Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Tiffany Chen April 10, 2023

The Effects of Chronic, Oral Deltamethrin Exposure on Nigrostriatal Dopamine Receptors: A Model for Pyrethroid Toxicity in Adults

by

Tiffany Chen

Timothy Sampson, PhD.
Adviser

Neuroscience and Behavioral Biology

Timothy Sampson, PhD.
Adviser

William Michael Caudle, PhD.
Committee Member

Robert Wyttenbach, PhD.
Committee Member

2023

The Effects of Chronic, Oral Deltamethrin Exposure on Nigrostriatal Dopamine Receptors: A Model for Pyrethroid Toxicity in Adults

By

Tiffany Chen

Timothy Sampson, PhD.
Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2023

Abstract

The Effects of Chronic, Oral Deltamethrin Exposure on Nigrostriatal Dopamine Receptors: A Model for Pyrethroid Toxicity in Adults

By Tiffany Chen

Pyrethroid pesticides have become one of the most commonly used pesticides across the globe due to their relatively low toxicity to mammals, and have been demonstrated to disrupt dopaminergic signaling in various animal models. The effects of long-term pyrethroid exposure in humans are poorly understood, but have been connected to the development of attention deficit/hyperactivity disorder, amyotrophic lateral sclerosis, and Parkinson's disease. Parkinson's disease is the second most common neurodegenerative disorder in the world and is characterized by degeneration of the nigrostriatal dopamine pathway. Dopamine receptors play an important role in the pathology and treatment of Parkinson's, as well as many other disorders such as attention deficit/hyperactivity disorder, schizophrenia, and Huntington's disease. However, there are few studies on the effects of pyrethroids on dopamine receptors, and none in an adult or neurodegenerative exposure model. Thus, this study is the first to our knowledge to demonstrate the effects of pyrethroid exposure in adult wild-type mice on nigrostriatal dopamine receptors. Chronic, oral deltamethrin exposure increased D1-like dopamine receptor levels in the striatum, which may be indicative of compensatory mechanisms and has the potential to become neurotoxic. Further scrutinization of the effects of adult pyrethroid exposure on dopamine receptors is imperative for understanding their roles in the development of neurodegenerative disorders.

The Effects of Chronic, Oral Deltamethrin Exposure on Nigrostriatal Dopamine Receptors: A Model for Pyrethroid Toxicity in Adults

By

Tiffany Chen

Timothy Sampson, PhD.
Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Neuroscience and Behavioral Biology

2023

Acknowledgements

Thank you to Dr. Sampson for his passion, time, and encouragement, and for always challenging me to think more critically about my research. Working under his guidance has been phenomenal.

Thank you to Dr. Caudle for readily offering his expertise and for directing me to the Sampson Lab. This incredible experience started in his office with one serendipitous question.

Thank you to Dr. Wyttenbach for his empathy and kindness as my professor and my growth as a writer. His wealth of knowledge and fantastic classes will forever remind me that the field of neuroscience is endlessly fascinating.

Thank you to Alexandria White for creating such an amazing project and for taking me under her wing. She has been a wonderful mentor, and her support and excitement at my progress have been invaluable to me.

Thank you to the entire Sampson Lab for sharing their knowledge whenever I needed it, and for being wonderful company during those long waits between washing steps.

This project would not have been possible without any of you.

Table of Contents

Tables and Figures

Abstract

Pyrethroid pesticides have become one of the most commonly used pesticides across the globe due to their relatively low toxicity to mammals, and have been demonstrated to disrupt dopaminergic signaling in various animal models. The effects of long-term pyrethroid exposure in humans are poorly understood, but have been connected to the development of attention deficit/hyperactivity disorder, amyotrophic lateral sclerosis, and Parkinson's disease. Parkinson's disease is the second most common neurodegenerative disorder in the world and is characterized by degeneration of the nigrostriatal dopamine pathway. Dopamine receptors play an important role in the pathology and treatment of Parkinson's, as well as many other disorders such as attention deficit/hyperactivity disorder, schizophrenia, and Huntington's disease. However, there are few studies on the effects of pyrethroids on dopamine receptors, and none in an adult or neurodegenerative exposure model. Thus, this study is the first to our knowledge to demonstrate the effects of pyrethroid exposure in adult wild-type mice on nigrostriatal dopamine receptors. Chronic, oral deltamethrin exposure increased D1-like dopamine receptor levels in the striatum, which may be indicative of compensatory mechanisms and has the potential to become neurotoxic. Further scrutinization of the effects of adult pyrethroid exposure on dopamine receptors is imperative for understanding their roles in the development of neurodegenerative disorders.

Introduction

Pesticides are defined by the United States Environmental Protection Agency (EPA) as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest," and include herbicides, insecticides, and fungicides (EPA, 2014; National Institute of Environmental Health Sciences, 2023). Worldwide pesticide use has grown exponentially since the 1940s in conjunction with the discovery of synthetic pesticides, with production increasing at a rate of ~11% per year (Carvalho, 2017). Pesticides are primarily used in agriculture to improve crop yield and to protect humans from vector-borne diseases such as malaria and dengue fever (Carvalho, 2023; Nicolopoulou-Stamati et al., 2016). However, they have many other common, lesser-known applications, such as flea and tick medications for pets, insect and rodent prevention in grocery stores, and mold prevention in plastics, paints, and caulks (Nicolopoulou-Stamati et al., 2016; Damalas, 2009).

There are several classes of regularly-used pesticides that each employ different mechanisms of physiological disturbance in their target organisms. However, it has been well documented that these pesticide classes all produce substantial toxic effects in non-target organisms ranging from crustaceans, fish, bees, birds, murine, and humans (WHO, 2022b; Canadian Centre for Occupational Health and Safety, 2017; Tudi et al., 2022). In humans, pesticide exposure has been linked to disrupted endocrine function, risk for cancers such as non-Hodgkin's lymphoma and breast cancer, neurodevelopmental disorders, neurodegenerative diseases, and death (Tudi et al., 2022; Alavanja et al., 2004; Nicolopoulou-Stamati et al., 2016). In 1990, the World Health Organization (WHO) estimated that around one million unintentional acute poisonings and 20,000 fatalities from pesticides occurred annually across the globe (Jeyaratnam, 1990). A more recent systematic review estimates that the number of global

unintentional acute pesticide poisonings have risen to 385 million per year, although fatalities have fallen to 11,000 (Boedeker et al., 2020).

Pyrethroids

One class of synthetic pesticide that has seen widespread use is pyrethroids, which are derived from *Chrysanthemum cinerariaefolium* (also known as *Tanacetum cinerariaefolium*) (Wakeling et al., 2012). The first synthetic pyrethroid was developed for commercial use in 1953 and prompted the creation of numerous other more stable, potent, and economically viable derivatives, although only about a dozen formulations are marketed in the United States (Matsuo, 2019).

Pyrethroid use has increased in the past decade in lieu of more toxic organophosphate pesticides and are now the second most-used pesticide in the world, making up more than 30% of global insecticide application (EPA, 2022; Lehmler et al., 2020). Pyrethroids sit at the forefront of disease vector control, with WHO recommending their use in mosquito nets, particularly for stable formulations that last over 3 years (Wilson et al., 2020; WHO 2015; WHO, 2017). Over 2000 registered pesticide products contain pyrethroids, some of which can be used in organic agriculture (Bond et al., 2014). Agriculturally, they are used on grains, nuts, vegetables, and cotton, among other crops (EPA, 2020b). They are also commonly found in many residential products such as household insecticides, shampoos, and clothing (Illinois Department of Public Health, 2007; Elwan et al., 2006).

Absorption can occur via inhalation, skin, or ingestion, with the most common route of exposure occurring through the ingestion of foods sprayed with pyrethroids (Todd, 2003; Saillenfait et al., 2015). Due to the lipophilic nature of pyrethroids, they are not easily washed away by water and are readily absorbed by waxy plant surfaces – an appealing trait for

commercial applications, but one that makes them difficult to remove before consumption (Rehman et al., 2014). Diet serves as a continuous source and predictor of exposure to pyrethroids across the world: adults in Italy who ate raw and cooked vegetables five or more times a week had significantly higher levels of pyrethroid metabolite in their urine; several bread and flour types in Iran had detectable levels of pyrethroid residue; and more frequent consumption of rice, pasta, fruit, and breakfast cereals was associated with higher levels of urinary metabolites for multiple formulations of pyrethroids in children in western France (Lu et al., 2008; Riederer et al., 2008; Morgan & Jones, 2013; Fortes et al., 2013; Pirsaheb et al., 2019; Glorennec et al., 2017). Furthermore, active pyrethroids – not their non-toxic metabolites – have been found in breast milk, even in women from countries that do not use pyrethroids for disease control, albeit in concentrations below the acceptable daily intake level specified by WHO (Sereda et al., 2005; Corcellas et al., 2012). Despite their usually quick metabolization in adults, pyrethroids can accumulate in body fat after long-term exposure, prolonging the time they remain in the body (Marei, 1982; Todd, 2003).

The primary mechanism of action for pyrethroids involves slowing the rate at which voltage-gated sodium channels close, although they also affect voltage-gated chlorine channels and calcium regulation (Wakeling et al., 2012; Rodríguez et al., 2016). These disruptions influence cell signaling and neurotransmission.

Pyrethroids are split into two categories: Type I, which lack an α-cyano group, and Type II, which contain an α-cyano group. Although their mechanisms of action are the same, effects of acute toxicity in mammals vary between Type I (e.g. permethrin, bifenthrin) and Type II pyrethroids (e.g. cypermethrin, deltamethrin). Type I pyrethroid toxicity symptoms, known as "T-syndrome," include fine tremor, aggressive behavior, and sensitive startle response. Type II

pyrethroid toxicity symptoms, known as “CS-syndrome,” include coarse tremor, sinuous writhing (choreoathetosis), salivation, and seizures (Todd, 2003; Wakeling et al., 2012). In humans, typical symptoms of exposure are limited to paresthesia, slight respiratory irritation, dizziness, headache, and nausea, and any abnormalities in mobility are often mild and short-term, ceasing after 24-48 hours (Wakeling et al., 2012; Illinois Department of Public Health, 2007).

Longer-lasting effects of pyrethroid exposure are less understood and have been a point of contention for some time, in part due to a lack of studies in humans. Using epidemiological data and animal studies available at the time, a toxicological profile for pyrethroids was compiled by the Agency for Toxic Substances and Disease Registry (ATSDR), in which they declared that there was little indication of any kind of risk to human health following chronic exposure (Todd, 2003). This position is still upheld by the EPA to this day (EPA, 2022).

