Neuromodulator and Olfaction Deficits in a MPTP Mouse Model of Parkinson's Disease

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Abstract: The aim of this project is to quantify the olfactory dysfunctions associated with Parkinson's Disease in a mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model. Parkinson's Disease, like many neurodegenerative diseases, gives rise to olfactory deficits during the early nonmotor stages of disease development. The MPTP neurotoxin model is a common method to study this early state progression due to the slow, realistic rate of neuronal degradation it causes throughout the brain. Even with the presence of such a model, a focus on the predominant motor aspects of the disease has left these smell losses inadequately explored from a neurochemical and behavioral level. Thus, the two objectives of this study are: to assess olfactory deficits in the MPTP model through discrimination/duration tests, and to correlate these behavioral results to previously observed changes of dopamine (DA) levels in the striatum. These results will help connect the behavioral progression of PD with the existing knowledge of olfactory processing networks. Beyond learning about the mechanisms themselves, the eventual hope is that such knowledge can be applied to the patient setting to develop a reliable disease marker before the onset of conventional PD symptoms.

Acknowledgments: This project would not have been possible without the help from the Linster lab and our collaboration with Dr. Libert's lab. I would specifically like to thank Matt Lewis and Adam Francisco for their advice and help in planning. I would also like to thank Kayla Brooks and Brandon Sepe for helping me conduct the behavioral and immunohistochemical experiments.

Abbreviations list

PD: Parkinson's Disease
MPTP:1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
OB: Olfactory bulb
MO: Mineral Oil
IHC: Immunohistochemistry
DAB: 3,3'-diaminobenzidine
Da: Dopamine
TH: Tyrosine Hydroxylase
AcH: Acetylcholine

Introduction

Parkinson's disease (PD) is a neurological disorder that leads to a gradual reduction in motor function. As a neurodegenerative disorder, these effects are caused by degeneration of dopaminergic neurons in the striatum and substantia nigra (Bove et al., 2005). These hallmark motor symptoms include tremors, bradykinesia, and general failures of voluntary muscle control (Bove et al., 2005). These are not the only areas and effects that PD has however.

The start of many neurodegenerative diseases is marked by non-motor deficits that precede the more obvious motor symptoms by a few years. Olfactory deficits in particular have been shown to be common during this stage (R. D. S. Prediger et al., 2009). While this pathology has started to be explored in cases of Alzheimer's Disease, not much advancement has been made on similar olfaction losses found in Parkinson's Disease (PD) patients (Ruan et al., 2012).

Animal models of PD present a reliable method to study such deficits. A PD-like state and progression can be induced in animals via chemical lesions targeting specific neural structures or general infusions that incite a degeneration of DA neurons. In order to explore the olfactory deficits of PD, the slower pathology that the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxin model offers would be most valuable for its early-state characterization (Bove et al., 2005). The chemical elicits its toxic effects as an MPP intermediate in the brain, where it is taken up by DA transporters into DA neurons and causes cell death by initiating oxidative stress pathways (Schober, 2004).

MPTP is effective on rodents, primates, and humans (Bove et al., 2005). Rodent models allow for simpler behavioral and molecular testing (Bove et al., 2005; Cleland, Morse, Yue, & Linster, 2002). Depending on the species, systematic injection or intranasal infusions of the drug can be used. When considering both techniques and the standard rat and mouse models however,

tests of the olfactory modality have not yielded a sensitive characterization of the drug's effects on olfactory processing. For example, the few studies that have been done all used simple testing methods such as a "cookie finding test" or simple odor bedding discrimination (Castro et al., 2012; Kurtenbach et al., 2013; R. Prediger et al., 2006; R. D. S. Prediger et al., 2009). Of the studies described here, the conclusions have not yet reached any consensus with some showing smell loss in the MPTP model, and others not showing any difference. None have used quantifiable discrimination tasks or alternate testing platforms to yield detailed results.

Similarly, an understanding of the chemical mechanisms underlying possible olfactory deficits is also incomplete. Though no clear picture of this progression has been established, some associations have been found with DA and other neuromodulator levels in the OB and midbrain (Bohnen et al., 2010; Mundiñano et al., 2013; Ruan et al., 2012). Previous studies in the Linster lab have at least partially substantiated the importance of DA in regulating odor perception intensity, the primary neurotransmitter associated with Parkinsonism (Wei, Linster, & Cleland, 2006). Generating more behavioral data could help to pinpoint how these changes could be tied to PD.

These thoughts led to the present experiment, which uses more specific odor discrimination and memory duration tests to determine any effects of MPTP injections in a mouse model. Unlike earlier tests, these methods used odorants with quantifiable carbon length differences that are proportional to the actual odor difference sensed by the nose (Cleland et al., 2002). Our results show that while non-associative memory formation and specificity is not affected by MPTP injections, memory duration is decreased in experimental mice.

Methods

Mice and Experimental Groups

24 B6 strain mice (12 female and 12 male) ranging from 8-12 weeks of age at the time of testing were used in this experiment. The experimental groups consisted of 12 subjects in the control group and 12 were in the PD group, with both groups being tested before and after injections. For ease of testing, these 24 animals were tested six groups of 4 mice based on sex and birth date.

The testing schedule for an individual group was determined based on the protocol for MPTP exposure. For each group, all four mice were first acclimated to the testing equipment the week before starting. The next week, the corresponding behavioral tests were administered for the first five days to establish baseline performance. On the fifth day, MPTP was administered to half the mice while saline was administered to the other half. After a 10 day waiting period, the mice were tested again with the relevant behavioral tests. The saline group served as the control group here.

