder data suggest that males given MPTP were still able to initiate species-appropriate responses to a social encounter even when severely impaired. Marmosets receiving both the full and partial MPTP doses were socially engaged with their female partner and responsive to the intruder during these trials. The full MPTP dose males were generally more attentive to their females prior to dosing and displayed a decline in attentiveness following dosing, but they still did attend the females following exposure to an

intruder. Partial dose individuals maintained a similar attentiveness to their females following an intruder trial after receiving the MPTP dose. Males were able to rally aggressive responses following dosage with MPTP such that there were no significant changes in the number of genital displays given for partial MPTP males, but while there was a decrease in the rate of genital displays in the full MPTP males, they still did the behavior even though their impairment scores were quite high. Males in the full MPTP model showed decreases in behavior following an intruder exposure as reflected by decreased scent marking. This is possibly explained by the high use of energy during these social encounters. Collectively, the social intruder data demonstrate that after receiving doses of MPTP, males were able to engage in socially appropriate behavior and continued to display territorial behavior. While males receiving a partial MPTP dose were somewhat impacted by motor deficit, males receiving the full MPTP dose were more prone to exhaustion-like behavior following the intrusion. These results are similar to those of Durand et al. [33], who reported changes in social relationships in female rhesus monkeys (*Macaca mulatta*) treated with MPTP (with both low and high dose regimens). Such changes occurred before motor or cognitive impairments were detected and were most common in subordinate animals.

Overall our study demonstrates that marmosets are a valid model for the evaluation of PD treatment and intervention as they exhibit both motor deficits and non-motor behavioral changes. Importantly, marmosets can and should be socially housed following treatment with MPTP; of note, even severely impaired animals engaged in socially appropriate behavior and received no negative treatment from their female mates. This is particularly important for evaluating cognitive and affective changes in PD that are often difficult to assess in humans and almost impossible in other animal models of PD.

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Highlights

- Behavioral phenotypes for a partial MPTP model in the marmoset are investigated.
- We identify non-motor symptoms including cognitive, olfactory, and social change.
- Socially housed marmosets present a valid model for evaluation of PD treatments.

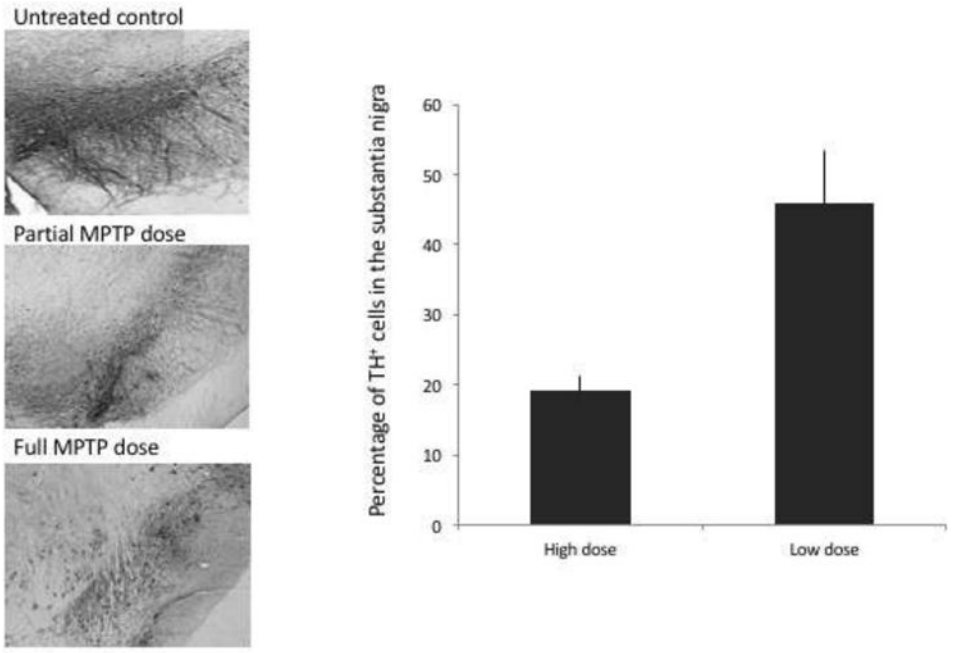


Figure 1.

(a) Midbrain sections of control, full MPTP model and partial MPTP model MPTP marmosets showing TH+-immunostaining in the substantia nigra. (b) Plots of stereologic data showing the percentage of TH+ neurons in full MPTP model ($n = 5$) and partial MPTP model ($n = 5$) individuals as a percentage of the number of TH+ neurons found in untreated healthy controls.