However, low-level exposure to pyrethroids, particularly deltamethrin, has been linked to lower verbal comprehension and working memory, behavioral difficulties, and attention deficit/hyperactivity disorder (ADHD) symptoms in children (Viel et al., 2015; Oulhote & Bouchard, 2013; Lee et al., 2022). Among Bolivian public health vector program spray men, those who were exposed to higher levels of pyrethroids exhibited decreased neurocognitive performance (Hansen et al., 2017). Neuronal death has also been observed after pyrethroid exposures, which may implicate them to certain neurodegenerative diseases (Ray & Fry, 2006). In fact, exposure has been associated with amyotrophic lateral sclerosis (ALS), albeit not significantly, as well as the development of Parkinson’s disease in separate populations (Kamel et al., 2012; Elbaz et al., 2009; Furlong et al., 2015; Stephenson, 2000).

Parkinson's Disease

Parkinson's disease (PD) is the second-most common neurodegenerative disorder in the world, and is most commonly recognized by motor impairments such as tremors, muscle stiffness, slow movement, and difficulty with balance and coordination. Non-motor symptoms such as anosmia, constipation, and rapid-eye movement sleep disorder also frequently occur in PD, and can manifest several years before motor symptoms appear (Schapira et al., 2017).

The disease is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) that extend into the striatum. Motor symptoms typically appear after ~70-80% of nigral dopaminergic neurons and ~50% of striatal dopamine have been lost (Bu et al., 2021; Seeman and Niznik, 1990; Kirby et al., 1999). Loss of dopamine transporter (DAT), which controls the reuptake of dopamine, and tyrosine hydroxylase (TH), an enzyme that catalyzes the formation of dopamine precursor levodopa (L-DOPA), is also seen in Parkinson's (Chotibut et al., 2012; Nutt et al., 2004; Miller et al., 1997; Tabrez et al., 2012). Abnormal aggregation of the protein α-synuclein (α-syn) into Lewy bodies is also a biological hallmark of the disease, and may play a role in substantia nigra neuron death (Poewe et al., 2017; Michel et al., 2016).

The prevalence of PD across the globe has doubled in the past 25 years, and rates of disability and death from the disease continue to increase faster than any other neurological disorder (WHO, 2022a). Thus, it is imperative to identify the factors that contribute to the disease's development and understand their role in its pathology. Although there are several risk genes associated with Parkinson's disease, only about 5-10% of PD cases are purely hereditary, suggesting that its etiology may be connected to environmental factors or gene-environment interactions (Jankovic & Tan, 2020; Poewe et al., 2017). Consequently, extensive research has

been conducted on exposure to various substances, such as heavy metals and psychostimulants, in relation to Parkinson's (Emamzadeh & Surguchov, 2018; Ball et al., 2019).

The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its ability to produce rapid-onset parkinsonian symptoms, combined with the pesticide paraquat's structural similarity to MPTP, prompted studies investigating the relation between pesticides and Parkinson's disease (Elbaz & Tranchant, 2007). Indeed, exposure to paraquat, organophosphates, carbamates, organochlorines, and pyrethroids has been linked to oxidative stress, mitochondrial dysfunction, conformational changes in α-syn, and structural damage to dopaminergic neurons (Islam et al., 2021; Elbaz & Tranchant, 2007).

Due to the loss of dopamine experienced in PD, a common treatment option is the administration of L-DOPA. Although it is initially highly effective, clinical efficacy often declines with long-term use alongside the appearance of drug-induced dyskinesias and dystonia that can be greatly disabling and difficult to treat (Thanvi & Lo, 2004). As such, alternate treatments that target dopamine receptors have emerged as frontline options for PD in young patients (Hisahara & Shimohama, 2011).

Dopamine Receptors

Dopamine controls a vast repertoire of functions, such as motor control, emotion, reward, and cognition. The diverse roles of dopamine are mediated by G-protein coupled dopamine receptors. There are five dopamine receptors, which are categorized into two families based on their structural, pharmacological, and biochemical characteristics: D1-like (D_1 and D_5 receptors) and D2-like (D_2, D_3, and D_4 receptors) (Missale et al., 1998).

D1-like receptors (DRD1) are most abundant in the striatum, nucleus accumbens, olfactory tubercle, and substantia nigra, but are also found to a lesser degree in other areas such

as the amygdala, hippocampus, and frontal cortex. They are located postsynaptically on non-dopaminergic neurons (Mishra et al., 2018; Ford, 2014). In the striatum, DRD1 protein and mRNA are expressed on DRD1-selective GABAergic medium spiny neurons (MSNs) and cholinergic interneurons (Martel & McArthur, 2020). In the midbrain, D_1 protein, but not mRNA, is densely expressed in the substantia nigra pars reticulata (SNr), but is not present in the substantia nigra pars compacta (SNc) (Missale et al., 1998). It is primarily present on nigrostriatal GABAergic axon terminals, but may also be expressed in GFAP-positive astrocytes (Nagatomo et al., 2017).

D1-like receptors couple to the stimulatory $G\alpha_{s/olf}$ family of G proteins, activating adenylyl cyclase and increasing cyclic AMP (cAMP) levels (Mishra et al., 2018). D_1 receptors in the prefrontal cortex also have an important role in working memory and DRD1 in the nucleus accumbens is important for maternal behavior (Sawaguchi & Goldman-Rakic, 1991; Numan et al., 2005). Activation of DRD1 produces an excitatory effect on locomotion (Beaulieu & Gainetdinov, 2011). Curiously, several studies note that DRD1 knockout mice exhibit behavioral hyperactivity (Xu et al., 1994; Centonze et al., 2003).

D2-like receptors (DRD2) are mainly found in the striatum, external globus pallidus, core of nucleus accumbens, and olfactory tubercle (Mishra et al., 2018). DRD2 is located presynaptically and postsynaptically, with D_2 and D_3 also acting as autoreceptors on dopamine neurons (Ford, 2014). In the striatum, D2-like receptors are primarily located on DRD2-selective medium spiny neurons, although a small subgroup of MSNs also co-express DRD1 (Missale et al., 1998; Beaulieu & Gainetdinov, 2011). Although it is less abundant, DRD2 protein and mRNA is also expressed on dopaminergic cell bodies in the substantia nigra pars compacta and ventral tegmental area (VTA), but is not seen in the substantia nigra pars reticulata. These D2-

like receptors are presumably autoreceptors and are colocalized with TH (Bertran-Gonzalez et al., 2008; Missale et al., 1998). Due to alternative gene splicing, D_2 receptors produce two isoforms: D_2-short (D2S) and D_2-long (D2L), which has 29 more amino acids than the short isoform (Lindgren et al., 2003). These isoforms appear to bind differentially to inhibitory G-proteins (Guirammand et al., 1995). The striatum primarily expresses D2L, whereas the SNc primarily expresses D2S (Khan et al., 1998).

D2-like receptors couple to the inhibitory $G\alpha_{i/o}$ family of G proteins, inhibiting adenylyl cyclase activity, cAMP production, and calcium channels, while activating G-protein regulated inwardly rectifying potassium channels (GIRK) (Mishra et al., 2018). Activation of DRD2 autoreceptors, particularly D_2, reduces dopamine neuron activity and dopamine release, which in turn decreases locomotion (Ford, 2014). However, postsynaptic DRD2 has a mild stimulatory effect on movement (Beaulieu & Gainetdinov, 2011). DRD2 autoreceptors in the striatum can regulate dopamine transmission by inhibiting calcium channels, increasing DAT activity, and reducing TH activity (Ford, 2014). Blocking DRD2 has been demonstrated to inhibit the reuptake of dopamine, while excessive stimulation of these receptors accelerates it (Cass & Gerhardt, 1994; Benoit-Marand et al., 2010). Interestingly, DRD2 knockout mice exhibit significant PD-like locomotive impairment (Hisahara & Shimohama, 2011).

Dopamine receptors play a role in multiple neurological disorders and are subsequently targets for numerous treatment methods. Polymorphisms in D_4 and D_5 receptor genes have been linked to ADHD (Coghill & Banaschewski, 2009). Low DRD2 levels and DRD1 hypersensitivity have been demonstrated in addiction and substance use disorder (Klein et al., 2018). Psychotic symptoms in schizophrenia are associated with elevated DRD2 levels, and DRD2 antagonism is the primary mechanism for antipsychotics (Martel & McArthur, 2020;

Seeman, 2013). Significant decreases in striatal DRD1 and DRD2 binding and density is consistently observed in Huntington's disease, although overactivation of DRD1 has been documented in early Huntington's disease before the onset of motor symptoms (Cepeda et al., 2014; Beaulieu & Gainetdinov, 2011; Moreno-Delgado et al., 2020).

Increased DRD1 and DRD2 densities are observed in the striatum in Parkinson's patients, particularly in early stages of the disease (Seeman & Niznik, 1990; Hisahara & Shimohama, 2011). Neuroimaging studies from later-stage PD patients show downregulation in striatal DRD2 – however, it is unclear if this is a result of the disease's progression or an effect of treatment (Kaasinen et al., 2021). PD treatments that target dopamine receptors are primarily DRD2 agonists, although some drugs, such as DHEC and apomorphine, also act on D1-like receptors (Hisahara & Shimohama, 2011). Receptor supersensitivity has also been proposed in the pathology of Parkinson's, which may explain the clinical success of L-DOPA (Lee et al., 1978).

Pyrethroids, Parkinson's, and Dopamine Receptors

Studies have demonstrated extensive effects of pyrethroid exposure on the dopaminergic system. Short-term type-II pyrethroid exposure elicits prolonged dopamine and acetylcholine release in the striatum, which is mediated by pre- and postsynaptic DRD2 receptors, respectively (Eells & Dubocovich, 1988). Pyrethroids can also trigger dopaminergic neuron death in the substantia nigra and decreases in striatal dopamine levels (Jiao et al., 2020; Mohammadi et al., 2019). Decreases in TH have also been documented following exposure to pyrethroids (Jiao et al., 2020; Liu et al., 2006). There have been mixed results on the effects of pyrethroids on DAT. Some studies observed higher levels of DAT, which may increase vulnerability to neurotoxins and is consistent with ADHD pathology (Elwan et al., 2006; Gillette and Bloomquist, 2003; Kirby et al., 1999; Richardson et al., 2015), whereas others demonstrated a decrease in DAT,

which is consistent with PD pathology (Pitzer et al., 2021; Pittman et al., 2003). Behaviorally, pyrethroid exposure in adult rodents induces tremor and mobility issues similar to those seen in Parkinson's (Pitzer et al., 2021; Rodríguez et al., 2016).

