The Rotenone Exposure Model of Parkinson's Disease Induces Progressive Locomotor Deficits in Mice

Nicole E. Yates

Colorado State University, Department of Biomedical Sciences

6 December 2024

Submitted to the Molecular, Cellular and Integrated Neuroscience Program, Colorado State University, in partial fulfillment of the requirements for a B.S. Degree (NB 499), Fall 2024

University Honors Program, Fall 2024

Abstract

Neurodegenerative diseases remain the leading cause of physical and cognitive disability worldwide. Within this group of diseases, Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder affecting an estimated 9.4 million people worldwide in 2020. It is characterized by the selective degeneration of dopaminergic neurons (DAn) in the substantia nigra pars compacta (SNpc), increased glial activation, and inflammatory signaling. Clinical motor symptoms include resting tremor, hunched posture, difficulties initiating movement, and a short shuffling gait. Exposure to environmental neurotoxins such as pesticides can recapitulate key pathological features of PD. Among such agents is the naturally occurring pesticide, rotenone, a mitochondrial complex I uncoupler that targets DAn in the SNpc, leading to parkinsonian phenotype in rodent models. However, it remains unknown how this toxin elicits gait disturbances throughout the course of exposure. In this study, inbred C57Bl/6 mice were exposed to 2.5 mg/kg/day of rotenone or respective vehicle control for 14 days, followed by a 7 day lesioning period. Using the Noldus CatWalk XT, a high-throughput gait tracking and analysis system, alterations in gait and locomotion were examined over the course of rotenone exposure and subsequent lesioning period. The resulting data demonstrates that rotenone exposure alters locomotion by decreasing stride lengths and speed of movements both in individual paws and with the animal's gait as a whole. The exposure induced changes in animals that progressed even after the cessation of exposure. This suggests that rotenone-induced neurotoxicity causes a sustained response in the brain leading to progressive neurologic and motor dysfunction, similar to that observed in patients with idiopathic PD.

Introduction

1. Neurodegenerative Disease

Neurodegeneration is the progressive irreversible loss of neurons occurring in the brain. Neurodegenerative diseases are pathologically characterized by cytotoxicity as well as protein misfolding and neuroinflammation that underlies the clinical presentation of each disorder (Dugger & Dickson, 2017; Zeydan et al., 2023). These conditions can be classified by the aggregative protein species (e.g. tau, α-synuclein, amyloid), by the major clinical presentation (e.g. motor, cognitive, behavioral), or by the anatomical location of pathology within the central nervous system (Dugger & Dickson, 2017).

The greatest singular risk factor for neurodegenerative diseases is advanced age. Alzheimer's disease and related dementias (ADRD) are the most prevalent age-related disorders with 6.9 million people ages 65 or older living with ADRD in the US in 2024 (Alzheimer's & dementia, 2024). Similarly, Parkinson's disease (PD) is currently the second most common age-related condition, despite being the most rapidly increasing in diagnoses. With the aging population, the number of people predicted to suffer from PD in 2030 is double what it was in 2005 (Dorsey et al., 2006) and the global disease burden being 9.4 million individuals in 2020 (Maserejian et al., 2020). The predicted increases in neurodegenerative diseases worldwide show a large need for continued research into the development, progression, and treatment of these currently irreversible diseases.

2. Parkinson's Disease Clinical Presentation and Treatment

Clinical presentation of PD varies by individual in symptomology and severity (Hayes, 2019). The majority of people affected by PD experience some form of tremors generally in the hands or jaw, increased muscle rigidity, a "mask-like" appearance caused by facial rigidity, and

alterations in gait and balance (Kouli et al., 2018; Hayes, 2019; Greenameyer et al., 2001). Muscular deficits may not only affect the individual's capability to care for themselves, but also create increased chances for injury from falls or other accidents that may be severely harmful or even deadly in the aging populations they affect. Furthermore, many individuals also experience anxiety, depression, or dementia as comorbidities; in recent years, hallucinations and paranoid delusions have become considered as possible PD indicators as well (Kouli et al., 2018; Hayes, 2019). With the worsening symptomology of PD, individuals experience a decrease in quality of life and may require intervention from family or medical caregivers.

Pathologically, PD is characterized by aggregation of α-synuclein protein and loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and striatum (Kouli et al., 2018; Hayes, 2019). Despite the novel PD literature emerging in recent years, the scientific understanding of PD mechanisms remains elusive and the majority of publicly available treatments focus on the treatment of symptoms rather than reversing the loss of DA neurons. The primary treatment is levodopa, the first effective treatment which remains the most prescribed pharmacological agent in this context. Levodopa is the precursor to dopamine and is capable of passing through the blood-brain barrier, unlike its metabolite (Hayes, 2019). The precursor allows remaining DA neurons to produce more dopamine with the intention of rescuing deficits in neurotransmission. Another drug known as carbidopa is used to block the levodopa metabolism in the peripheral nervous system to both increase availability to the central nervous system and to reduce peripheral side effects. The resulting increase in dopamine relieves symptoms; however, it does nothing to stop the continued loss of neurons and may result in worsened neuropsychiatric presentations with prolonged use (Hayes, 2019).