All experimental procedures followed guidelines established by NIH and were conducted under the auspices of a protocol approved by the Cornell IACUC.

MPTP Administration

MPTP diluted in saline before injection. A stock 2 mg/mL solution in saline was made first. After this, the appropriate volume was drawn so that a 20mg MPTP per Kg of animal bodyweight would be administered in a single injection. No volume was greater than 10 uL/g body mass.

Injections were done intraparietally on mice on the day of injections. A single cycle of injections consisted of 4 injections, done two hours apart. After injection, mice were allowed a

10 day recovery period as required by Cornell IACUC. Food and water were provided throughout.

Odorants

The planned experiments required multiple odors or odor sets for use. The odor discrimination tests used four different functional group sets: carboxylic acids, alcohols, aldehydes, and esters (Fig 1). Each odor set here had 3 chemicals 1C difference apart each. The memory duration tests used four single odors.

The odorant solutions were made in a mineral oil solvent. To ensure equal diffusion rates, all of the solutions were made at an appropriate vapor partial pressure of 1.0 Pa. These dilutions can be seen in Table 1.

The odors were presented individually to the animals through metal teaballs. 60 uL of the odorant or MO was placed onto a clean filter paper square. This was then placed into a clean teaball that was used in the testing cage for only one trial.

Odorant dilutions for 1.0 Pa	
Odor (1.0 Pa)	Volume (uL) per 50 ml MO
Acetic acid	3.9
Propanoic acid	16.6
Butanoic acid	63.6
Propanol	2.8
Butanol	10.4
Pentanol	37.2
Pentanal	3.3
Hexanal	11.1
Heptanal	35.3
Propyl acetate	3.1
Butyl acetate	10.9
Amyl acetate	36.1
Butyl hexanoate	813.5
1,8-cineole	97.7
2-furyl methyl ketone	129.5
Trans-2-heptenal	71.4

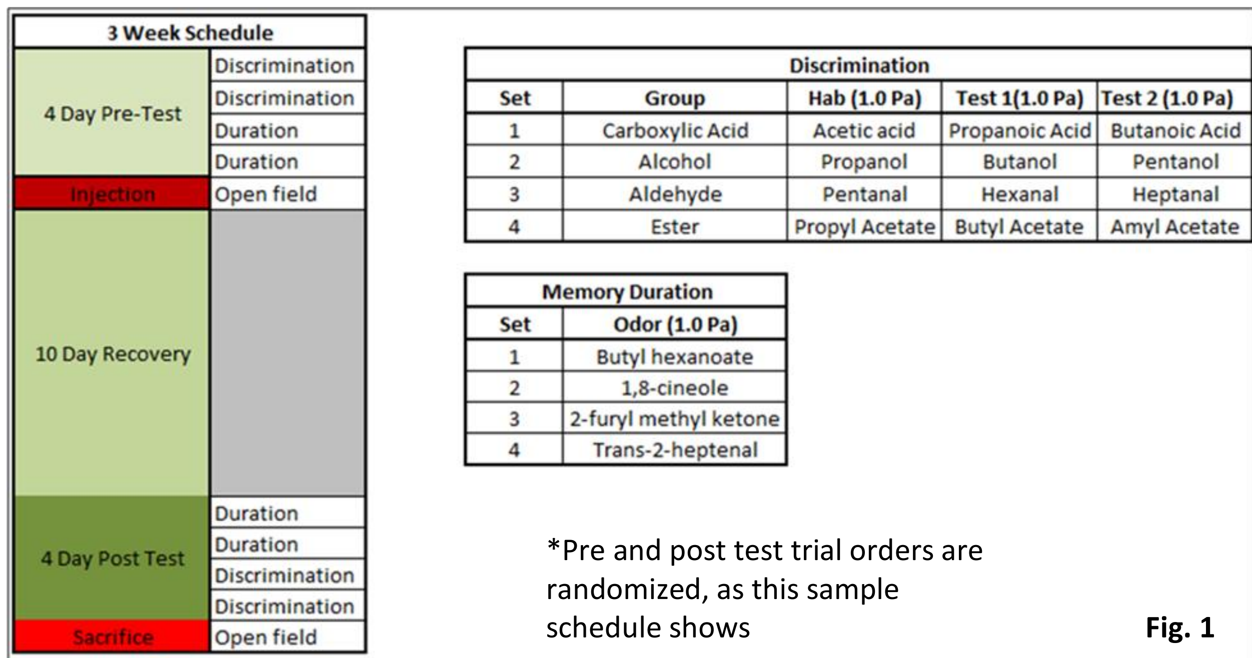
Table 1: Dilutions of each odorant to achieve 1.0 Pa vapor pressure. Each odor was diluted in a mineral oil (MO) solvent, creating 50 mL solutions.

Behavioral testing schedule

Two olfactory tests and a general open field test were the three behavioral tasks used in this study. The olfactory tests consisted of odor discrimination or duration tests (Freedman et al., 2013). Four odor sets or odors were used in each test respectively (Fig 1). This ensured that no mouse had a repeated set based on the four repetitions/test schedule, preventing the influence of odor experience.