Given their importance in modulating dopaminergic function and roles in various disorders, there is a necessity to understand the effects of pyrethroids on dopamine receptors. However, observed results from current literature are rather mixed. Kung et al. (2015) reported a decrease in DRD1 and DRD2 mRNA in zebrafish exposed as embryos, Crago and Schlenck (2015) found a decrease in DRD2 mRNA expression in juvenile rainbow trout, and Pitzer et al. (2019) saw a decrease in DRD1 mRNA expression in treated rat pups. Another study conducted in zebrafish embryos demonstrated no difference in DRD1 nor DRD2 mRNA levels (Wang et al., 2020), and unpublished data cited by Pitzer et al. (2021) also saw no change in striatal DRD1 or DRD2 protein levels in rat pups. Conversely, Patel & Pitzer (2019), an undergraduate student in Pitzer's lab, observed an increase in DRD1 protein in rat pups. In the offspring of exposed rodents, Richardson et al. (2015) similarly saw an increase in DRD1 protein but no change in DRD2 for mice, whereas Singh et al. (2014) reported a decrease in striatal DRD2 protein in rats (Table 1).

Table 1. Summary of pyrethroid effects on DA receptors in previous studies

Study	Model	DA Receptor Effect	Dosing paradigm
Crago & Schlenk (2015)	Juvenile rainbow trout	↓DRD2 mRNA	0.15, 1.5 µg/L, 96 hrs or 2 weeks, bifenthrin (Type I)
Kung et al. (2015)	Zebrafish embryo	↓DRD1 mRNA; ↓DRD2 mRNA	0.25–0.50 µg/l, 3 hpf-72 hpf, deltamethrin (Type II)
Wang et al. (2020)	Zebrafish embryo	No change in DRD1 or DRD2 mRNA	.02, .2, 2, 20 µg/L, 96 hrs, esfenvalerate (Type II)

Singh et al. (2014)	Prenatally exposed rats	↓DRD2 protein, striatum	1.25, 2.5, 5 mg/kg, gestation days 5-21, cypermethrin (Type II)
Richardson et al. (2015)	Undosed offspring of dosed female mice	↑DRD1 protein, striatum; no change in DRD2	0.3, 1, 3 mg/kg, all of gestation until postnatal day 22, deltamethrin (Type II)
Pitzer et al. (2019)	Adult rats (exposed as pups)	↓DRD1 mRNA	.25, 0.5, 1 mg/kg/day, postnatal days 3-20, deltamethrin (Type II)
Pitzer et al. (2021) (citing unpublished data)	Developmentally exposed rats	No change in DRD1 or DRD2 protein, striatum	Unspecified dose, postnatal exposure, deltamethrin (Type II)
Patel & Pitzer (2019)	Developmentally exposed rats	↑DRD1 protein, striatum	1 mg/kg, postnatal days 3-20, deltamethrin (Type II)

It should be noted that some of the previous literature for the effects of pyrethroid exposure on dopamine receptors uses fish models, but it has been established that pyrethroids have greater toxicity in aquatic life than in mammals (Biscoe et al., 2016). Therefore, a mammalian species ought to be used to provide a more relevant model for human exposures. Furthermore, all prior studies center around the neurodevelopmental impacts of pyrethroids. However, given that dopamine receptors are an integral part of the pathology and treatment of Parkinson's disease, there is a necessity to understand how pyrethroids may impact dopamine receptors in adults. Herein, we demonstrate that chronic oral pyrethroid exposure in adult wild-type mice increases D1-like dopamine receptor protein levels in the striatum, which not only carries potential implications for early Parkinson's disease pathology, but may also indicate the utilization of compensatory mechanisms in response to pyrethroids. Furthermore, our wild-type mouse model opens the door for considerations of other neurological disorders in which dopamine receptors and environmental exposures play a role.

Methods and Materials

Animal Models

Male C57BL/6 (wild-type) mice purchased from Jackson Labs were co-housed based on treatment group (n=6-12 per group) in the Whitehead Biomedical Research Building animal facility. Sample sizes were based on a statistical power analysis at >80% power, allowing 20% tolerance for type 2 error. Starting at 8-10 weeks of age, mice were orally gavaged with deltamethrin (3 mg/kg) dissolved in sterilized corn oil or sterile corn oil as a vehicle control once per week for 12 weeks. Mice were monitored for signs of acute toxicity, none of which were observed during preliminary studies. Mice were humanely euthanized and perfused within 72 hours to 1 week after the final dose of deltamethrin. Brain tissue was collected, dissected, and halved. One half was allocated for immunofluorescence and the other allocated for protein to facilitate optimal extraction conditions. Animal handling and sample preparation was completed by the Sampson lab. All animal use protocols were reviewed and accepted by the Institutional Animal Care and Use Committee at Emory University under Protocol #201900030.

Immunofluorescence

Fixed and perfused brains were mounted in Tissue-Tek® OCT compound (performed by the lab) and sectioned at 40 µm via cryostat. Sections were blocked in PBS with 0.01% sodium azide, 3% normal goat serum (NGS), 0.3% Triton-X 100, and 1 drop/mL of M.O.M.® blocking reagent (Vector Laboratories) for 2 hours at room temperature. Slices were then incubated in anti-DRD1 (1:500 rabbit; Abcam ab279713), anti-DRD2 (1:500 guinea pig; Synaptic Systems 376 205), and anti-TH (1:300 mouse; Millipore MAB318) primary antibodies diluted in blocking buffer overnight at 4 °C. Following three 5-minute washing steps in PBS, sections were incubated for one hour at room temperature with corresponding anti-rabbit IgG-AF488, anti-

rabbit IgG-AF594, anti-guinea pig IgG-AF594, and anti-mouse IgG-AF657 (1:1000; Invitrogen) secondaries. After three 5-minute washing steps, sections were mounted onto glass slides in ProLong™ Diamond Antifade mountant with DAPI (Thermo Fisher). All antibodies used are in Table 2.

Samples were stained in batches of four (two controls, two treated) to ensure consistent staining conditions within-batch when imaging. DRD1 and DRD2 were stained separately, and all samples were stained with TH. The first batch of DRD1 samples was stained with anti-rabbit IgG-AF488, and all subsequent staining for DRD1 used anti-rabbit IgG-AF594.

DAPI, TH, striatal dopamine receptors, and midbrain DRD1 were imaged with a KEYENCE B2-X710 fluorescence microscope. Exposure times were consistent within-batch. Images were analyzed by tracing the fluorescent region of interest was and measuring for integrated density, then measuring background signal from areas without staining. Corrected total fluorescence intensity was determined using the following formula: Integrated Density of ROI – (Area of ROI * mean fluorescence of background) (Fitzpatrick, 2014).

DRD2, DAPI, and TH in the substantia nigra pars compacta were imaged with an inverted Olympus FV-1000 confocal microscope at 60x objective. DRD2 levels were measured using particle analysis.

Western Blot

Protein was homogenized with 1x MSD buffer (5x homogenization buffer, deionized water, 1% Triton X-100, and protein/phosphatase inhibitor tablet) and centrifuged at 30,130 x *g* at 4 °C. Protein was quantified with a Pierce™ BCA protein assay kit and spectroscope. Protein stock was then combined with a proportionate amount of 1x MSD buffer and 4x NuPAGE™ Buffer with 50 mM DTT. 13.3µL of protein for each sample was loaded onto Novex™ 4-20%

tris-glycine gels, WedgeWell format, electrophoresed at 150 V for 60 minutes, then transferred to PVDF membranes. Membranes were blocked in 5% bovine serum albumin (BSA) for one hour at room temperature, then incubated with anti-DRD1 antibody (1:750 rat; Sigma Aldrich D2944), anti-DRD2 antibody (1:750 rabbit; Merck Millipore AB5084P) or anti-GAPDH (1:1000 rabbit; Cell Signaling Technology 5174S) primary antibody diluted in 5% BSA overnight at 4 °C. Following three 5-minute washing steps in 1x Tris-buffered saline with 0.1% Tween-20, membranes were probed with anti-rat (1:1000; Jackson Labs) or anti-rabbit IgG HRP (1:1000-5000; Cell Signaling Technology) diluted in 5% BSA at room temperature for one hour. All antibodies used are listed in Table 2. DRD1 and DRD2 blots were detected using SuperSignal™ West Femto Maximum Sensitivity Substrate and GAPDH blots were detected using SignalFire™ ECL reagent (Cell Signaling Technology). All blots were imaged using Azure Biosystems c400 western blot imaging system.

Anti-DRD1 and anti-DRD2 primary antibodies for western blot were selected in accordance with validation results by Stojanovic et al. (2017).

Table 2. Antibodies

Antibody	Application	Concentration	Product Number
Primary Antibodies			
Anti-dopamine receptor D1, rabbit monoclonal	Immunofluorescence	1:500	Abcam AB279713
Anti-dopamine receptor D2, guinea pig polyclonal	Immunofluorescence	1:500	Synaptic Systems 376 205
Anti-tyrosine hydroxylase, mouse monoclonal	Immunofluorescence	1:300	Millipore MAB318
Anti-dopamine receptor D1, rat monoclonal	Western blot	1:750	Sigma Aldrich D2944

Anti-dopamine receptor D2, rabbit polyclonal	Western blot	1:750	Merck Millipore AB5084P
Anti-GAPDH, rabbit monoclonal	Western blot	1:1000	Cell Signaling Technology 5174S
Secondary Antibodies			
Anti-rabbit IgG-Alexafluor 488	Immunofluorescence	1:1000	Invitrogen A-11008
Anti-rabbit IgG Alexafluor 594	Immunofluorescence	1:1000	Invitrogen A-11012
Anti-guinea pig IgG Alexafluor 594	Immunofluorescence	1:1000	Invitrogen A-10076
Anti-mouse IgG 647	Immunofluorescence	1:1000	Invitrogen A-21240
Anti-rat IgG-HRP	Western Blot	1:1000	Jackson Labs 112035003
Anti-rabbit IgG-HRP	Western blot	1:1000-1:5000	Cell Signaling 7074

Statistical Analyses

Immunofluorescence images and western blots were quantified with Fiji (Fiji Is Just ImageJ) software (Schindelin et al., 2012). Tests for normality followed by unpaired two-tailed t-tests were performed with Graphpad Prism version 9.5.1 for macOS. Results were considered statistically significant when $p \leq 0.05$.

Results

Deltamethrin exposure increased striatal DRD1 protein levels, but not DRD2

Striatal DRD1 and DRD2 protein levels are increased in early-stage Parkinson's disease patients, and some studies have observed changes in striatal dopamine receptor protein levels following pyrethroid exposure (Seeman & Niznik, 1990; Hisahara & Shimohama, 2011; Singh et al., 2014; Richardson et al., 2015). Immunofluorescence and western blot were used to measure

potential changes in striatal DRD1 and DRD2 protein levels after exposure to deltamethrin. Western blots were used to corroborate findings from immunofluorescence and vice versa. Imaging confirmed the location of both receptor families in the mouse striatum (caudoputamen, nucleus accumbens) and olfactory tubercle (Figures 1B and 2B). Fluorescence intensity was analyzed in the striatum. Corrected total fluorescence analysis yielded a significant increase in striatal DRD1 (Figure 1A), which was confirmed by western blot analysis (Figure 1C). However, neither corrected total fluorescence intensity nor western blot analysis yielded differences between treatments for striatal DRD2 protein, although there was a very slight trending increase in the deltamethrin treated group in both analyses (Figures 2A & 2C). These findings indicate that deltamethrin exposure differentially increases DRD1 but not DRD2 in the striatum.