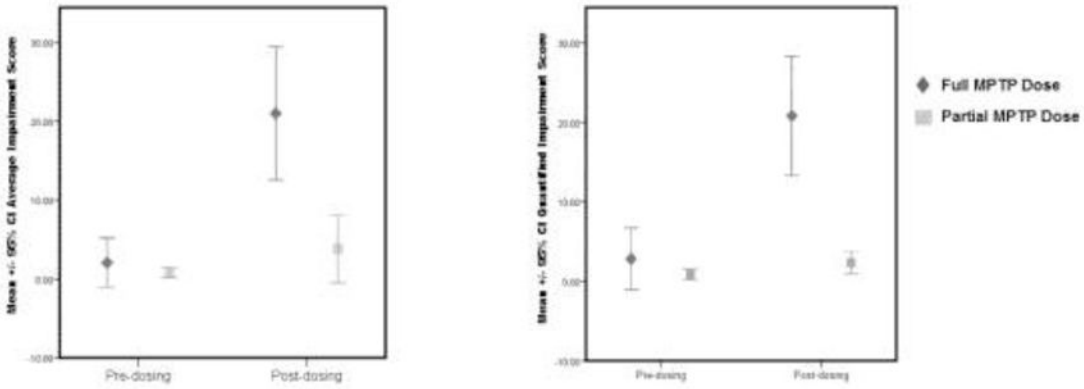


Figure 2.

Mean gross impairment scores (+/− 95% CI), pre- and post-dosing, for the full (n = 5) and partial (n = 5) marmoset MPTP subjects; a) PD score and b) Quantified PD score.

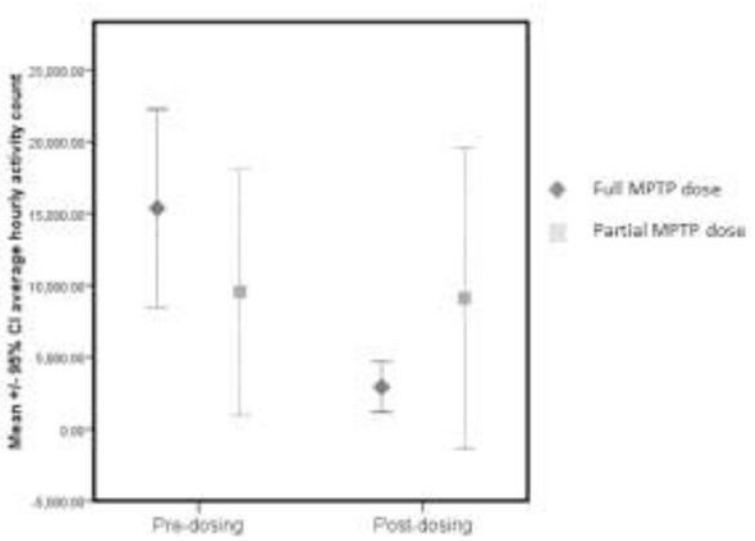


Figure 3.

The full MPTP model subjects ($n = 5$) displayed significantly lower activity counts post-dosing. Activity counts of the partial MPTP subjects ($n = 5$) did not change at the end of the 12 week post-doing period.