Aside from pharmacologic treatments, there are more invasive treatments also used for Parkinson's disease. In the 1960s, this used to refer to intentional thalamic lesioning that was soon abandoned due to its imprecise nature and the development of dopamine supplementation. However in more recent years, a new surgical treatment called deep brain stimulation (DBS) was developed (Hayes, 2019). It involves the implantation of an electrode into the ventral intermediate nucleus of the thalamus to allow a focus stimulation to that region of the brain. The average tremor reduction of people who have undergone DBS is over 80% (Fariba & Gupta, 2023). However, this more invasive method of treatment has its own faults. This surgical treatment is only intended to decrease the severity of motor symptoms, and similarly to pharmacological treatments, has no capability to slow the continued progression of cell death or reverse any of the damage already done to the dopaminergic neural circuits. With surgery comes the dangers of hemorrhage, hardware maintenance, and the possibility of infection in crucial regions of the brain. Furthermore, cost has a hand in making this treatment inaccessible to the majority of those afflicted with PD.

Currently, we do not have an effective method of treatment that is both effective in reducing symptoms and halting further progression of PD pathology. Without understanding how the disease develops in the central nervous system, it is impossible to target the root causes of PD and prevent the damages to the neural pathways.

2a. Etiology of PD

There are many risk factors that must be considered when examining the development of PD, the most primary of them being age and sex. Most populations experience significant rates of PD expression after 65 years of age and at higher rates in males compared to females (Willis et al., 2022; Kouli et al., 2018; Hayes, 2019). However, these are only two factors out of many

that have been linked to rates of PD, proving it to be a multifaceted disease. Other factors linked to PD onset include genetic mutations and environmental toxin exposures.

Familial genetic PD makes up a minority of diagnosed cases, with only 10-15% attributed to family history of the disease and 5% to direct inheritance of a mutation that results in parkinsonian phenotypes. Familial inheritance has been found in several genes such as SNCA, PINK1, and PRKN have served as a focal point for genetic PD studies (Kouli et al., 2018; Ye et al., 2023). SNCA is the gene that codes for the α-synuclein protein, which appears as misfolded protein aggregates that are a hallmark of PD pathology. Mutations to PINK1 and PRKN affect mitochondrial biology. PINK1 is a serine-threonine kinase that identifies mitochondrial damage and recruits the ubiquitin ligase PRKN. These proteins together are used to target damaged mitochondria for destruction and the loss of this function will lead to increased oxidative stress on the cells (Ye et al., 2023). However, these are not the only genes affecting risk of PD progression. There have been over a hundred genes linked to PD susceptibility and that range of possibilities is one feature of PD that makes it both difficult to detect the genetic risk in patients and difficult to replicate in laboratory studies. As a result, much research also examines toxin-induced models of PD to examine the more heterogeneous effects of the disease seen in the majority of clinical cases.

With the minority of cases being attributed to genetics, the majority of PD cases are due to assorted risk factors altering biological functions. In particular, certain environmental exposures have been linked to increased rates of PD in humans. These exposures include pesticides, herbicides, and heavy metals such as manganese. Manganese exposures and other heavy metal exposures are a common occurrence in metallurgic occupations as they are commonly used in welding and related tasks (Bauburich et al., 1988). Similarly, pesticides are a

frequent occupational exposure for agricultural workers because of their frequently essential use to the success of agriculture; worldwide there is an estimated 3.6 billion kilograms of pesticides used per year (Richardson et al., 2019). The concern with pesticide use comes with the effect it has on non-target species, namely humans. Many pesticides' methods of function are not limited to the pests they are used on and as a result may affect the humans or surrounding environment. Most importantly, pesticides are used on foods and have a much higher degree of contact with the general public compared to welding exposures and as a result, may have a far greater impact on public health than we currently understand.

2b. PD Pathology

Parkinson's disease has a large variety of biological pathways that have been implicated in the development of the disease, however, how these pathways interact with each other and the fine details in what order they progress is largely unknown. How the disease progresses may also be affected by which risk factors were involved in each case and what biological pathways may not yet be linked to PD progression. Despite these large unknowns, many studies have found significant hallmarks to PD expression.

Prior to the severe loss of DA neurons, microglial activation and inflammatory complement cascades have been linked to PD expression. Microglia and complement cascades serve as the initial immune defense for the brain and when activated against foreign stressors, result in pro-inflammatory cytokine signaling and increased microglial activity. PD patients have been found to present both chronic microglial activity and higher rates of inflammatory cytokines than healthy controls (Kouli et al., 2018; Rocha et al., 2022). This microglial pro-inflammatory signaling functions as an attenuating factor for neuronal cell death (He et al, 2001) and reveals glial inflammation to be a pathogenic feature of PD.

Protein aggregates have been noted as a feature of many neurodegenerative diseases, including Parkinson's disease, although the protein in question varies by disease. PD is a synucleinopathy and is characterized by aggregation of the presynaptic protein α-synuclein. In its native form, α-synuclein remains mostly unfolded until coming into contact with negatively charged lipids upon which it will adopt an α-helical structure. In PD, a β-sheet form is adopted instead. These sheets are capable of densely slotting together, making the proteins more prone to aggregation as a result (Dugger & Dickson, 2017; Hays, 2019; Kouli et al., 2018). These protein aggregates have been determined to be a major component of Lewy Bodies - neuronal inclusions that are found in clinical cases of PD among other neurodegenerative diseases. α-synuclein protein function remains somewhat unknown although it is hypothesized to have a role in synaptic vesicle trafficking (Dugger & Dickson, 2017); the decreased available functional protein affects cell function in a way that has yet to be fully explored.