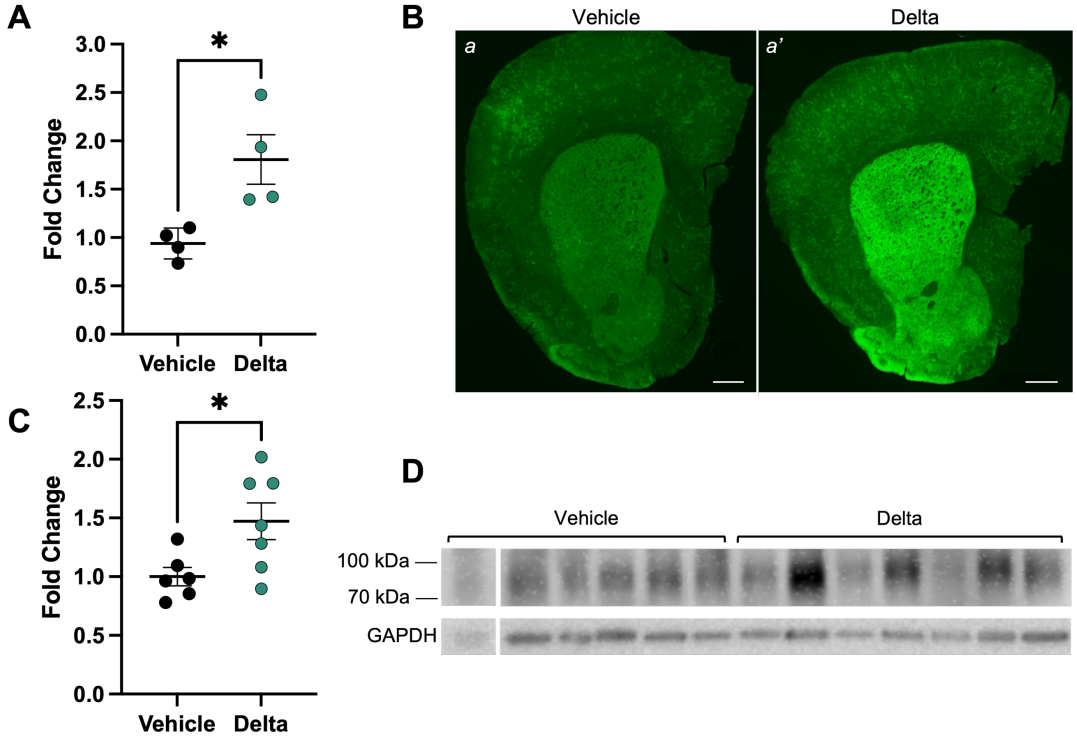

Figure 1. Deltamethrin exposure significantly increased DRD1 protein levels in striatum. A) Corrected total fluorescence intensity analysis for striatal DRD1. B) Representative images of immunofluorescent staining for DRD1 in striatum between corn oil vehicle (a) and deltamethrin (a') treatments. Scale bars are 500 µm. C) Western blot analysis of striatal DRD1 protein. D) Western blot of striatal DRD1 protein showed bands between ~70 kDa and ~100 kDa. One vehicle sample was excluded due to protein levels being too low to quantify. GAPDH was used as a housekeeping protein. *$p \leq 0.05$.

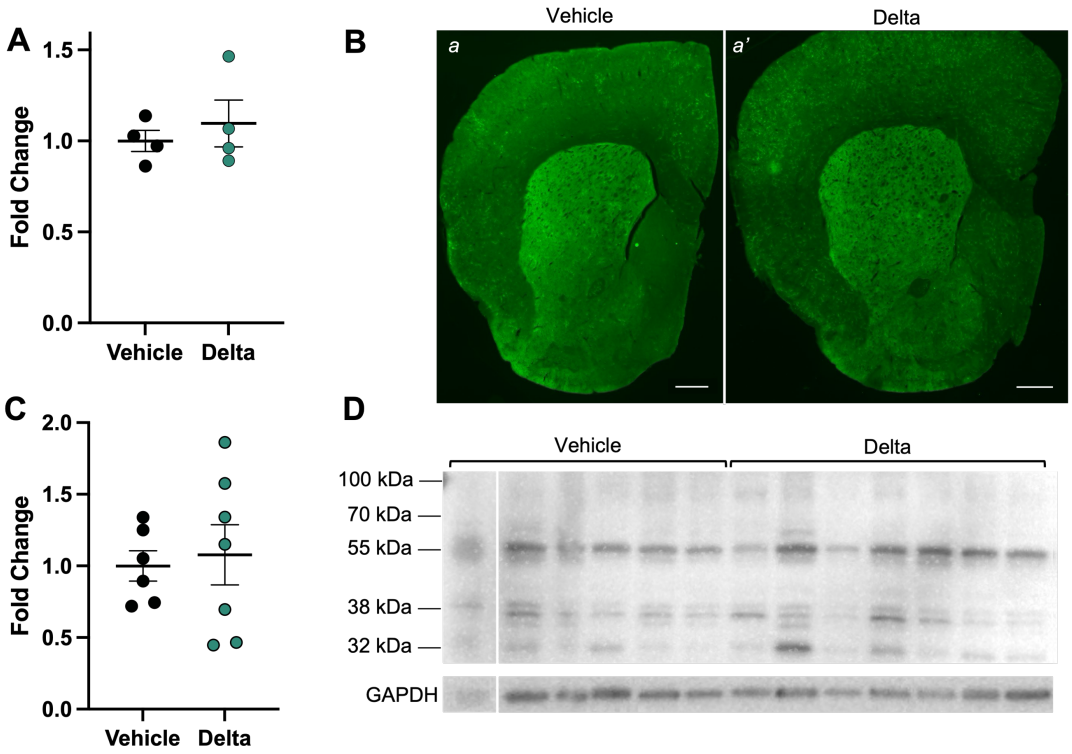

Figure 2. Deltamethrin exposure did not significantly alter DRD2 protein levels in striatum. (A) Corrected total fluorescence intensity analysis for striatal DRD2. (B) Representative images of immunofluorescent staining for DRD1 in striatum between corn oil vehicle (a) and deltamethrin (a') treatments. Scale bars are 500 µm. (C) Western blot analysis of striatal DRD2 protein. (D) Western blot of striatal DRD2 protein showed prominent bands at ~32 kDa, ~38 kDa, and ~55 kDa, and faint bands between ~70 kDa and ~100 kDa. Densitometry was performed on bands at ~55 kDa . One vehicle sample was excluded due to protein levels being too low to quantify. GAPDH was used as a housekeeping protein. *$p \leq 0.05$.

Deltamethrin exposure did not alter midbrain DRD1 or DRD2 levels

Given the crucial role of the substantia nigra pars compacta and nigrostriatal pathway in the pathophysiology of Parkinson's disease, protein levels for midbrain DRD1 and DRD2 were measured using immunofluorescence and western blot (Poewe et al., 2017). Corrected total fluorescence intensity analysis of SNr saw a trending, but nonsignificant, increase in DRD1 levels (Figure 3A). Midbrain slices were costained with TH as an anatomical marker to confirm that DRD1 was expressed in the substantia nigra pars reticulata (Figure 3B). Indeed, TH was

expressed on dopaminergic cells in the SNc and VTA, but did not overlap with DRD1 in the SNr (Figure 3Bc & 3Bc').

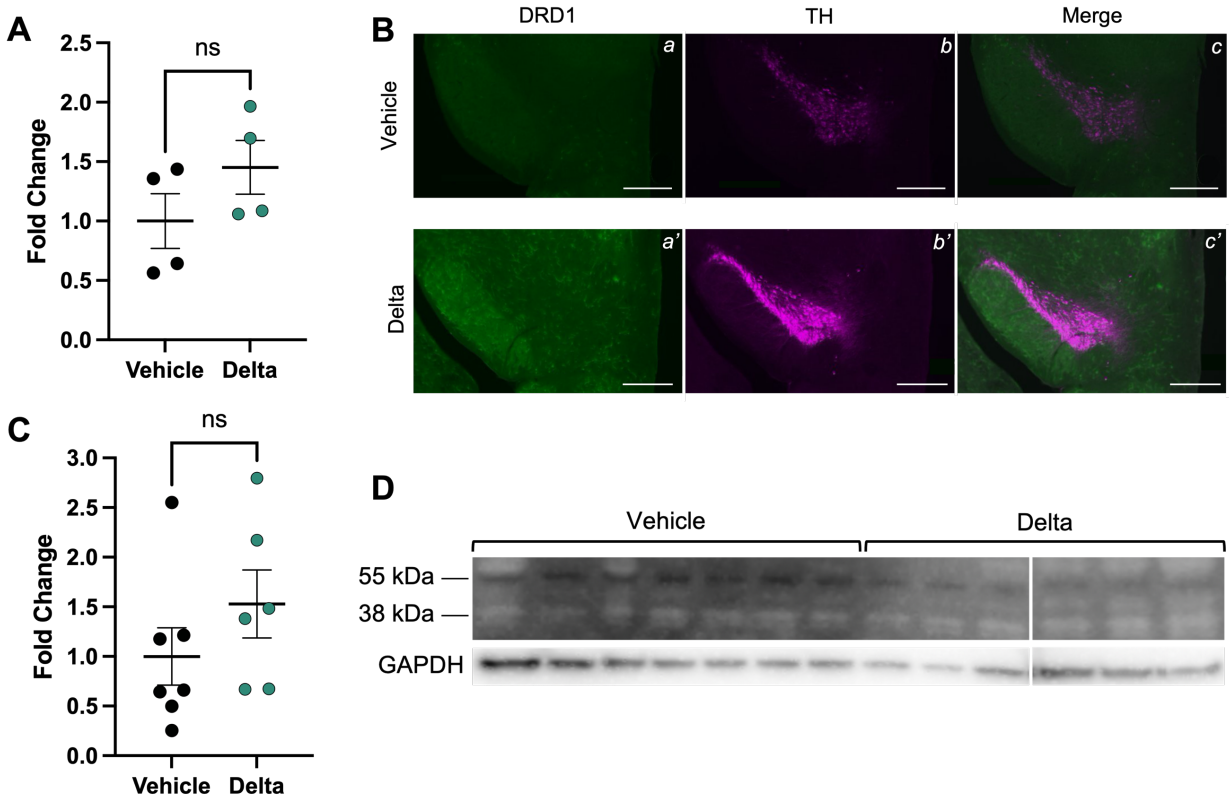

Figure 3. Deltamethrin exposure did not significantly alter midbrain DRD1 protein levels. (A) Corrected total fluorescence intensity analysis for DRD1 in substantia nigra pars reticulata. (B) Representative images of DRD1 (green) and tyrosine hydroxylase (magenta) in the midbrain between corn oil vehicle (a, b, & c) and deltamethrin (a', b', c') treatments. DRD1 was exclusively seen in the SNr, whereas TH was exclusively seen in the SNC (c & c'). Scale bar are 500 µm. (C) Western blot analysis of midbrain DRD1 protein. (D) Western blot of midbrain DRD1 protein showed bands at ~55 kDa and ~38 kDa. Densitometry was performed in ~55 kDa bands. One deltamethrin sample was excluded from analysis due to protein levels being too low to quantify. GAPDH was used as a housekeeping protein. *$p \leq 0.05$.