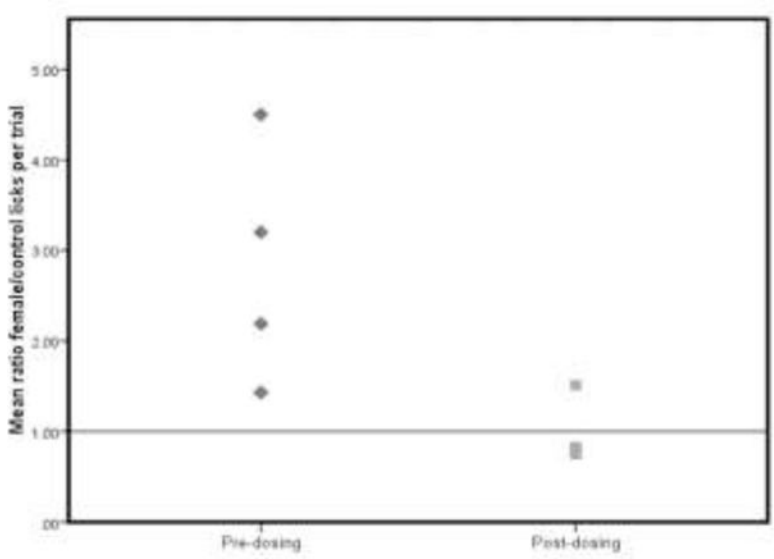
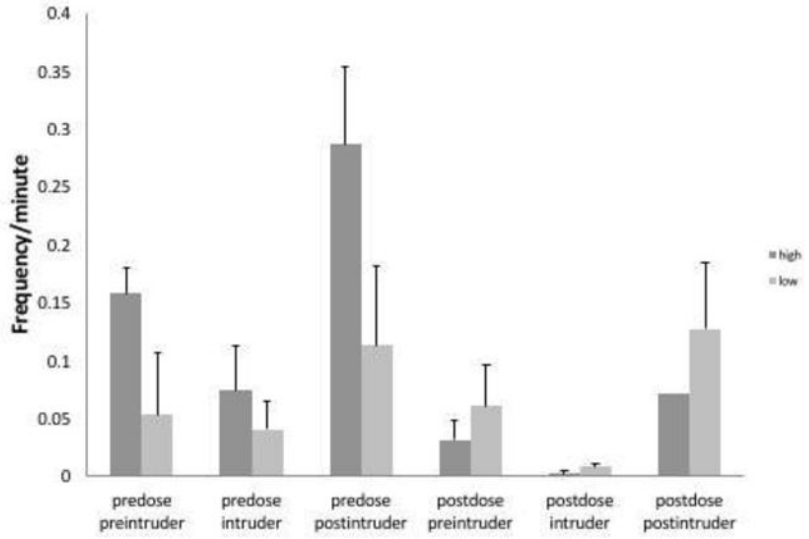


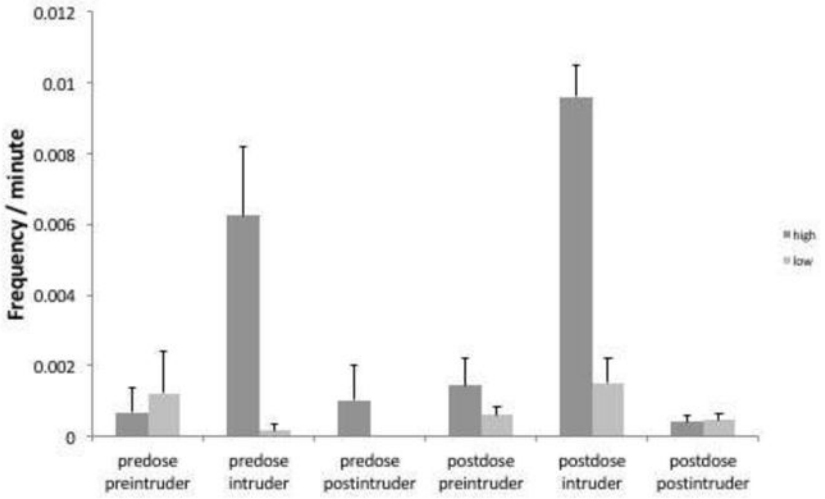
Figure 4.

The mean ratio of licks to the female scented:control disks for males ($n = 5$) in the partial MPTP dosing regimen. Prior to treatment with MPTP subjects displayed a preference for the female scented disks with ratios higher than 1. This preference is diminished following dosing with MPTP with ratios closer to 1.

(a)



(b)