Loss of dopaminergic (DA) neurons is one of the defining features of PD's distinctive motor deficits. The SNpc is a region of the midbrain populated with a high number of DA neurons that project along the nigrostriatal pathway to the putamen region of the dorsal striatum. These regions are largely responsible for voluntary motor function, and influences both the initiation of desired voluntary movement and the inhibition of unwanted motor output via dopamine signaling (Sonne et al., 2024). DA neurons are responsible for production of large quantities of dopamine neurotransmitter used in these regions of the brain (Kouli et al., 2018; Hays, 2019; Zeng et al., 2018). The loss of the cells in turn causes deficits in voluntary movement signaling that induces the clinical locomotor deficits that are characteristic of PD.

3. Rotenone

Rotenone is a potent lipophilic isoflavonoid that is naturally occurring in plants from the *Leguminosae* family which functions as a very selective mitochondrial complex I inhibitor. It is a known neurotoxic compound that has been used as a pesticide and picicide in multiple countries worldwide despite its observed toxicity (Richardson et al., 2019; Ferrati et al., 2023). The rotenone compound is known to have a short half-life period and oxidizes quickly when exposed to air. Despite this, decades of reported PD data have shown that people who were chronically exposed to rotenone, such as agricultural workers, experienced a greater risk of developing PD (Tanner et al., 2011; Richardson et al., 2019). Rotenone administration in animal models has revealed selective degeneration of dopaminergic neurons in the nigra and striatum and has been used as a model for PD research for several decades (Heinz et al., 2017; Richardson et al., 2019; Zeng et al., 2018).

3a. Electron Transport Chain Interactions

The electron transport chain (ETC) is a series of protein complexes that function as couplers for redox reactions designed to form an electrochemical gradient between the intermembrane space and the matrix of the mitochondria. This electrochemical gradient is utilized in the process of oxidative phosphorylation to produce ATP (Ahmad et al., 2024). The enzyme complexes that make up the ETC are complex I, complex II, coenzyme Q, complex III, cytochrome C, and complex IV. Complexes I and II each serve as initiation points for the ETC and accept electrons from NADH and succinate respectively (Ahmad et al., 2024).

Mitochondrial complex I, also called ubiquinone oxidoreductase, is embedded in the inner membrane of the mitochondria and protrudes into the innermost matrix. The protein is made up of numerous protein subunits to perform two main functions: oxidoreductase activity

and transmembrane proton pumping, which together make up the NADH electron donation to the proton gradient (Greenamyre et al., 2001; Ahmad et al., 2024).

Rotenone's method of action is an inhibition of the electron transfer from the redox-active iron-sulfide complexes to the ubiquitinone. In isolated mitochondria, rotenone exposure has been observed to decrease complex I activity by 70-80% of its original function (Heinz et al., 2017). This incomplete transfer of electrons to oxygen can induce the formation of reactive oxygen species (ROS), which can lead to mitochondrial DNA damage, mitochondrial death, and cellular apoptosis with severe enough ROS production (Ahmad et al., 2024; Heinz et al., 2017; Richardson et al., 2019). Despite the extensive research performed with rotenone, the precise molecular mechanism of the toxin remains unknown; however, its effects on the cell and organism as a whole can still be explored.

3b. Mitochondrial Damage

Reactive oxygen species is an umbrella term that refers to superoxide radicals, peroxides, hydroxyl radicals, and hydrogen peroxides. Although ROS can be produced by other cellular processes, around 90% of ROS production is caused by mitochondrial oxidative phosphorylation (Checa & Aran, 2020). The ROS production induced by mitochondrial complex I dysfunction causes alterations to biological pathways as it functions like secondary messengers. As a result, it can indirectly alter autophagy, proinflammatory signaling, and apoptosis which have all been noted as PD pathology. Furthermore, the production of ROS in the mitochondria reduces the mitochondrial membrane potential and also induces Ca^{2+} entry. Excessive Ca^{2+} in the mitochondria can lead to mitochondrial outer membrane permeability, which in turn leads to mitochondrial death (Checa & Aran, 2020). Post-mortem studies of PD patients reported deficiencies of mitochondrial complex I (Kouli et al., 2018), which aligns with the understood

effects of mitochondrial damage. With enough mitochondrial death, cells would be unable to keep up the energy requirements for essential cell function and induce apoptosis, thereby causing a parkinsonian phenotype with the loss of DA neurons. With this process of mitochondrial damage leading to cellular death, the goal of my experimentation was to determine if rotenone toxin produces progressive locomotor deficits that would function as an *in vivo* parkinsonian mouse model.

Methods

Animal Care

Animal protocols were approved by the Institutional Animal Use Committee at Colorado State University (IACUC), mice were handled in compliance with PHS Policy and Guide for the Care and Use of Laboratory Animals, and procedures were performed under National Institutes of Health (NIH) guidelines. Mice were housed in microisolator cages (2-4 animals/cage), kept on a 12-hour reverse light/dark cycle, and had access to both food and water ad libitum. Male C57Bl/6 background mice were used in the study at 3 months of age (n = 20/control, n = 20/rotenone).

Rotenone Preparation and Dosing

Rotenone was first prepared in a 50× stock solution that was diluted in 100% dimethyl sulfoxide (DMSO). The rotenone solution was then diluted in medium-chain triglyceride, Miglyol 812 to obtain final working concentrations of 2.5 mg/kg at a dosage of 2 µL/g body weight in 98% miglyol and 2% DMSO (Rocha et al., 2022; Rocha et al., 2023). The rotenone was prepared fresh every other day and stored in amber septa vials. Mice were dosed with 2µL/g body weight of respective treatment via intraperitoneal injection and were weighed daily before injection to ensure accurate dosing. A 50 µL Hamilton syringe was used to measure the dosage

and then transferred to a 22G insulin syringe to administer the treatment. The dosing regimen of 2.5 mg/kg was administered once per day for 14 days. Mice were terminated at 7 days post initial injection (n = 10/control, n = 10/rotenone) and 21 days post initial injection (n = 9/control, n = 10/rotenone). One mouse from the control cohort was sacrificed at 4 days post initial injection due to a punctured intestine leading to declining health.