Confocal imaging of the midbrain revealed that dopaminergic neurons, which expressed TH (red), were present in the substantia nigra pars compacta (4b & 4b'). DRD2 (yellow) was also present in the midbrain (4a & 4a'). DRD2 was present on the soma of all SNc dopaminergic neurons, presumably as autoreceptors (4c & 4c'), but also appeared in areas that did not contain TH-staining, indicating that non-dopaminergic neurons in the midbrain also expressed DRD2 (4d

& 4d'). Because DRD2 was seen on dopaminergic and non-dopaminergic neurons, particle analysis was performed to measure levels of DRD2 in areas with TH staining and areas without TH staining. There was no change between treatments in the number of DRD2 receptors per dopaminergic neuron (5A), nor in non TH-positive areas (5B).

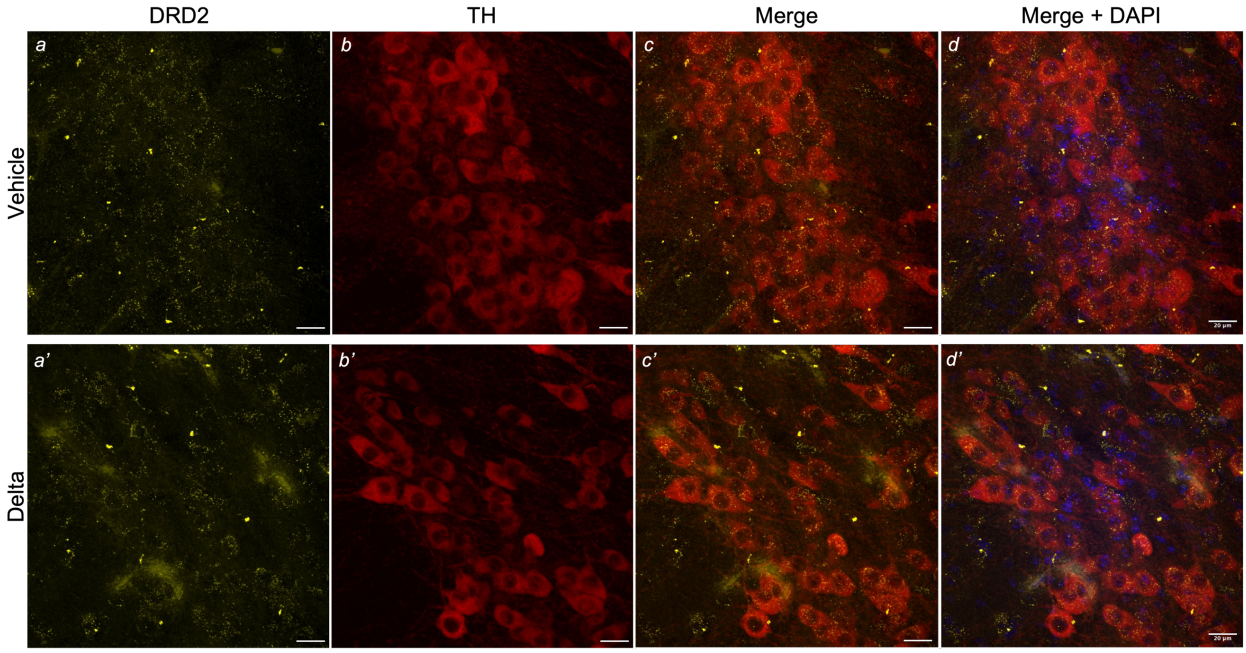

Figure 4. D2-like receptors were present on dopaminergic and non-dopaminergic neurons in midbrain. Fluorescent confocal images were taken in the substantia nigra at 60x objective. D2-like receptors (yellow) were widely expressed (a & a'). Tyrosine hydroxylase (red) was seen in the substantia nigra pars compacta and represented dopaminergic neurons (4b & 4b'). DRD2 was present on soma of TH-positive cells but also seen in non TH-positive areas (4c & 4c'). Scale bars are 20 µm.

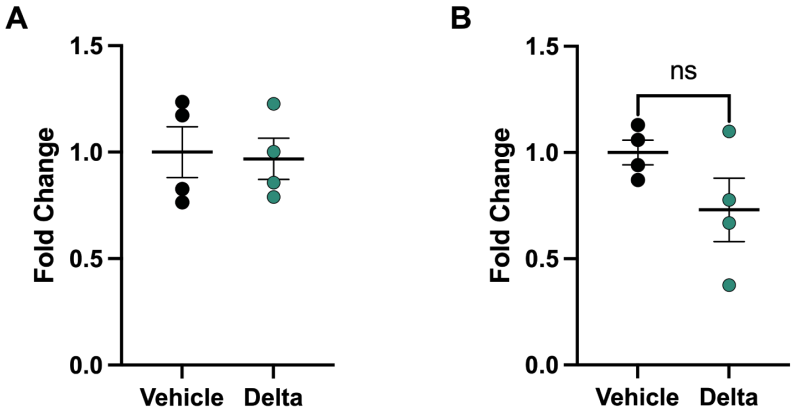

Figure 5. Deltamethrin exposure did not significantly alter midbrain DRD2 levels on dopaminergic or non-dopaminergic neurons. A) Particle analysis of average number of D2-like receptors per dopaminergic neuron. Measured by creating ROI around TH-positive neurons and dividing number of DRD2 particles within ROI by number of TH-positive cells. B) Particle analysis for average number of D2-like receptors not on dopaminergic

neurons. Measured using (total DRD2 particle count - DRD2 particle count in ROI)/(total image area - TH-positive ROI area).*$p \leq 0.05$.

Discussion

Pesticide exposure has been connected to the development of Parkinson's disease, a movement disorder characterized by the loss of dopamine and dopaminergic neurons in the nigrostriatal pathway (Islam et al., 2021; Ball et al., 2019). Synthetic pyrethroids, one of the most commonly used classes of pesticides, have been demonstrated to disrupt dopaminergic signaling in ways that are consistent with disorders such as ADHD and Parkinson's depending on age during exposure (Richardson et al., 2015; Pitzer et al., 2019; Stephenson, 2000; Jiao et al., 2020). Dopamine receptors are important aspects in the pathology and treatment in many neurodevelopmental and neurodegenerative disorders (Coghill & Banaschewski, 2009; Seeman, 2013; Moreno-Delgado et al., 2020; Hisahara & Shimohama, 2011). However, current studies on the effects of pyrethroids on dopamine receptors only model developmental exposures (Crago & Schlenk, 2015; Kung et al., 2015; Wang et al., 2020; Singh et al., 2014; Richardson et al., 2015; Pitzer et al., 2019; Patel & Pitzer, 2019; Pitzer et al., 2021). Thus, our study is the first to our knowledge to investigate how pyrethroid exposure in an adult mouse model affects dopamine receptors.

Oral deltamethrin exposure significantly increased DRD1 protein levels in the striatum, but did not affect striatal DRD2, which was consistent with findings from Richardson et al. (2015) and Patel & Pitzer (2019), despite differences in age when exposed. These findings disagree with other literature, with the disparity primarily existing between studies on dopamine receptor mRNA transcription levels (Crago & Schlenk, 2015; Kung et al., 2015; Wang et al., 2020; Pitzer, 2019). However, correlation levels between mRNA levels and expression of corresponding proteins has been demonstrably poor and cannot reliably be used to predict one

another – thus, it may be inaccurate to make a direct comparison to these studies (Koussounadis et al., 2015). Furthermore, some of these studies used developmental fish models, but it has been established that pyrethroids are differentially more toxic to aquatic life than to mammals (Crago & Schlenk, 2015; Kung et al., 2015; Wang et al., 2020; Biscoe et al., 2016).

DRD2 in the midbrain was observed in non TH areas, indicating that non-dopaminergic neurons in the substantia nigra also expressed D2 receptors. It is possible these neurons were D1/D2-coexpressing medium spiny neurons, which project from the striatum into the substantia nigra (Deng et al., 2006).

The effects of exposure on dopamine receptor levels were not entirely consistent with pathology for Parkinson's disease. Although the rise in DRD1 striatal protein levels was in agreement with studies in PD, patients also exhibit increased DRD2 in the striatum, which contrasts our findings (Seeman & Niznik, 1990; Hisahara & Shimohama, 2011). Furthermore, no changes were found in dopamine receptor levels in the midbrain. There is evidence that Parkinson's progresses through retrograde axonal degeneration beginning in the striatum, which, given that no change in DRD2 was observed in the striatum, may explain why the substantia nigra was not affected (Tagliaferro & Burke, 2016).

It should be noted that mice were exposed starting at 8-10 weeks of age and were sacrificed 12 weeks later. At these ages, mice are not considered particularly old, and are equivalent to a roughly 20-30 year old human (Hagan, 2017). Considering that age is ultimately the greatest risk factor for Parkinson's, with the average age of onset around 60 years old, it is possible that the age of our models poses a limitation in our model, as younger organisms are less susceptible to neurodegeneration than older ones (Hindle, 2010). Alternatively, we could be

seeing compensatory mechanisms that are representative of the preclinical stages of disease (Bezard et al., 2003; Borgonovo et al., 2017).

Given that our exposure paradigm was in wild-type mice, it is relevant to consider these findings outside the lens of Parkinson's. Surmeier et al. (1992) demonstrated that activation of D1 receptors on nigrostriatal neurons reduces Na^+ current in voltage gated sodium channels, which are the primary target for pyrethroids. Since pyrethroids prolong the activation of voltage gated sodium channels, it is therefore possible that the higher expression of DRD1 observed after exposure is a compensation to correct the overactivation of sodium channels in the presence of deltamethrin (Wakeling et al., 2012). Pyrethroids are also known to decrease striatal dopamine levels (Rodríguez et al., 2016; Mohammadi et al., 2019). D1-like receptors have a much lower affinity for dopamine than D2-like receptors, which may explain why DRD1, but not DRD2, levels were altered to compensate for a loss of dopamine (Martel & McArthur, 2020). A rise in striatal DRD1 has also been implicated in levodopa-induced dyskinesia (LID), and application of DRD1 antagonists protects against or reverses LID symptoms (Aubert et al., 2004; Mela et al., 2012; Jones-Tabah et al., 2020). DRD1 stimulation mediates the activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), which is overactive in levodopa-induced dyskinesia (Fieblinger et al., 2014). This D1-mediated ERK signaling cascade is cytotoxic and may also be responsible for the striatal neurodegeneration observed in Huntington's disease (Chen et al., 2004; Paoletti et al., 2008; Tang et al., 2007). Thus, the increased expression of DRD1 protein may be a mechanism of neurotoxicity that is catalyzed by pyrethroid exposure.