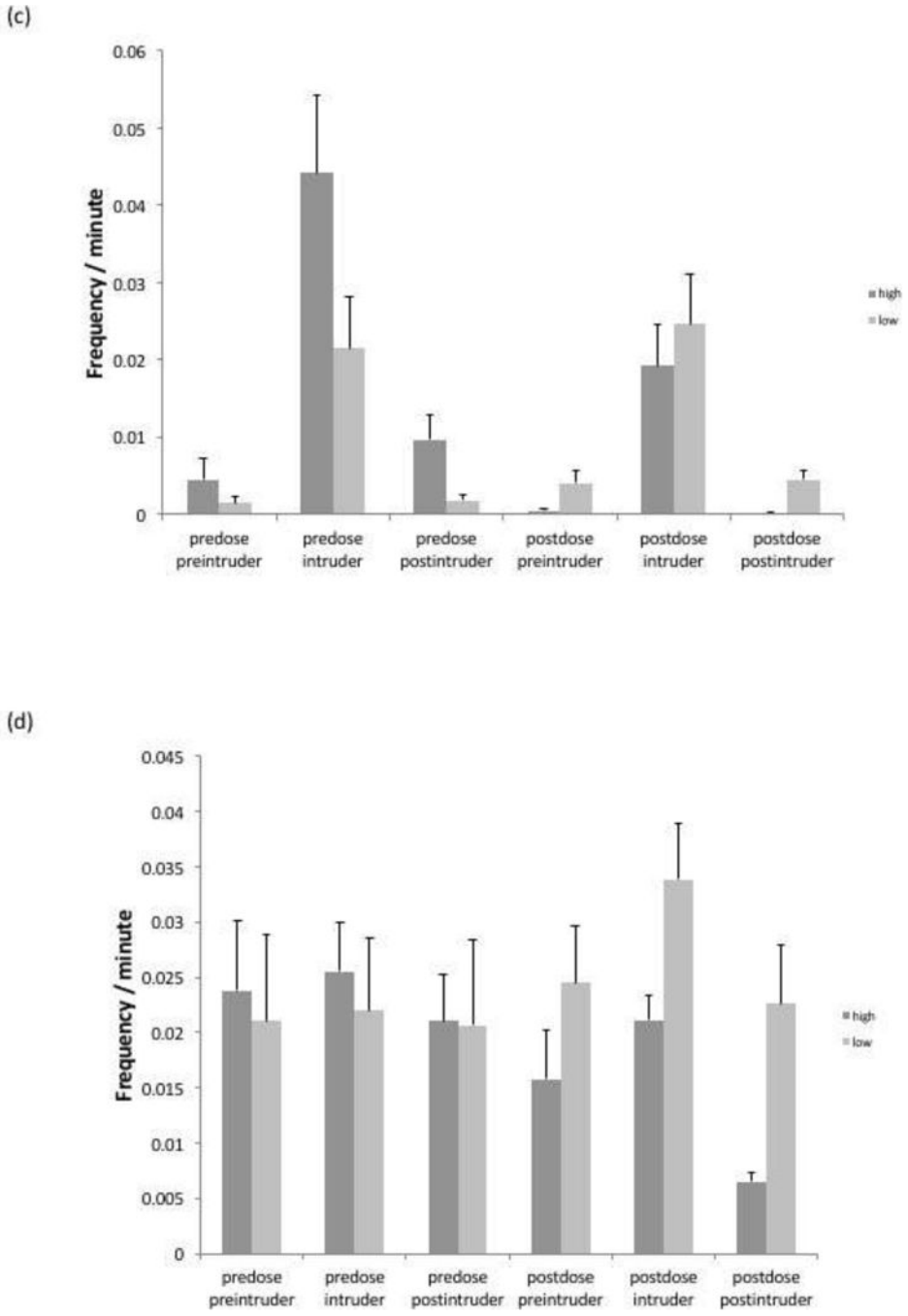


Figure 5.

Responses to a social intruder in subjects receiving full ($n = 5$) and partial ($n = 5$) MPTP dose. (a) Female attend: males were more attentive to females during the intruder trial than during the pre- or post-dose phase. Males that received the full dose were less attentive after receiving the dose. (b) Twitter calls: full dose males had higher rates of twitter calls during an intruder encounter. (c) Genital display: males exhibited more genital display behavior during the intruder trial and rates were not suppressed following dosing. (d) Scent marking:

following a full dose males scent marking behavior dropped significantly following an intruder trial. Error bars represent standard error.