During both the pre and post injection weeks of the three week testing period, the overall schedule of tests were very similar. In both weeks, two odor discrimination tests and two odor duration tests were run individually over the first four days. An open field test was run on the

fifth day. The olfactory tests were scheduled so that only one test was run per day and the open field test was always run on the last day. The order of the olfactory tests was randomized and counterbalanced with respect to the type of test, the odor set, and the discrimination test odors (Figure 1). This ensured that no one mouse received the same odor twice throughout the two weeks of testing in either test type.



Discrimination				
Set	Group	Hab (1.0 Pa)	Test 1(1.0 Pa)	Test 2 (1.0 Pa)
1	Carboxylic Acid	Acetic acid	Propanoic Acid	Butanoic Acid
2	Alcohol	Propanol	Butanol	Pentanol
3	Aldehyde	Pentanal	Hexanal	Heptanal
4	Ester	Propyl Acetate	Butyl Acetate	Amyl Acetate

Memory Duration	
Set	Odor (1.0 Pa)
1	Butyl hexanoate
2	1,8-cineole
3	2-furyl methyl ketone
4	Trans-2-heptenal

Fig 1: Overall testing cycle per group and odor sets used in each olfactory test. Each group of mice underwent testing in three week cycles, as shown in the schedule. This consisted of randomized single duration or discrimination olfactory tests for the first four days. On the fifth day, open field tests were done preceding injections or brain extractions. The individual odor sets used during the olfactory tests are represented in the two tables. Discrimination had classes of odors differing by carbon number while duration had single odors. Odorants were randomized and counterbalanced based on the test, odor set, and discrimination test order. In the end, each animal was exposed to each odor set once over the three weeks.

Olfactory test protocols

The individual protocols of the olfactory tests are based on the ideas of habituation and discrimination between odor stimuli (Freedman et al., 2013; McNamara et al., 2008). Both of the

olfactory tests used here start with a habituation task. This creates a non-associative odor memory through repeated exposure to the desired odorant. The marker of this is decreasing investigations by the animals with each repeated trial. After this, either discrimination or memory duration can be tested. Discrimination measures the ability of the animals to differentiate this habituated odorant from test odorants that are one and two carbons different, but in the same functional group (Fig 1). Memory duration uses the same test odorant used in habituation for that day, but modifies the trial interval times to measure how long mice can stay habituated to the last habituation trial (Fig 1). In either case, the investigation time in response to the test odor, as compared to the last habituation trial, is the variable of interest.

The timings of each test can be seen in Figure 2(a), which includes a mineral oil (blank) start, four habituation trials, and several test trials. All olfactory tests were done in plastic testing cages without bedding. Each day, the mice were acclimated for 10 minutes in these cages before starting that day's trials. The odor discrimination test consisted of 50 second trials, interspersed by 5 minute intervals. The odor duration test also used the 50s/5 minute setup, but had longer intertrial intervals for later test trials.

Both olfactory tests were manually and video recorded. Regardless of the method, the criteria for recording the "sniffing" or investigation time of the odorant during each trial was the same. At the start of the trial, the teaball containing the odorant for that trial was hung from the top of the cage (Fig 2b). After this, any time the nose of the mouse came within 1 cm of the teaball was counted as part of the investigation time. The total count of this time within the 50 s trial was the investigation time for that trial.