The alterations observed in response to deltamethrin are therefore quite concerning considering the chronic daily exposures experienced by the general population. A human health risk assessment for deltamethrin released by the EPA estimated that children ages 3-5 have the

highest dietary chronic exposure to deltamethrin at approximately 1 µg/kg/day. Estimated chronic dietary exposure levels in the general US population is around 0.3 µg/kg/day (Collantes et al., 2017). The no-adverse-effect-level (NOEL) for oral deltamethrin exposure is 1 mg/kg/day (Barlow et al., 2001). Both the NOEL and human exposure estimates are lower than the dose we used in our study. However, fat, skin, and skeletal muscle have been demonstrated to accumulate large amounts of deltamethrin and act as slow-release depots, so it is unknown what levels of deltamethrin may truly be in the body after long-term daily exposure (Kim et al., 2007).

Most toxicological studies for pyrethroids use pure forms of the pesticide dissolved into a vehicle of choice. The vehicle used to administer pyrethroids is important, as toxicity can vary greatly depending on the vehicle used. For example, for deltamethrin, the oral LD_{50} in vegetable oil or polyethylene glycol ranges from 19 to 34 mg/kg in mice, whereas doses up to 5000 mg/kg did not produce lethality in aqueous vehicles (Barlow et al., 2001). However, pesticide products marketed for commercial and residential applications use formulations that combine a variety of inert ingredients in combination with the active pyrethroid. The EPA places all inert ingredients under four categories: inert ingredients of toxicological concern, potentially toxic inert ingredients, inert ingredients of unknown toxicity, and inert ingredients of minimal concern. Under federal United States law, only inert ingredients of toxicological concern are required to be disclosed on pesticide labels -- all other inert ingredients do not need to be specified, meaning the identities and quantities in specific formulations remain confidential (Todd, 2003). Thus, the effects of vehicle on pyrethroid toxicity becomes an important concern when applying understandings from research studies to everyday life. Williamson et al. (1989) demonstrated that the commercial fenvalerate formulation Pydrin significantly enhanced the toxicity of fenvalerate via oral and intraperitoneal routes, lowering its LD_{50} from 190 mg/kg in corn oil to 72

mg/kg in Pydrin. Their findings indicate that inert ingredients in trade formulations that are not inherently toxic may have toxic interactions with pyrethroids, which should be considered when determining the safety of their real-world applications.

Pyrethroid pesticides have become one of the most commonly used pesticides across the world. However, the longer-term effects of pyrethroid exposure in humans are poorly understood, but have been connected to disorders such as ADHD, ALS, and Parkinson's disease. We observed that chronic oral deltamethrin exposure in adult wild-type mice increased DRD1 levels in the striatum, which may be indicative of compensatory mechanisms associated with preclinical Parkinson's and other diseases. Such changes are concerning given that increases in striatal DRD1 are involved in nigrostriatal dopamine neuron degeneration seen in disorders such as Huntington's disease, and may signify how pyrethroids can influence the progression and treatment of other neurological disorders (Paoletti et al., 2008; Tang et al., 2007; Pearson et al., 2006). Further scrutinization of the effects of adult pyrethroid exposure on dopamine receptors is imperative for understanding their roles in the development of neurodegenerative disorders.

Future Directions

There are several genes associated with PD that increase an individual's susceptibility to developing the disease in combination with environmental insults (Cannon & Greenamyre, 2013; Angelopoulou et al., 2022). In fact, one genetic variant, the single-nucleotide polymorphism *rs3129882* in HLA-DRA, has been demonstrated to increase susceptibility to Parkinson's specifically in response to pyrethroid exposure (Kannarkat et al., 2015). Therefore, it may be worthwhile to investigate how dopamine receptors in PD mouse models could be affected by pyrethroids to better understand if and how these genes interact with such exposures.

Additionally, it is necessary to consider potential synergistic interactions of pyrethroid exposure and other toxic exposures. For example, organophosphates are still commonly used alongside pyrethroids, and their combined effects are notably more toxic than exposure to either pesticide alone (Simaremare et al., 2020; Iyyadurai et al., 2014). As such, comparing the influence of several pesticides together on dopamine receptors would provide a more realistic model for exposure in humans.

References

Alavanja, M. C. R., Hoppin, J. A., & Kamel, F. (2004). Health effects of chronic pesticide exposure: Cancer and neurotoxicity. *Annual Review of Public Health*, *25*(1), 155–197. https://doi.org/10.1146/annurev.publhealth.25.101802.123020

Angelopoulou, E., Paudel, Y. N., Papageorgiou, S. G., & Piperi, C. (2022). Environmental impact on the epigenetic mechanisms underlying parkinson's disease pathogenesis: A narrative review. *Brain Sciences*, *12*(2), 175. https://doi.org/10.3390/brainsci12020175

Aubert, I., Guigoni, C., Håkansson, K., Li, Q., Dovero, S., Barthe, N., Bioulac, B. H., Gross, C. E., Fisone, G., Bloch, B., & Bezard, E. (2004). Increased D1 dopamine receptor signaling in levodopa-induced dyskinesia. *Annals of Neurology*, *57*(1), 17–26. https://doi.org/10.1002/ana.20296

Ball, N., Teo, W.-P., Chandra, S., & Chapman, J. (2019). Parkinson's disease and the environment. *Frontiers in Neurology*, *10*. https://doi.org/10.3389/fneur.2019.00218

Barlow, S. M., Sullivan, F. M., & Lines, J. (2001). Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. *Food and Chemical Toxicology*, *39*(5), 407–422. https://doi.org/10.1016/s0278-6915(00)00152-6

Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacological Reviews*, *63*(1), 182–217. https://doi.org/10.1124/pr.110.002642

Benoit-Marand, M., Ballion, B., Borrelli, E., Boraud, T., & Gonon, F. (2010). Inhibition of dopamine uptake by D2 antagonists: An in vivo study. *Journal of Neurochemistry*, *116*(3), 449–458. https://doi.org/10.1111/j.1471-4159.2010.07125.x

Bertran-Gonzalez, J., Bosch, C., Maroteaux, M., Matamales, M., Hervé, D., Valjent, E., & Girault, J.-A. (2008). Opposing patterns of signaling activation in dopamine d1and d2receptor-expressing striatal neurons in response to cocaine and haloperidol. *The Journal of Neuroscience*, *28*(22), 5671–5685. https://doi.org/10.1523/jneurosci.1039-08.2008

Bezard, E., Gross, C. E., & Brotchie, J. M. (2003). Presymptomatic compensation in Parkinson's disease is not dopamine-mediated. *Trends in Neurosciences*, *26*(4), 215–221. https://doi.org/10.1016/s0166-2236(03)00038-9

Biscoe, M., Britton, C., Jacob, A., Jones, R. D., Page, J., Sell, N., & Waleko, G. (2016). *Ecological Risk Assessment Rationale for Pyrethroids in Registration Review [EPA-HQ-OPP-2010-0384-0048]*. United States Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPP-2010-0384-0048

Boedeker, W., Watts, M., Clausing, P., & Marquez, E. (2020). The global distribution of acute unintentional pesticide poisoning: Estimations based on a systematic review. *BMC Public Health*, *20*(1). https://doi.org/10.1186/s12889-020-09939-0

Bond, C., Buhl, K., & Stone, D. (2014). *Pyrethrins general fact sheet*. National Pesticide Information Center; Oregan State University Extension Services. http://npic.orst.edu/factsheets/pyrethrins.html

Borgonovo, J., Allende-Castro, C., Laliena, A., Guerrero, N., Silva, H., & Concha, M. L. (2017). Changes in neural circuitry associated with depression at pre-clinical, pre-motor and early motor phases of Parkinson's disease. *Parkinsonism & Related Disorders*, *35*, 17–24. https://doi.org/10.1016/j.parkreldis.2016.11.009

Bu, M., Farrer, M. J., & Khoshbouei, H. (2021). Dynamic control of the dopamine transporter in neurotransmission and homeostasis. *Npj Parkinson's Disease*, *7*(1). https://doi.org/10.1038/s41531-021-00161-2

Canadian Centre for Occupational Health and Safety. (2017, April 3). *Pesticides*. CCOHS. https://www.ccohs.ca/oshanswers/chemicals/pesticides/general.html

Cannon, J. R., & Greenamyre, J. T. (2013). Gene–environment interactions in Parkinson's disease: Specific evidence in humans and mammalian models. *Neurobiology of Disease*, *57*, 38–46. https://doi.org/10.1016/j.nbd.2012.06.025

Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security*, *6*(2), 48–60. https://doi.org/10.1002/fes3.108

Cass, W. A., & Gerhardt, G. A. (1994). Direct in vivo evidence that D2 dopamine receptors can modulate dopamine uptake. *Neuroscience Letters*, *176*(2), 259–263. https://doi.org/10.1016/0304-3940(94)90096-5

Centonze, D., Grande, C., Saulle, E., Martín, A. B., Gubellini, P., Pavón, N., Pisani, A., Bernardi, G., Moratalla, R., & Calabresi, P. (2003). Distinct roles of D1 and D5 dopamine receptors in motor activity and striatal synaptic plasticity. *The Journal of Neuroscience*, *23*(24), 8506–8512. https://doi.org/10.1523/jneurosci.23-24-08506.2003

Cepeda, C., Murphy, K. P. S., Parent, M., & Levine, M. S. (2014). The role of dopamine in Huntington's disease. In *Progress in Brain Research* (pp. 235–254). Elsevier. http://dx.doi.org/10.1016/b978-0-444-63425-2.00010-6

Chen, J., Rusnak, M., Luedtke, R. R., & Sidhu, A. (2004). D1 dopamine receptor mediates dopamine-induced cytotoxicity via the ERK signal cascade. *Journal of Biological Chemistry*, *279*(38), 39317–39330. https://doi.org/10.1074/jbc.m403891200

Chotibut, T., Apple, D. M., Jefferis, R., & Salvatore, M. F. (2012). Dopamine transporter loss in 6-OHDA Parkinson's model is unmet by parallel reduction in dopamine uptake. *PLoS ONE*, *7*(12), e52322. https://doi.org/10.1371/journal.pone.0052322

Coghill, D., & Banaschewski, T. (2009). The genetics of attention-deficit/hyperactivity disorder. *Expert Review of Neurotherapeutics*, *9*(10), 1547–1565. https://doi.org/10.1586/ern.09.78

Collantes, M., Dotson, D. A., & Craig, E. (2017, June 5). *Deltamethrin: Draft human health risk assessment for registration review [EPA-HQ-OPP-2009-0637-0036]*. United States Environmental Protection Agency. https://regulations.gov/EPA-HQ-OPP-2009-0637-0036/content.pdf

Corcellas, C., Feo, M. L., Torres, J. P., Malm, O., Ocampo-Duque, W., Eljarrat, E., & Barceló, D. (2012). Pyrethroids in human breast milk: Occurrence and nursing daily intake estimation. *Environment International*, *47*, 17–22. https://doi.org/10.1016/j.envint.2012.05.007

Crago, J., & Schlenk, D. (2015). The effect of bifenthrin on the dopaminergic pathway in juvenile rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicology*, *162*, 66–72. https://doi.org/10.1016/j.aquatox.2015.03.005

Damalas, C. (2009). Understanding benefits and risks of pesticide use. *Scientific Research and Essays*, *4*(10), 945–949.