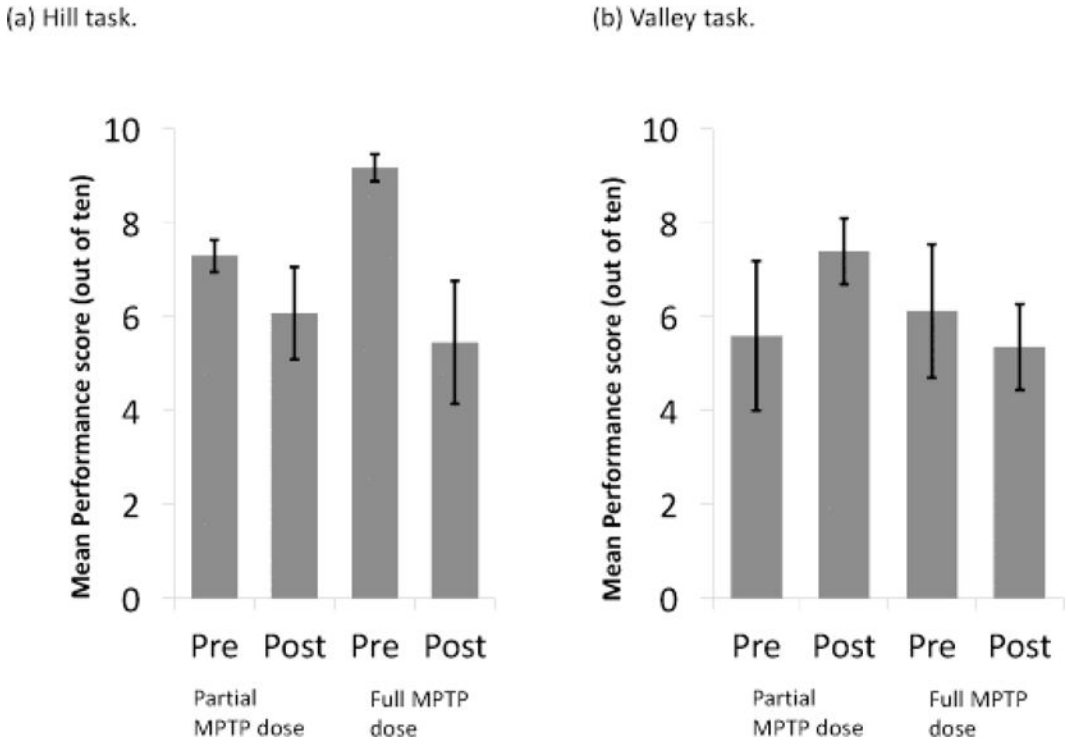


Figure 6.

Staircase. No main effect of dose on performance of the staircase tasks was found. Performance on the hill task (Figure 6a) declined post-dosing for subjects receiving the full ($n = 5$) and partial ($n = 5$) MPTP dose. No significant effects were found for performance on the valley task (Figure 6b). Error bars represent standard error.

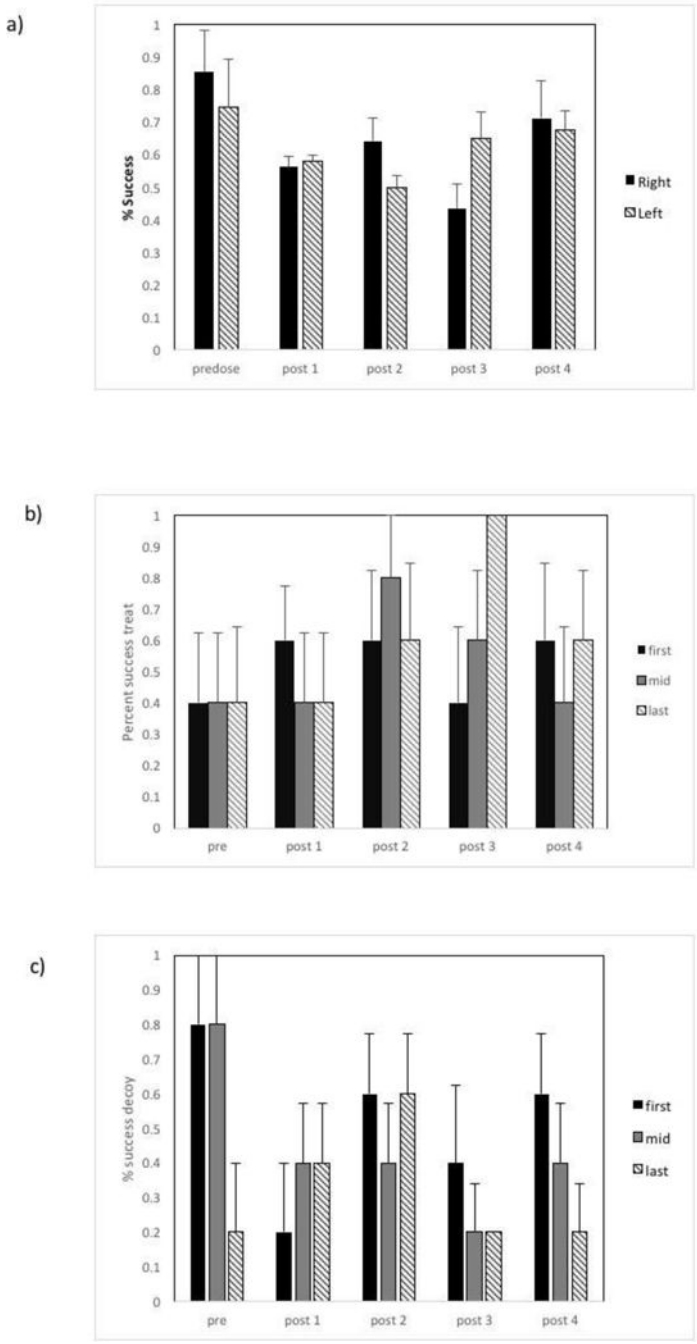


Figure 7.