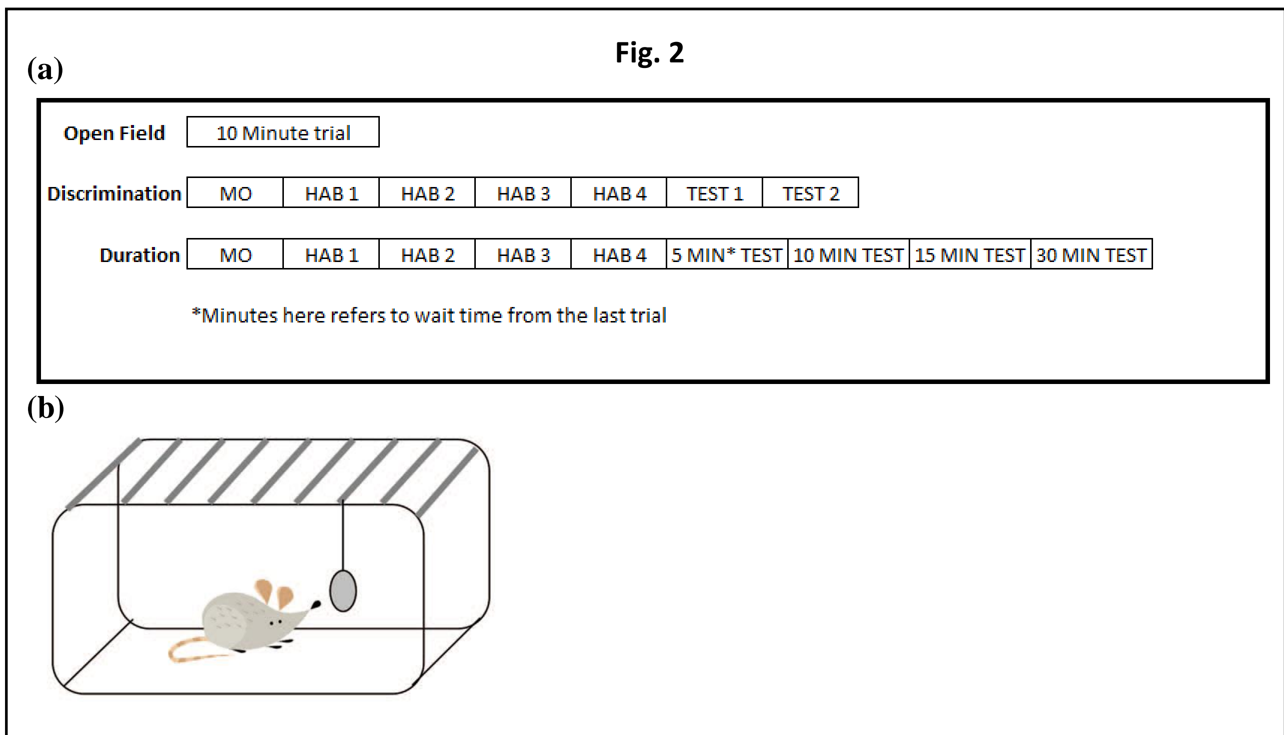


Fig 2: (a) Representation of trial timings in behavioral tests. First, the open field test is represented as a single 10 minute trial inside a large square stage. This was done once before injections and once after injections. Next, the discrimination test is represented with in initial MO (blank) trial, followed by habituation and test trails. All the trials were 50 s long with 5 minute intervals. Finally, the memory duration test is shown with an initial MO trial, four habituation trials, then four test trials. The MO and habituation trials follow the 50s/5min timings, but the test trials have variable intertrial intervals as designated by the trial name. **(b)** A representation of the testing cage in which discrimination and duration olfaction tests were done. The hanging ball represents a teaball in which odorants are placed (Freedman et al., 2013).

Open field test

The open field test was used to assess general activity and motor function of the mice (Prut & Belzung, 2003). The consisted of a 10 minute video recording in a well lit white, plexiglass, square stage of 61x61 cm dimension(size of arena). Data for the open field tests were analyzed through Stoelting Any Maze software for distance, speed, and outside/center zone time measurements.. This was done by overlaying a grid of both zones onto the recorded videos (Fig 3a).

Immunohistochemical (IHC) analysis

On the last day of the three week testing cycle (after the open field test was finished), the mice in that testing group were sacrificed and transcardially perfused with 4% peraformaldehyde. After storing overnight in the same peraformaldehyde solution at 4°C, the tissue was transferred to a 30% sucrose solution to stabilize. 48 hours later, after the brains were no longer floating, they were frozen. Freezing was done by supercooling methylbutane in a liquid nitrogen bath, then submerging the tissue in the methylbutane. These specimens were then stored at -80°C until future use.

A cryostat was used to obtain coronal sections of 14 um thickness from these samples. The main regions of interest in these sections were the olfactory bulb, olfactory tubercule, piriform cortex, and striatum. Sections were collected in PBS, but the desired ones were mounted on Fisherbrand Superfrost Plus Microscope Slides.

Immunohistochemical DAB staining of these regions were carried out on slide mounted sections to detect DA. The primary antibody was rabbit anti-tyrosine hydroxylase (AbCam ab112) at 1:50 dilution, which was allowed to stain overnight at 4°C. The DAB staining was accomplished with an anti-rabbit VectaStain ABC/DAB kit. The standard rapid staining protocol from this kit was followed for the stain. The end slides were scanned using an Aperio ScanScope.

Staining analysis was done using ImageJ software compare expression levels in different regions. The TH staining was focused on the striatum, so medial sections from three control and three MPTP mice were used. The measurement was represented as integrated density. This measured the staining intensity of the region, but subtracted out the background intensity. This accounted for differences in the size of the selected area and the mean fluorescence of the

background (set as the density reading from external striatum). Three slices were analyzed per group, but one outlier in the MPTP group was not included in calculations.

Statistics

Data for different tests were measured using different units: olfactory tests with investigation time (seconds), open field tests with multiple measures, and IHC staining density with integrated density. Results were compiled with Microsoft Excel and statistical software (SPSS) was used to perform ANOVA tests. The group (control or MPTP) and testing session (pre or post injection) were the independent variables explored in all experiments.

In the habituation model, a non-associative memory of the odor is formed over multiple odor presentations. This is evidence by a significant decrease in investigation of the odor across these presentations. We used ANOVA testing to ask if memory formation changes as a function of experimental group or test session (pre or post); this test used the investigation times of all four habituation trials as the dependent variable and experimental group (control or MPTP injection) and test session (pre vs post) as main effects.

Discrimination of novel odors was tested by comparing investigation times during the last habituation trial to investigation of new test odors that were 1C and 2C different. We used ANOVA testing with investigation times as dependent variables and experimental group and test session as main effects.

Memory duration was tested by asking for how long mice stay habituated to an odor after the last habituation trial. To test this we first determine if mice habituate (established previously) and then test if they investigate the habituated odor significantly longer after a given delay to the next trial. An ANOVA with investigation times during the last habituation trial, and 5, 10, 15 and

30 minute intervals was used. Posthoc tests check which groups were significantly habituated to the odor after each delayed test trial.

During this calculation, two mice that died after the MPTP injection did not contribute data in their post-injection period. 20 olfaction trials (for four individual mice) on days where those mice refused to approach the teaball were not included as well.

Results

Open field

The Any-Maze software was used to measure multiple data from each open field trial. The distance travelled, average speed, and line crossings were used to estimate the movement and motor function of the animals (Fig 3). ANOVA analysis was done separately on each variable, with the experimental group and session as the main effects. All of these tests showed that the open field activity was not significantly different between the MPTP/control groups ($p > 0.5$) or the pre/post sessions ($p > 0.5$).