Deng, Y.-P., Lei, W.-L., & Reiner, A. (2006). Differential perikaryal localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. *Journal of Chemical Neuroanatomy*, *32*(2–4), 101–116. https://doi.org/10.1016/j.jchemneu.2006.07.001

Eells, J. T., & Dubocovich, M. L. (1988). Pyrethroid insecticides evoke neurotransmitter release from rabbit striatal slices. *The Journal of Pharmacology and Experimental Therapeutics*, *246*(2), 514–521. PMID: 3404444.

Elbaz, A., Clavel, J., Rathouz, P. J., Moisan, F., Galanaud, J.-P., Delemotte, B., Alpérovitch, A., & Tzourio, C. (2009). Professional exposure to pesticides and Parkinson disease. *Annals of Neurology*, *66*(4), 494–504. https://doi.org/10.1002/ana.21717

Elbaz, A., & Tranchant, C. (2007). Epidemiologic studies of environmental exposures in Parkinson's disease. *Journal of the Neurological Sciences*, *262*(1–2), 37–44. https://doi.org/10.1016/j.jns.2007.06.024

Elwan, M. A., Richardson, J. R., Guillot, T. S., Caudle, W. M., & Miller, G. W. (2006). Pyrethroid pesticide-induced alterations in dopamine transporter function. *Toxicology and Applied Pharmacology*, *211*(3), 188–197. https://doi.org/10.1016/j.taap.2005.06.003

Emamzadeh, F. N., & Surguchov, A. (2018). Parkinson's disease: Biomarkers, treatment, and risk factors. *Frontiers in Neuroscience*, *12*. https://doi.org/10.3389/fnins.2018.00612

EPA. (2006). *US EPA - Pesticides - Fact sheet for permethrin [EPA 738-F-06-012]*. United States Environmental Protection Agency. https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-109701_1-Jun-06.pdf

EPA. (2013, February 21). *Permethrin, Resmethrin, d-Phenothrin (Sumithrin®): Synthetic Pyrethroids For Mosquito Control*. United States Environmental Protection Agency.

https://www.epa.gov/mosquitocontrol/permethrin-resmethrin-d-phenothrin-sumithrinr-synthetic-pyrethroids-mosquito

EPA. (2014, October 3). *What is a Pesticide?* United States Environmental Protection Agency. https://www.epa.gov/minimum-risk-pesticides/what-pesticide

EPA. (2020a, September). *Permethrin Interim Registration Review Decision Case Number 2510 [EPA-HQ-OPP-2011-0039]*. United States Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPP-2011-0039-0144

EPA. (2020b, October 22). *EPA addresses human health and ecological risks posed by 13 pyrethroids*. United States Environmental Protection Agency. https://www.epa.gov/pesticides/epa-addresses-human-health-and-ecological-risks-posed-13-pyrethroids

EPA. (2022, April 28). *Registration Review of Pyrethrins and Pyrethroids [EPA-HQ-OPP-2008-0331-0084]*. United States Environmental Protection Agency. https://www.regulations.gov/document/EPA-HQ-OPP-2008-0331-0084

Fieblinger, T., Sebastianutto, I., Alcacer, C., Bimpisidis, Z., Maslava, N., Sandberg, S., Engblom, D., & Cenci, M. A. (2014). Mechanisms of dopamine D1 receptor-mediated ERK1/2 activation in the parkinsonian striatum and their modulation by metabotropic glutamate receptor type 5. *The Journal of Neuroscience*, *34*(13), 4728–4740. https://doi.org/10.1523/jneurosci.2702-13.2014

Fitzpatrick, M. (2014). *Measuring cell fluorescence using ImageJ — The Open Lab Book v1.0*. The Open Lab Book. https://theolb.readthedocs.io/en/latest/imaging/measuring-cell-fluorescence-using-imagej.html

Ford, C. P. (2014). The role of D2-autoreceptors in regulating dopamine neuron activity and transmission. *Neuroscience*, *282*, 13–22. https://doi.org/10.1016/j.neuroscience.2014.01.025

Fortes, C., Mastroeni, S., Pilla, M. A., Antonelli, G., Lunghini, L., & Aprea, C. (2013). The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. *Food and Chemical Toxicology*, *52*, 91–96. https://doi.org/10.1016/j.fct.2012.10.035

Furlong, M., Tanner, C. M., Goldman, S. M., Bhudhikanok, G. S., Blair, A., Chade, A., Comyns, K., Hoppin, J. A., Kasten, M., Korell, M., Langston, J. W., Marras, C., Meng, C., Richards, M., Ross, G. W., Umbach, D. M., Sandler, D. P., & Kamel, F. (2015). Protective glove use and hygiene habits modify the associations of specific pesticides with Parkinson's disease. *Environment International*, *75*, 144–150. https://doi.org/10.1016/j.envint.2014.11.002

Gillette, J. S., & Bloomquist, J. R. (2003). Differential up-regulation of striatal dopamine transporter and α-synuclein by the pyrethroid insecticide permethrin. *Toxicology and*

Applied Pharmacology, *192*(3), 287–293. https://doi.org/10.1016/s0041-008x(03)00326-0

Glorennec, P., Serrano, T., Fravallo, M., Warembourg, C., Monfort, C., Cordier, S., Viel, J.-F., Le Gléau, F., Le Bot, B., & Chevrier, C. (2017). Determinants of children's exposure to pyrethroid insecticides in western France. *Environment International*, *104*, 76–82. https://doi.org/10.1016/j.envint.2017.04.007

Guiramand, J., Montmayeur, J.-P., Ceraline, J., Bhatia, M., & Borrelli, E. (1995). Alternative splicing of the dopamine D2 receptor directs specificity of coupling to G-proteins. *Journal of Biological Chemistry*, *270*(13), 7354–7358. https://doi.org/10.1074/jbc.270.13.7354

Hagan, C. (2017, November 7). *When are mice considered old?* The Jackson Laboratory. https://www.jax.org/news-and-insights/jax-blog/2017/november/when-are-mice-considered-old

Hansen, M. R. H., Jørs, E., Lander, F., Condarco, G., Debes, F., Tirado Bustillos, N., & Schlünssen, V. (2017). Neurological deficits after long-term pyrethroid exposure. *Environmental Health Insights*, *11*, 117863021770062. https://doi.org/10.1177/1178630217700628

Hindle, J. V. (2010). Ageing, neurodegeneration and Parkinson's disease. *Age and Ageing*, *39*(2), 156–161. https://doi.org/10.1093/ageing/afp223

Hisahara, S., & Shimohama, S. (2011). Dopamine receptors and Parkinson's disease. *International Journal of Medicinal Chemistry*, *2011*, 1–16. https://doi.org/10.1155/2011/403039

Illinois Department of Public Health. (2007, June). *Pyrethroid insecticides*. http://www.idph.state.il.us/envhealth/factsheets/pyrethroid.htm

Islam, M. S., Azim, F., Saju, H., Zargaran, A., Shirzad, M., Kamal, M., Fatema, K., Rehman, S., Azad, M. A. M., & Ebrahimi-Barough, S. (2021). Pesticides and Parkinson's disease: Current and future perspective. *Journal of Chemical Neuroanatomy*, *115*, 101966. https://doi.org/10.1016/j.jchemneu.2021.101966

Iyyadurai, R., Peter, J. V., Immanuel, S., Begum, A., Zachariah, A., Jasmine, S., & Abhilash, K. P. P. (2014). Organophosphate-pyrethroid combination pesticides may be associated with increased toxicity in human poisoning compared to either pesticide alone. *Clinical Toxicology*, *52*(5), 538–541. https://doi.org/10.3109/15563650.2014.909933

Jankovic, J., & Tan, E. K. (2020). Parkinson's disease: Etiopathogenesis and treatment. *Journal of Neurology, Neurosurgery & Psychiatry*, *91*(8), 795–808. https://doi.org/10.1136/jnnp-2019-322338

Jeyaratnam, J. (1994). Acute pesticide poisoning. In *Occupational Health in National Development* (pp. 14–26). World Health Organization. http://dx.doi.org/10.1142/9789814354349_0002

Jiao, Z., Wu, Y., & Qu, S. (2020). Fenpropathrin induces degeneration of dopaminergic neurons via disruption of the mitochondrial quality control system. *Cell Death Discovery*, *6*(1). https://doi.org/10.1038/s41420-020-00313-y

Jones-Tabah, J., Mohammad, H., Hadj-Youssef, S., Kim, L., Martin, R. D., Benaliouad, F., Tanny, J. C., Clarke, P. B. S., & Hébert, T. E. (2020). *Dopamine D1 receptor signalling in dyskinetic Parkinsonian rats revealed by fiber photometry using FRET-based biosensors*. Cold Spring Harbor Laboratory. http://dx.doi.org/10.1101/2020.03.12.989103

Kaasinen, V., Vahlberg, T., Stoessl, A. J., Strafella, A. P., & Antonini, A. (2021). Dopamine receptors in Parkinson's disease: A meta-analysis of imaging studies. *Movement Disorders*, *36*(8), 1781–1791. https://doi.org/10.1002/mds.28632

Kamel, F., Umbach, D. M., Bedlack, R. S., Richards, M., Watson, M., Alavanja, M. C. R., Blair, A., Hoppin, J. A., Schmidt, S., & Sandler, D. P. (2012). Pesticide exposure and amyotrophic lateral sclerosis. *Neurotoxicology*, *33*(3), 457–462. https://doi.org/10.1016/j.neuro.2012.04.001

Kannarkat, G. T., Cook, D. A., Lee, J.-K., Chang, J., Chung, J., Sandy, E., Paul, K. C., Ritz, B., Bronstein, J., Factor, S. A., Boss, J. M., & Tansey, M. G. (2015). Common genetic variant association with altered HLA expression, synergy with pyrethroid exposure, and risk for Parkinson's disease: An observational and case–control study. *Npj Parkinson's Disease*, *1*(1). https://doi.org/10.1038/npjparkd.2015.2