Real-Time Gait Analysis

The Noldus Catwalk XT is a gait analysis system used for quantitative locomotor assessment of rats and mice by collecting nonrestrictive movement patterns across a one-meter trackway equipped with contrasting illumination patterns allowing for pawprint visualization and pressure sensing (Rocha et al., 2023). Mice were not acclimated to the trackway prior to baseline measurement acquisition. Mice were permitted 10 seconds to cross the trackway and were rerun for 3 compliant runs per time point. Parameters analyzed included run duration in seconds, run average speed in cm/s, percentage of time spent on diagonal stance support, percentage of time spent on three-point stance support, swing speed for individual paws in cm/s, stride length for individual paws in cm, and the mean intensity collected when the paw is at its maximum contact with the trackway for individual paws.

Statistical Analysis

All data analyzed was composed of an average between the three runs per time point and normalized to baseline prior to statistical analysis. Experimental values from each group were analyzed with a ROUT (Q = 1%) test to identify significant outliers for exclusion from the final data set. Variance differences between two-parameter variables were identified using a mixed-model two-way ANOVA. Significance was identified as *$p < .05$; **$p < .01$; ***$p < .001$;

and ****p < .0001. All statistical analysis was performed using Prism (version 10.2.3; Graph Pad Software, San Diego, CA, USA).

Results

Representative images acquired from the CatWalk XT of paw placement on the trackway (Fig. 1A,B) show visible differences in the gait of mice treated with control versus rotenone at 19 days post initial injection. The control mouse displays a diagonal footfall pattern in which a front paw is on the track at the same time its opposing hind paw is on the track (e.g. right front and left hind paw), while the rotenone mouse experienced both a loss of the clear diagonal footfall pattern and visibly increased the number steps taken to cross the trackway. Another set of representative images from the trackway (Fig. 1C,D) show the pressure and area of the pawprints on the track based on the intensity of the illumination picked up by the camera. In these representative images, the rotenone mouse shows a greater area of its paws on the trackway and a wider spread on the amount of intensity on the trackway.

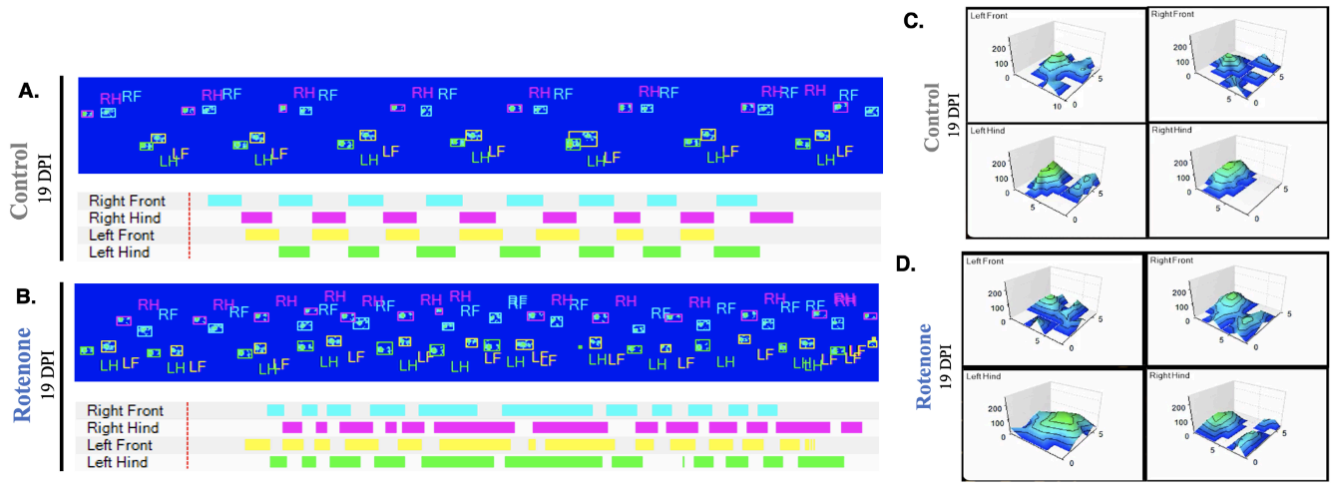

Figure 1. Animals exposed to rotenone display irregular footfall patterns. Locomotion of mice exposed to vehicle or rotenone was analyzed using the Noldus CatWalk XT system. Representative diagrams show the footfall patterns of the mice as they moved across the platform at 19 days post exposure of vehicle (A) or rotenone (B) and representations of footprint area and intensity are shown at 19 days post exposure in a run from control mice (C) and a run from rotenone mice (D).