The other measures of time in the outside zone and center distance were used to assess outside vs. center zone movement. ANOVA analysis also showed that these measures were not significantly different between the MPTP/control groups ($p > 0.1$) or the pre/post sessions ($p > 0.1$). In all cases, animals moved more within the outside zone than the center zone. This suggests that general anxiety and activity levels were not much different between the groups or sessions.

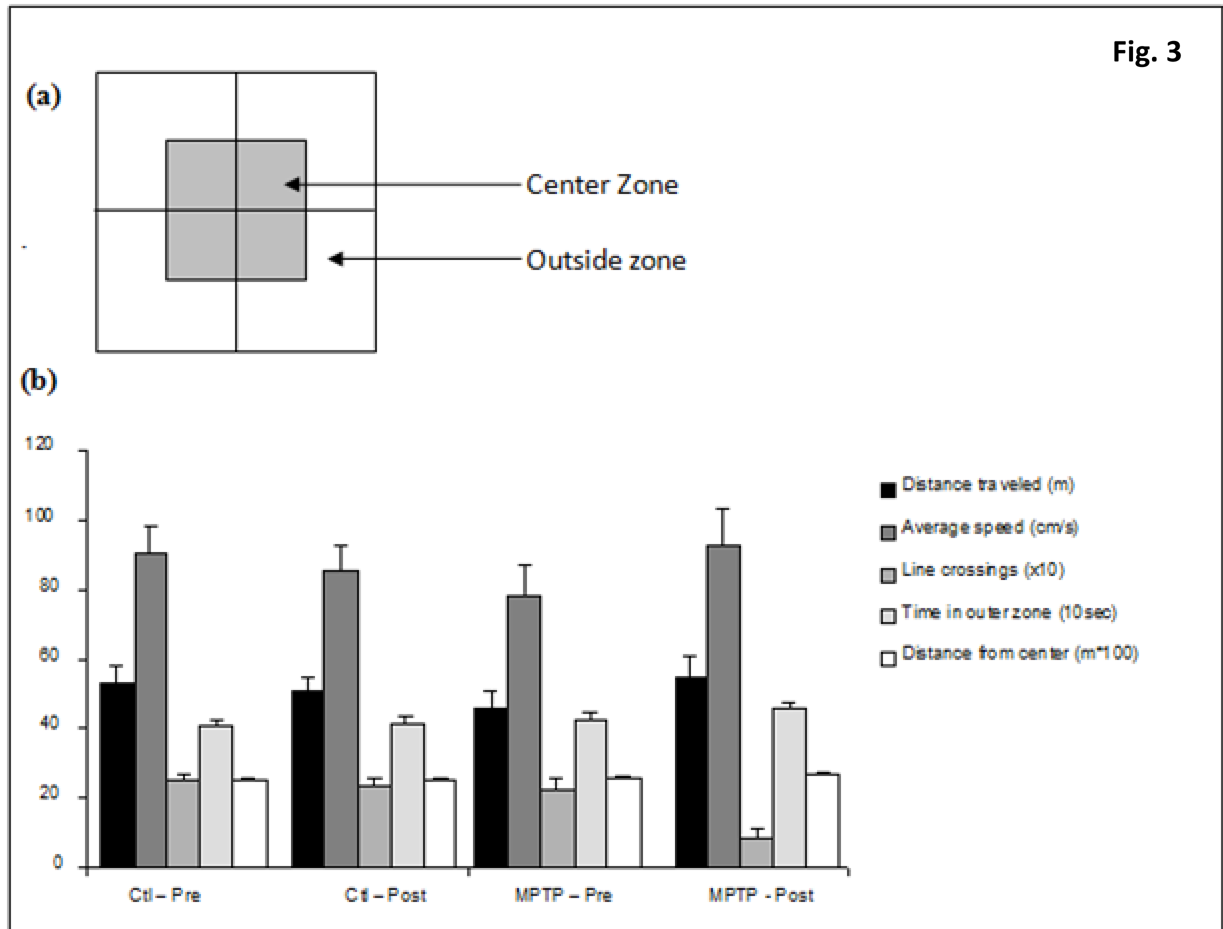


Fig 3: (a) This represents the overlay grid for measurements. After video recording, AnyMaze software was used to divide the recorded open field video stage into the quadrants shown. A center and outside zone, as well as two axes, were established as the "lines".(b) Mean measurements from open field trials of MPTP and control groups pre and post injection are shown. Analysis showed that activity in all categories was not significantly different between the groups or sessions: distance travelled/average speed/line crossings in relation to motor function ($p > 0.5$) and time in outside zone/center distance in relation to anxiety levels ($p > 0.1$). This suggests that motor function and anxiety levels were constant in all situations.

Olfactory behavioral tests

Olfactory habituation tasks allow us to test memory formation (habituation), discrimination and memory duration. Each of these is assessed by comparing the investigation times on different trials: habituation compares responses to the first and last habituation trials,

discrimination compares responses to novel odors and the last habituation trial and duration compares responses the last habituation trial to those to the same odor after different delays.

(1) Overall investigation behavior. The mean investigation time across the control/MPTP groups during pre and post injection sessions were analyzed (Fig 4). ANOVA testing showed that there was no significant difference in these times between the pre/post injection periods or the control/MPTP groups rats (F_{group} (1, 75) = 2.282; $p > 0.1$; F_{trial} (1, 75) = 2.554; $p > 0.1$).