Percent success of retrieval from a conveyor belt for males receiving a partial dose of MPTP (n = 5). (a) Retrieval of treats from the right and left side recovered following treatment. (b) There were no significant changes in the ability to successfully retrieve a reward during the dual treat presentation. (c) There was no significant increase in the cognitive error of retrieval of the decoy during the dual treat presentation following treatment.

Table 1

Scoring system used to evaluate the clinical signs of Parkinson's disease in the marmoset

Scores for each of the four components were summed to provide a total Parkinsonian disability score.

Characteristic	Score	Ranking
Range of Movement	0	walking on the floor and other substrates (ceiling, walls, perches)
	1	walking on the floor of the cage only
	2	movement of limbs and/or trunk, without locomotion (in this case movement trumps location, so this is an animal sitting not locomoting, with movement of limbs in any portion of the cage space)
	3	movement of head only, without locomotion (in this case movement trumps location, so this is an animal sitting not locomoting, with movement of head only in any portion of the cage space)
	4	no movement (in this case movement trumps location, so this is an animal sitting not locomoting, with no movement in any portion of the cage space)
Bradykinesia score	0	normal speed and initiation of movement
	1	mild slowing of movement
	2	moderate slowing, difficulty initiating and maintaining movement, freezing
	3	marked slowing, or unable to move, with prolonged freezing episodes
Postural abnormality score	0	normal, upright, holds head up, normal balance
	1	hunched body, holds head up
	2	hunched body and neck, face down, may lose balance
Checking behavior (attention)	0	present, looking around, observant
	1	absent

Note: Johnson & Fox, 2015.

Table 2
Behavior recorded during the social intruder test

Behavior	Scoring	Description
Location – front of cage	Instantaneous	Subject is at front of cage
Location – nestbox	Instantaneous	Subject is in the nestbox
Location – other	Instantaneous	Subject is located somewhere other than nestbox or front of cage
Attend female	Instantaneous	Focal animal is attending the social partner
Attend intruder	Instantaneous	Focal animal is attending the intruder
Attend other	Instantaneous	Focal animal is attending an item other than female or intruder
Attend none	Instantaneous	Focal animal is not specifically attending anything, appears “zoned out”
Arch walk	All occurrences	The subject walks with back arched high
Scent mark	All occurrences	Genital rub on branches or other surfaces
Erection	All occurrences	Subject has penile erection
Sniff	All occurrences	Subject sniffs social partner
Mount	All occurrences	Subject sexually mounts his partner
Copulate	All occurrences	Engaged in mating behavior
Genital Display	All occurrences	Exposing the genital area by lifting the tail
Attack	All occurrences	Subject physically aggresses against intruder
Long call	All occurrences	High amplitude vocalization, considered a long distance contact call
Chuck	All occurrences	Aggressive short staccato vocalizations
Twitter	All occurrences	Low amplitude vocalization, typical social contact call

Table 3

Behavioral phenotypes associated with full and partial MPTP dose in the marmoset.

Assessment	Full MPTP dose	Partial MPTP dose
Gross-motor impairment	Mean increase of 18.93 (PD score)	Mean increase of 3.03 (PD score)
	Mean increase of 18.00 (Quantified PD score)	Mean increase of 1.44 (Quantified PD score)
Hourglass	No change	No change
Activity level	Lower activity counts	No change in activity counts
	Increased number of daily rest periods	No change in number of daily rest periods
	Increased number of daily arousals	No change in number of daily arousals
	More time spent inactive	No change in time spent inactive
	No circadian shift	No circadian shift
Olfactory test	Insufficient data to analyze	Loss of preference to female scent
Social intruder	Decreased attentiveness to female	No change in social or aggressive response
	Decreased scent mark behavior	
Staircase	Performance declined on hill	Performance declined on hill
	No change in performance on valley	No change in performance on valley
Conveyor	Insufficient data to analyze	No change in cognitive decision making