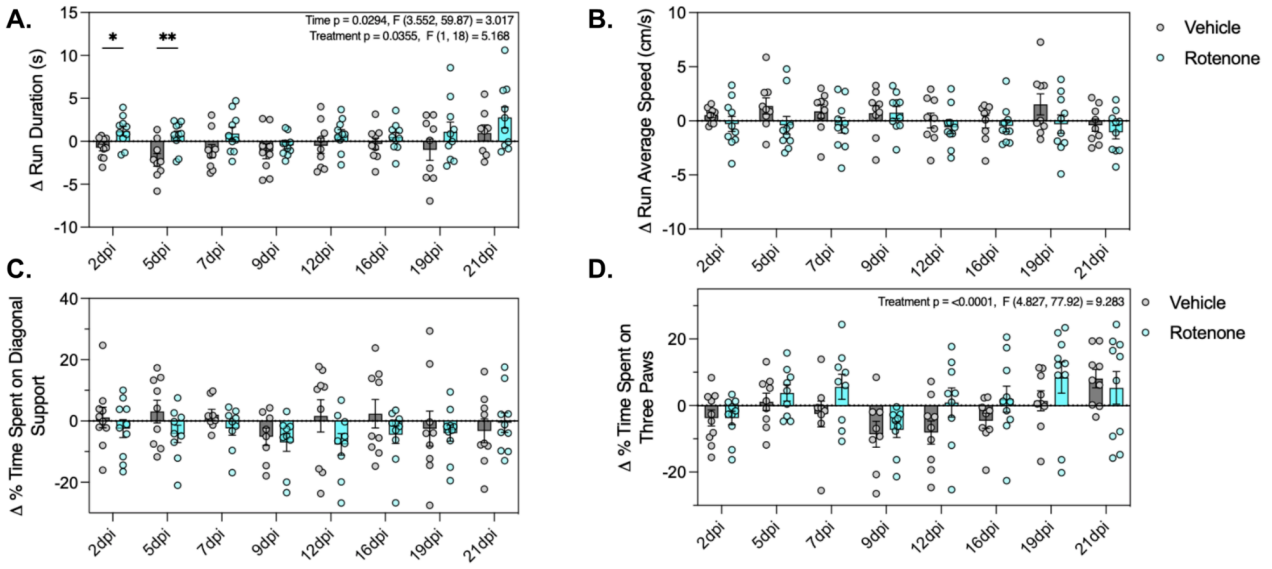

Figure 2: Animals exposed to rotenone display irregular footfall patterns and slowed movement. Locomotion of mice exposed to vehicle or rotenone was analyzed using the Noldus CatWalk XT system. Analysis was performed on change in duration of the run (A), average speed of the run (B), the percentage of time during the run the mice spent standing on diagonal front and hind limbs (C), and the percentage of time during the run the mice spent standing on any three limbs simultaneously (D); mixed-model statistical test performed; N = 10 mice/group, * = $p<0.05$, ** = $p<0.01$

To determine the alterations in overall motor function and balance, the data for run duration, average speed, the percentage of time spent on diagonal limb support, and percentage of time spent on three limb support were analyzed. Both rotenone and control treated mice saw a trend in increasing time taken to cross the trackway and significance across times and treatment groups (Fig. 2A). Average speed of the run shows no apparent difference across rotenone and control mice (Fig. 2B). Similarly, the percentage of time spent on diagonal paw support shows a slight trending decrease in rotenone treated mice, but no statistical significance (Fig. 2C); however, the percentage of time spent on three paw support shows an increased percentage in rotenone mice compared to control (Fig. 2D).

To examine finer locomotor effects of rotenone, parameters specific to individual paws were examined. This includes the speed at which a paw moves from the previous step to the next

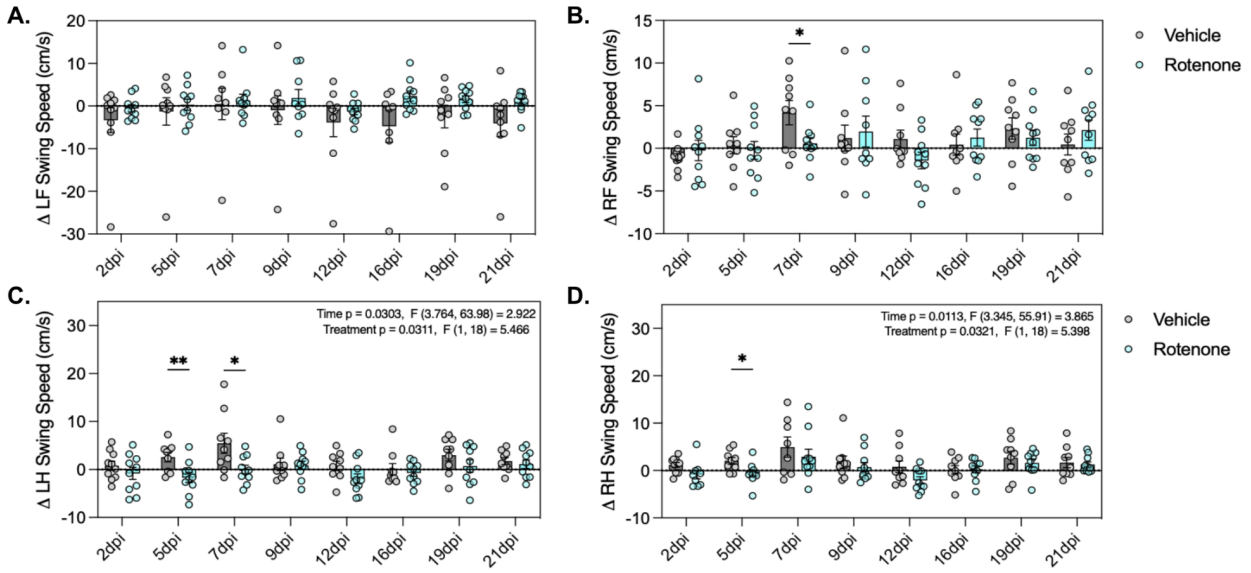

Figure 3: Animals exposed to rotenone experience decreased swing speed most severely in the hind limbs. Analysis performed with Noldus CatWalk XT system on change in swing speed against baseline for individual paws (A-D) as an average across three compliant runs per animal per time point. *N* = 10 mice/group. A mixed-model statistical approach was utilized to assess significance across repeat measures; * = p<0.05, ** = p<0.01