(2) Memory formation. Next, the ability of the mice to habituate in general was analyzed. Both the control and the MPTP group show the ability to habituate, as measured by the significant difference between the first and fourth habituation trial (Fig 5 and 6). These were the trials done at the start of the discrimination and duration tests. There was also no significant effect of pre vs post testing (F_{test} (1, 281) = 0.766; $p > 0.05$). Interestingly however, there was a significant effect of the experimental group (F_{group} (1, 281) =8.326; $p < 0.01$), the actual habituation trial ($F(3, 281) = 10.170$; $p < 0.001$), and the interaction between the experimental group and habituation rate ($F(3, 281) = 3.836$; $p < 0.01$). This suggests that the habituation rate differs between the control and MPTP group in some way, but the exact correlation could not be determined.

(3) Discrimination. Since the role of the discrimination task was to see how well mice could differentiate between the habituation odor and novel test odors differing from the habituation odor by one or two carbons, an ANOVA analysis was performed on these trials (Fig 5). This showed that neither the experimental group ($F(1, 190) =$ 1.3.1; $p > 0.05$) or the pre/post period $F(1, 190) = 1.282$; $p > 0.05$) significantly

affected discriminatory ability. Only the odors themselves showed any significant effects ($F(2, 190) = 20.216$; $p < 0.001$), supporting the idea that MPTP does not affect odor discrimination. Both groups were able to discriminate the 2C test odor ($p < 0.05$), but not the 1C test odor ($p > 0.05$) as shown by LSD posthoc analysis.

(4) Memory duration. The memory duration depends on the initial habituation period functioning, after which the length of this habituation can be tested. As shown above, it was seen that both groups could habituate to the given odor sets. ANOVA analysis of the fourth habituation trials and the 5, 10, 15, and 30 min interval trials were then done next (Fig 6). The longer intertrial intervals were shown to have a significant general effect ($F(4, 347) = 9.782$; $p < 0.001$), showing that the test itself was working. The MPTP group alone was strongly affected in investigation time post-injection ($F(4, 184) = 5.143$; $p < 0.005$), in contrast to the control group which did not show any session differences in investigation time. Posthoc testing detailed which particular trials were significant: the post-MPTP group could remember the habituated odor at the 5 and 10 minute trials only ($p > 0.05$). In the pre control session, the post control session, and the pre MPTP session, mice had memory of the habituated odor in the 5, 10, and 15 minute interval trials ($p > 0.05$). Overall, this supports that MPTP caused a 5 minute duration reduction from 15 to 10 minutes.

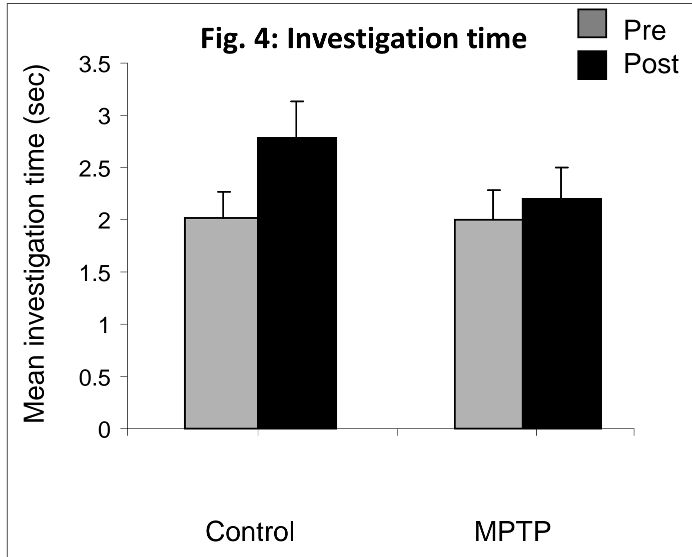


Fig 4: Mean investigation time of both discrimination and duration, compared between Control and MPTP groups pre and post injection. This data included all olfactory trials regardless of test type. Analysis showed that there was not a significant difference in either experimental groups or pre/post injection sessions ($p > 0.1$)

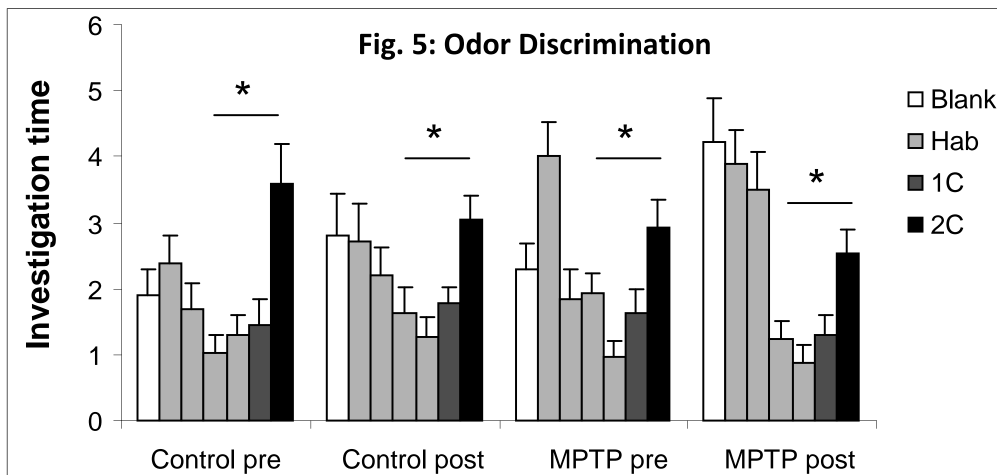


Fig 5: Effects on odor discrimination. The graph shows mean investigation time of all the trials of the discrimination test, organized by the tested variables. (*) indicates test trials that were significantly different from the last habituation trial, showing discrimination. N=12 each for the control and MPTP groups.

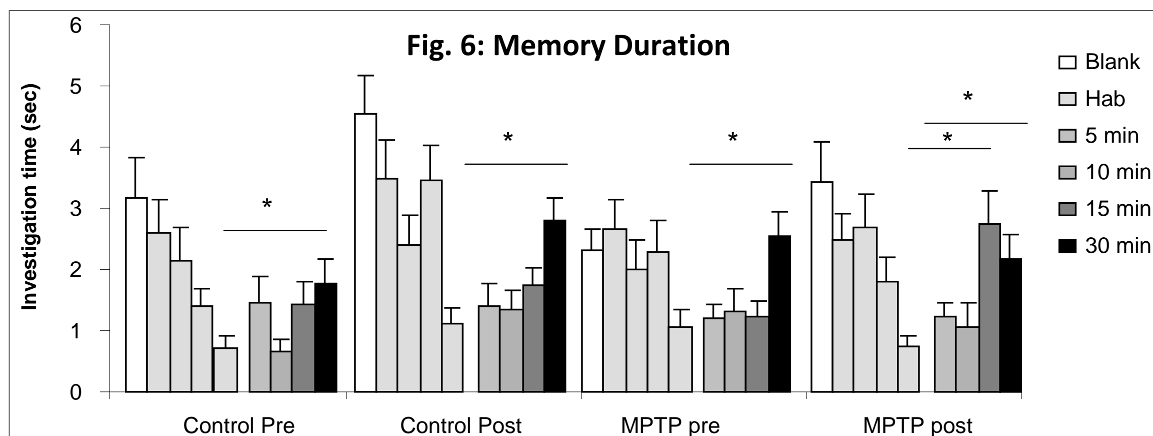


Fig 6: Memory duration experiment. These graphs show mean investigation times. (*) indicates test trials that were significantly different from the last habituation trial ($p < 0.05$). Significantly higher investigation during test trials indicates loss of memory for the habituation odor. N=12 each for the control and MPTP groups.

IHC

Immunohistochemical analysis involves staining DA levels within the striatum of perfused brains. Results showed that there was a significant decrease in TH staining in the MPTP group as compared to the control group (Fig 7). This represented a mean integrated density decrease of 72%. Student t-test analysis confirmed this significance ($p < 0.5$).