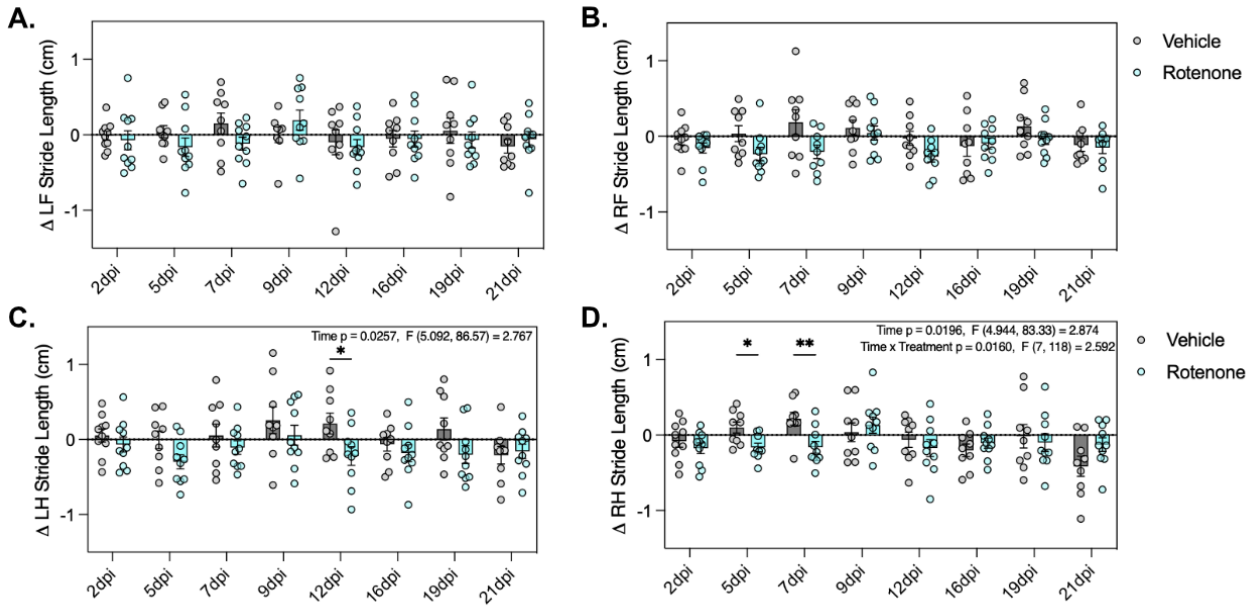

Figure 4: Animals exposed to rotenone experience decreased stride length. Analysis performed with Noldus CatWalk XT system on change in stride length against baseline for individual paws (A-D) as an average across three compliant runs per animal per time point**.** *N* = 10 mice/group. A mixed-model statistical approach was utilized to assess significance across repeat measures; * = p<0.05, ** = p<0.01, **** = p<0.0001

one, referred to as swing speed, the length of the paw's stride, and the mean intensity of illumination from the paw when it is at maximum contact with the trackway. While the front paws show minimal differences in swing speed between rotenone and control mice, (Fig. 3A,B), the hind paws show a greater decrease in swing speed in rotenone treated mice and across the time points (Fig. 3C,D). Similarly, the front paws show no statistical differences in stride length (Fig. 4A,B), but again the hind paws show significant changes in stride length of the hind paws. The left hind paw shows a significant decrease in stride length only (Fig. 4C), while the right hind paw shows significance in both time and time X treatment (Fig. 4D). The max contact mean intensity of the mice is decreased with time and between the rotenone and control mice in all paws (Fig. 5A-D).