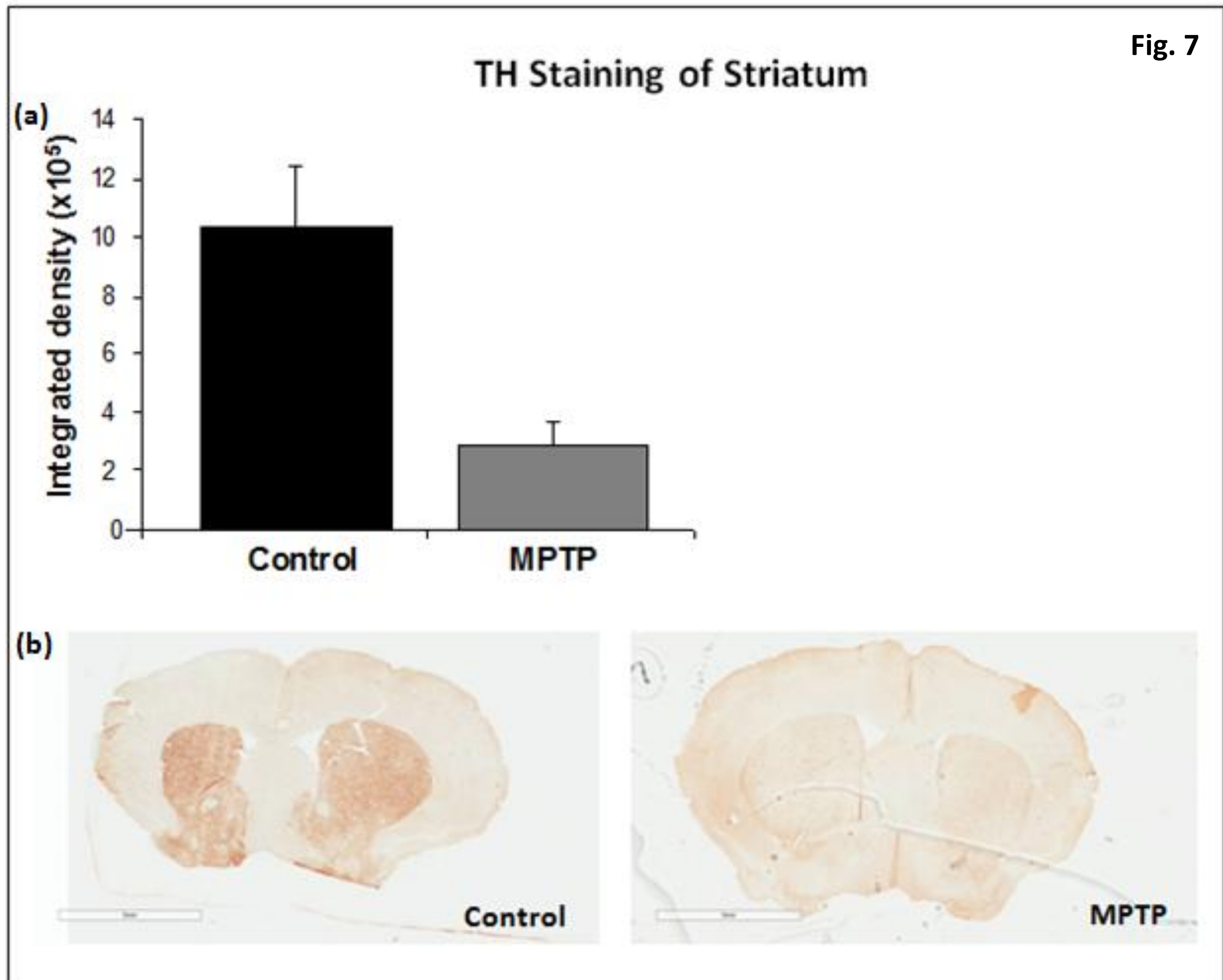


Fig 7: Visual and mean integrated density measurements of striatal TH staining using DAB IHC. (a) This graph shows the mean integrated density measurements of three control and three MPTP striatal regions of mouse brains. Integrated density is the background corrected unit used for IHC staining intensity. Analysis showed that there was a significant 72% decrease in staining in the MPTP mice, as compared to the control (student t-test $p < 0.5$). (b) These images of cortically sliced brain complement the numerical staining differences between the groups. The darker staining in the striatum of the control group represents how the DA rich striatum normally stains. The absence of this intensity can be seen in the MPTP slice. N=3 each for the control and MPTP groups.

Discussion

This study accomplished the initial goal to better characterize olfactory deficits in the MPTP PD model. Our study tested for deficits in memory formation, discrimination and memory duration. The habitation trials across both tests showed that both groups had the ability to

habituate even after injections. There seems to be a significant difference in the habituation rate correlated to individual control and MPTP groups, but the specific relationship could not be measured. The odor duration test showed that the post-MPTP group had a short duration memory (< 15 minutes) compared to the control group who remembered for longer than 15 minutes but not 30 minutes. This was at an earlier time than the control mice and the pre-injection MPTP mice, suggesting that the PD pathology decreased memory duration. There were no significant effects in discrimination, showing that MPTP did not influence odor discrimination ability as tested by our paradigm. The overall investigation time was not influenced by the groups or the testing session.

The IHC and the open field tests support the idea that these differences are derived from olfactory/memory pathways because no overall differences in activity levels were observed. First of all, the IHC of MPTP vs. control mice showed that MPTP did decrease the number of striatal dopaminergic neurons by 72%. This follows the expected histology of a PD model and could explain some of the diminished memory abilities seen here. The open field test showed that this degeneration did not affect the motor function or anxiety of the animals in any significant way. This means that differences in the olfaction tests were likely not affected by the inability of the MPTP mice to move close to the odorants. It also adds on past evidence that nonmotor deficits can occur before any apparent motor dysfunction.

As mentioned previously, one test that showed no significant difference between the experimental group or session was odor discrimination. This is also the olfactory ability tested in prior studies, albeit with less sensitive methods and different PD models. Studies using an intranasal MPTP rat model and a transgenic alpha-synuclein mouse model showed discrimination deficiencies in the PD group as measured by “own” vs. novel scents (Castro et al.,

2012; Fleming et al., 2008). However, discrimination tests done with fruit extract odorants rather than social odors showed that the transgenic PD mice could still discriminate odors with a lower sensitivity than *wt* mice (Fleming et al., 2008). Our conclusions can only be made about the i.p. injected mouse MPTP model, for which these results show no significant discrimination differences.

It is difficult to correlate the detailed positive and negative results here to a human timeline. There has been hardly any olfactory testing along early PD progression in humans. This is probably due to the fact that olfaction starts well before the disease is recognized anyway. The few studies done so far show a 75+% age adjusted olfaction loss in patients who already have the common disease stages (Haehner et al., 2009; Sobel et al., 2001). If the MPTP model is representative of the human progression, the deficits seen here would most likely occur at least 4 years before physical or imaging detection of disease.

Our results encourage further studies. The first thing that should be considered is to analyze acetylcholine levels using the remaining perfused mouse brains. The Linster lab has shown that nicotinic type AcH plays an important role in modulating the receptive field of the OB (Devore & Linster, 2012). This is another neuromodulator that has been correlated to decrease within cholinergic OB inputs in human and animal PD models (Bohnen et al., 2010; Mundiñano et al., 2013).Specifically looking at expression levels within the OB could help further link the behavioral results with the actual histology. This analysis was originally planned for this study, but was unable to be done due to time constraints.

Another useful analysis would be using alternate olfactory tests with the same model and testing cycle. Instead of discrimination and duration tests, olfactory generalization could be measured (Cleland et al., 2002). This test also measures discrimination between odors, but

accomplishes this with a specific bedding marked odor associated with a food reward. After training, the ability of the mice to dig and choose the correct odorant versus an incorrect choice can be recorded. This could help to find more intricate olfaction changes occurring in this PD model.

Conclusion

Thus, the behavioral olfaction tests performed in this study supports the idea that odor duration, not discrimination or habituation, is impaired in the MPTP B6 mouse model of Parkinson's Disease. Although replications with longer trained mice, different olfactory tests, and more IHC are needed, these findings represent an important advancement in the knowledge of the nonmotor aspects of PD. Future research will hopefully characterize this further to develop earlier disease markers in humans.

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