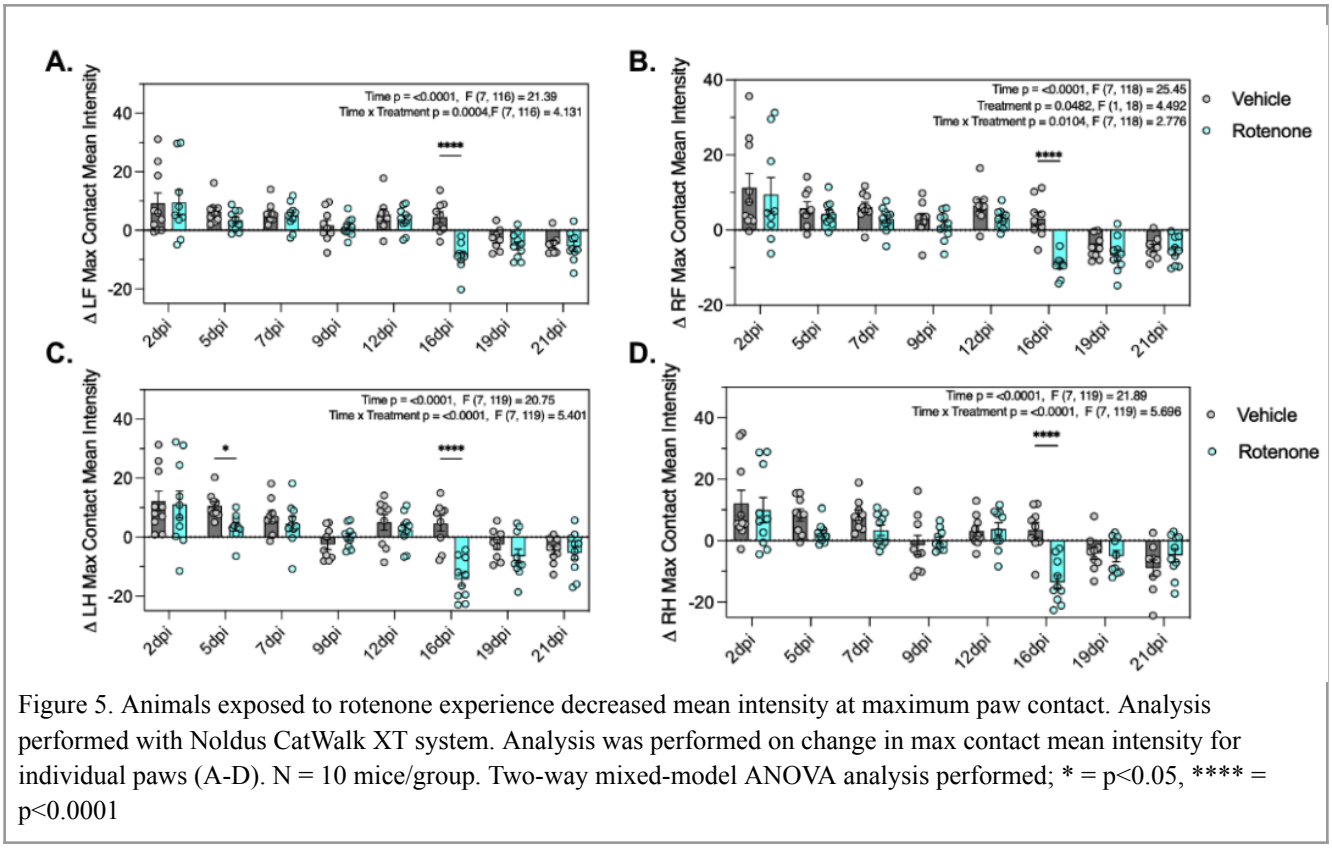

Figure 5. Animals exposed to rotenone experience decreased mean intensity at maximum paw contact. Analysis performed with Noldus CatWalk XT system. Analysis was performed on change in max contact mean intensity for individual paws (A-D). N = 10 mice/group. Two-way mixed-model ANOVA analysis performed; * = $p<0.05$, **** = $p<0.0001$

Discussion

Some of the deficits of the study may have affected the data collected. Mice were run on the trackway a total of nine times, which is over double the recommended amount by Noldus as increased exposure to the trackway creates acclimation over time that may affect the behavior of the mice. Furthermore, weight changes of the mice over the course of the experiment may change the intensity of paw illumination and may affect the max contact mean intensity data due to its reliance on intensity measurements taken with the paws' contact to the trackway. Finally, anxiety-like behaviors induced by dosing and mouse handling may alter behavior and thus change some characteristics in stride and speed of movements.

Despite the deficits, mice exposed to rotenone neurotoxin demonstrated PD-like motor symptoms and gait instability over the course of treatment and post-lesioning. This included increased duration of the run, stand time, and time spent on three limbs as well as decreased cadence and stride length. Individual paws and limbs demonstrate variability in the onset and severity of motor symptoms. This is similar to the disease in humans, where the presentation of clinical motor symptoms is asymmetric. Despite differences in onset and severity, initial alterations in gait appear to occur around 5 days post initial injection and maintains statistical significance in changes across time in run duration, hind paw swing speed, and hind paw stride length, showing a progressive effect in the worsening of rotenone-induced locomotor deficits. The parameter of max contact mean intensity shows a steadily decreasing trend in both control and rotenone mice with statistical significance across time and treatment. There is also a feature at 16 days post initial injection in which rotenone mice's normalized max contact mean intensity switches to negative while the control mice remain positive until 19 days post initial injection. This could be a point at which the mice rotenone mice are spreading their weight over a larger

area of the paws, leading to a lower mean intensity reading, however with the trending decrease in the control mice as well, there may also be acclimation occurring at some rate to cause the overall trending decrease. No conclusion regarding maximum contact mean intensity can be made based on the current data, but it is a unique and significant trend held across all four paws and could be explored further. In conclusion, progressive locomotor deficits characteristic of parkinsonian phenotypes are found in mice exposed to the neurotoxin rotenone.

References

2024 Alzheimer's disease facts and figures. (2024). *Alzheimer's & dementia : the journal of the Alzheimer's Association*, *20*(5), 3708–3821. https://doi.org/10.1002/alz.13809

Ahmad, M., Wolberg, A., Kahwaji, C.I. (2024). Biochemistry, Electron Transport Chain. *StatPearls Publishing.* https://www.ncbi.nlm.nih.gov/books/NBK526105/

Baburich, S.B., Brown, M.A., Cooper, B.J. (1988). Welding, brazing, and thermal cutting. *NIOSH Numbered Publications, No 88-110.* Retrieved from CDC Stacks Public Health Publications ID# 19474. https://stacks.cdc.gov/view/cdc/19474

Checa, J., & Aran, J. M. (2020). Reactive Oxygen Species: Drivers of Physiological and Pathological Processes. *Journal of inflammation research*, *13*, 1057–1073. https://doi.org/10.2147/JIR.S275595

Dorsey, E.R., Constantinescu, R., Thompson, J.P., Biglan, K.M., Holloway, R.G., Kieburtz, K., Marshall, F.J., Ravina, B.M., Schifitto, G., Siderowf, A., & Tanner, C.M. (2006). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology, 68*(5), 384-386. https://doi.org/10.1212/01.wnl.0000247740.47667.03

Dugger, B. N., & Dickson, D. W. (2017). Pathology of Neurodegenerative Diseases. *Cold Spring Harbor perspectives in biology*, *9*(7). https://doi.org/10.1101/cshperspect.a028035

Fariba, K.A., Gupta, V. (2023). Deep Brain Stimulation. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. https://www.ncbi.nlm.nih.gov/books/NBK557847/

Ferrati, M., Maggi, F., Spinozzi, E. (2023). Rotenone: Advances on Resources, Biosynthetic Pathway, Bioavailability, Bioactivity, and Pharmacology. In: Xiao, J. (eds) Handbook of Dietary Flavonoids. Springer, Cham. https://doi.org/10.1007/978-3-030-94753-8_92-1

Garrick, J. M., Costa, L. G., Cole, T. B., & Marsillach, J. (2021). Evaluating Gait and Locomotion in Rodents with the CatWalk. *Current protocols*, *1*(8), e220. https://doi.org/10.1002/cpz1.220

Greenamyre, J.T., Sherer, T.B., Betarbet, R. and Panov, A.V. (2001). Complex I and Parkinson's Disease. *IUBMB Life, 52*: 135-141. https://doi.org/10.1080/15216540152845939

Hayes M. T. (2019). Parkinson's Disease and Parkinsonism. *The American journal of medicine*, *132*(7), 802–807. https://doi.org/10.1016/j.amjmed.2019.03.001

He, Y., Appel, S., Le, W. (2001). Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain Res. (3)*909(1–2):187–93. https://doi.org/10.1016/S0006-8993(01)02681-6

Heinz, S., Freyberger, A., Lawrenz, B., Schladt, L., Schmuck, G., & Ellinger-Ziegelbauer, H. (2017). Mechanistic Investigations of the Mitochondrial Complex I Inhibitor Rotenone in the Context of Pharmacological and Safety Evaluation. *Scientific reports*, *7*, 45465. https://doi.org/10.1038/srep45465

Kouli, A., Torsney, K.M., Kuan, W.L. (2018). Parkinson’s Disease: Etiology, Neuropathology, and Pathogenesis, Chapter 1. *Parkinson’s Disease: Pathogenesis and Clinical Aspects*. Brisbane (AU): Codon Publications. PMID: 30702842

Maserejian, N., Vinikoor-Imler, L., & Dilley, A. (2020). Estimation of the 2020 Global Population of Parkinson’s Disease (PD) [abstract]. *Movement Disorders, 35*. https://www.mdsabstracts.org/abstract/estimation-of-the-2020-global-population-of-parkinsons-disease-pd/

Richardson, J. R., Fitsanakis, V., Westerink, R. H. S., & Kanthasamy, A. G. (2019). Neurotoxicity of pesticides. *Acta neuropathologica*, *138*(3), 343–362. https://doi.org/10.1007/s00401-019-02033-9

Rocha, S. M., Bantle, C. M., Aboellail, T., Chatterjee, D., Smeyne, R. J., & Tjalkens, R. B. (2022). Rotenone induces regionally distinct α-synuclein protein aggregation and activation of glia prior to loss of dopaminergic neurons in C57Bl/6 mice. *Neurobiology of disease*, *167*, 105685. https://doi.org/10.1016/j.nbd.2022.105685

Rocha, S. M., Kirkley, K. S., Chatterjee, D., Aboellail, T. A., Smeyne, R. J., & Tjalkens, R. B. (2023). Microglia- specific knock-out of NF-κB/IKK2 increases the accumulation of misfolded α-synuclein through the inhibition of p62/sequestosome-1-dependent autophagy in the rotenone model of Parkinson's disease. *Glia*, *71*(9), 2154–2179. https://doi.org/10.1002/glia.24385

Sonne, J., Reddy, V., Beato, M.R. (2024). Neuroanatomy, Substantia Nigra. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK536995/

Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestly, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston, J.W. (2011). Rotenone, paraquat and Parkinson’s disease. Environ Health Perspect. doi:10.1289/ehp.1002839

Willis, A.W., Roberts, E., Beck, J.C., Fiske, B., Ross, W., Savica, R., & Van Den Eeden, S.K., Marras, C. on behalf of the Parkinson’s Foundation P4 Group. (2022). Incidence of

Parkinson Disease in North America. *Npj Parkinson's Disease, 8*(170). https://doi.org/10.1038/s41531-022-00410-y

Ye, H., Robak, L. A., Yu, M., Cykowski, M., & Shulman, J. M. (2023). Genetics and Pathogenesis of Parkinson's Syndrome. *Annual review of pathology*, *18*, 95–121. https://doi.org/10.1146/annurev-pathmechdis-031521-034145

Zeng, X. S., Geng, W. S., & Jia, J. J. (2018). Neurotoxin-Induced Animal Models of Parkinson Disease: Pathogenic Mechanism and Assessment. *ASN neuro*, *10*, 1759091418777438. https://doi.org/10.1177/1759091418777438

Zeydan, B., Kantarci, K. (2023). What Is Neurodegeneration?. In: Cross, D.J., Mosci, K., Minoshima, S. (eds) Molecular Imaging of Neurodegenerative Disorders. Springer, Cham. https://doi.org/10.1007/978-3-031-35098-6_1