Khan, Z. U., Mrzljak, L., Gutierrez, A., de la Calle, A., & Goldman-Rakic, P. S. (1998). Prominence of the dopamine D2 short isoform in dopaminergic pathways. *Proceedings of the National Academy of Sciences*, *95*(13), 7731–7736. https://doi.org/10.1073/pnas.95.13.7731

Kim, K.-B., Anand, S. S., Kim, H. J., White, C. A., & Bruckner, J. V. (2007). Toxicokinetics and tissue distribution of deltamethrin in adult sprague–dawley rats. *Toxicological Sciences*, *101*(2), 197–205. https://doi.org/10.1093/toxsci/kfm277

Kirby, M. L., Castagnoli, K., & Bloomquist, J. R. (1999). In vivo effects of deltamethrin on dopamine neurochemistry and the role of augmented neurotransmitter release. *Pesticide Biochemistry and Physiology*, *65*(3), 160–168. https://doi.org/10.1006/pest.1999.2440

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., & Correa, R. G. (2018). Dopamine: Functions, signaling, and association with neurological diseases. *Cellular and Molecular Neurobiology*, *39*(1), 31–59. https://doi.org/10.1007/s10571-018-0632-3

Koussounadis, A., Langdon, S. P., Um, I. H., Harrison, D. J., & Smith, V. A. (2015). Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. *Scientific Reports*, *5*(1). https://doi.org/10.1038/srep10775

Kung, T. S., Richardson, J. R., Cooper, K. R., & White, L. A. (2015). Developmental deltamethrin exposure causes persistent changes in dopaminergic gene expression, neurochemistry, and locomotor activity in zebrafish. *Toxicological Sciences*, *146*(2), 235–243. https://doi.org/10.1093/toxsci/kfv087

Lee, K.-S., Lim, Y.-H., Lee, Y. A., Shin, C. H., Kim, B.-N., Hong, Y.-C., & Kim, J. I. (2022). The association of prenatal and childhood pyrethroid pesticide exposure with school-age ADHD traits. *Environment International*, *161*, 107124. https://doi.org/10.1016/j.envint.2022.107124

Lee, T., Seeman, P., Rajput, A., Farley, I. J., & Hornykiewicz, O. (1978). Receptor basis for dopaminergic supersensitivity in Parkinson's disease. *Nature*, *273*(5657), 59–61. https://doi.org/10.1038/273059a0

Lehmler, H.-J., Simonsen, D., Liu, B., & Bao, W. (2020). Environmental exposure to pyrethroid pesticides in a nationally representative sample of U.S. adults and children: The National Health and Nutrition Examination Survey 2007–2012. *Environmental Pollution*, *267*, 115489. https://doi.org/10.1016/j.envpol.2020.115489

Lindgren, N., Usiello, A., Goiny, M., Haycock, J., Erbs, E., Greengard, P., Hökfelt, T., Borrelli, E., & Fisone, G. (2003). Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. *Proceedings of the National Academy of Sciences*, *100*(7), 4305–4309. https://doi.org/10.1073/pnas.0730708100

Liu, G.-P., Ma, Q., & Shi, N. (2006). Tyrosine hydroxylase as a target for deltamethrin in the nigrostriatal dopaminergic pathway . *Biomedical and Environmental Sciences*, *19*(1), 27–34. PMID: 16673815.

Lu, C., Barr, D. B., Pearson, M. A., Walker, L. A., & Bravo, R. (2008). The attribution of urban and suburban children's exposure to synthetic pyrethroid insecticides: A longitudinal assessment. *Journal of Exposure Science & Environmental Epidemiology*, *19*(1), 69–78. https://doi.org/10.1038/jes.2008.49

Marei, A. E. S. M., Ruzo, L. O., & Casida, J. E. (1982). Analysis and persistence of permethrin, cypermethrin, deltamethrin and fenvalerate in the fat and brain of treated rats. *Journal of Agricultural and Food Chemistry*, *30*(3), 558–562. https://doi.org/10.1021/jf00111a037

Martel, J. C., & McArthur, S. G. (2020). Dopamine receptor subtypes, physiology and pharmacology: New ligands and concepts in schizophrenia. *Frontiers in Pharmacology*, *11*. https://doi.org/10.3389/fphar.2020.01003

Matsuo, N. (2019). Discovery and development of pyrethroid insecticides. *Proceedings of the Japan Academy, Series B*, *95*(7), 378–400. https://doi.org/10.2183/pjab.95.027

Mela, F., Marti, M., Bido, S., Cenci, M. A., & Morari, M. (2012). In vivo evidence for a differential contribution of striatal and nigral D1 and D2 receptors to l-DOPA induced dyskinesia and the accompanying surge of nigral amino acid levels. *Neurobiology of Disease*, *45*(1), 573–582. https://doi.org/10.1016/j.nbd.2011.09.015

Michel, P. P., Hirsch, E. C., & Hunot, S. (2016). Understanding dopaminergic cell death pathways in Parkinson disease. *Neuron*, *90*(4), 675–691. https://doi.org/10.1016/j.neuron.2016.03.038

Miller, G. W., Staley, J. K., Heilman, C. J., Perez, J. T., Mash, D. C., Rye, D. B., & Levey, A. I. (1997). Immunochemical analysis of dopamine transporter protein in Parkinson's disease. *Annals of Neurology*, *41*(4), 530–539. https://doi.org/10.1002/ana.410410417

Mishra, A., Singh, S., & Shukla, S. (2018). Physiological and Functional Basis of Dopamine Receptors and Their Role in Neurogenesis: Possible Implication for Parkinson's disease. *Journal of Experimental Neuroscience*, *12*, 117906951877982. https://doi.org/10.1177/1179069518779829

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., & Caron, M. G. (1998). Dopamine receptors: From structure to function. *Physiological Reviews*, *78*(1), 189–225. https://doi.org/10.1152/physrev.1998.78.1.189

Mohammadi, H., Ghassemi-Barghi, N., Malakshah, O., & Ashari, S. (2019). Pyrethroid exposure and neurotoxicity: A mechanistic approach. *Archives of Industrial Hygiene and Toxicology*, *70*(2), 74–89. https://doi.org/10.2478/aiht-2019-70-3263

Moreno-Delgado, D., Puigdellívol, M., Moreno, E., Rodríguez-Ruiz, M., Botta, J., Gasperini, P., Chiarlone, A., Howell, L. A., Scarselli, M., Casadó, V., Cortés, A., Ferré, S., Guzmán, M., Lluís, C., Alberch, J., Canela, E. I., Ginés, S., & McCormick, P. J. (2020). Modulation of dopamine D1 receptors via histamine H3 receptors is a novel therapeutic target for Huntington's disease. *eLife*, *9*. https://doi.org/10.7554/elife.51093

Morgan, M. K., & Jones, P. A. (2013). Dietary predictors of young children's exposure to current-use pesticides using urinary biomonitoring. *Food and Chemical Toxicology*, *62*, 131–141. https://doi.org/10.1016/j.fct.2013.08.029

Nagatomo, K., Suga, S., Saitoh, M., Kogawa, M., Kobayashi, K., Yamamoto, Y., & Yamada, K. (2017). Dopamine D1 receptor immunoreactivity on fine processes of GFAP-positive astrocytes in the substantia nigra pars reticulata of adult mouse. *Frontiers in Neuroanatomy*, *11*. https://doi.org/10.3389/fnana.2017.00003

National Institute of Environmental Health Sciences. (2023, March 7). *Pesticides*. National Institute of Environmental Health Sciences; National Institutes of Health. https://www.niehs.nih.gov/health/topics/agents/pesticides/index.cfm

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical pesticides and human health: The urgent need for a new concept in agriculture. *Frontiers in Public Health*, *4*. https://doi.org/10.3389/fpubh.2016.00148

Numan, M., Numan, M. J., Pliakou, N., Stolzenberg, D. S., Mullins, O. J., Murphy, J. M., & Smith, C. D. (2005). The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal behavior in rats. *Behavioral Neuroscience*, *119*(6), 1588–1604. https://doi.org/10.1037/0735-7044.119.6.1588

Nutt, J. G., Carter, J. H., & Sexton, G. J. (2004). The dopamine transporter: Importance in Parkinson's disease. *Annals of Neurology*, *55*(6), 766–773. https://doi.org/10.1002/ana.20089

Oulhote, Y., & Bouchard, M. F. (2013). Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. *Environmental Health Perspectives*, *121*(11–12), 1378–1384. https://doi.org/10.1289/ehp.1306667

Paoletti, P., Vila, I., Rifé, M., Lizcano, J. M., Alberch, J., & Ginés, S. (2008). Dopaminergic and glutamatergic signaling crosstalk in Huntington's disease neurodegeneration: The role of p25/cyclin-dependent kinase 5. *The Journal of Neuroscience*, *28*(40), 10090–10101. https://doi.org/10.1523/jneurosci.3237-08.2008

Patel, N., & Pitzer, E. (2019). The effects of developmental deltamethrin exposure on glutamate and dopamine receptor expression in rats [AM_ATRIUM05]. *Undergraduate Scholarly Showcase*.

Pearson, B. L., Simon, J. M., McCoy, E. S., Salazar, G., Fragola, G., & Zylka, M. J. (2016). Identification of chemicals that mimic transcriptional changes associated with autism, brain aging and neurodegeneration. *Nature Communications*, *7*(1). https://doi.org/10.1038/ncomms11173

Pirsaheb, M., Fakhri, Y., Karami, M., Akbarzadeh, R., Safaei, Z., Fatahi, N., Sillanpää, M., & Asadi, A. (2019). Measurement of permethrin, deltamethrin and malathion pesticide residues in the wheat flour and breads and probabilistic health risk assessment: A case study in Kermanshah, Iran. *International Journal of Environmental Analytical Chemistry*, *99*(13), 1353–1364. https://doi.org/10.1080/03067319.2019.1622009

Pittman, J. T., Dodd, C. A., & Klein, B. G. (2003). Immunohistochemical changes in the mouse striatum induced by the pyrethroid insecticide permethrin. *International Journal of Toxicology*, *22*(5), 359–370. https://doi.org/10.1177/109158180302200504

Pitzer, E. M., Sugimoto, C., Gudelsky, G. A., Huff Adams, C. L., Williams, M. T., & Vorhees, C. V. (2019). Deltamethrin exposure daily from postnatal day 3–20 in Sprague-Dawley rats causes long-term cognitive and behavioral deficits. *Toxicological Sciences*, *169*(2), 511–523. https://doi.org/10.1093/toxsci/kfz067

Pitzer, E. M., Williams, M. T., & Vorhees, C. V. (2021). Effects of pyrethroids on brain development and behavior: Deltamethrin. *Neurotoxicology and Teratology*, *87*, 106983. https://doi.org/10.1016/j.ntt.2021.106983

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A.-E., & Lang, A. E. (2017). Parkinson disease. *Nature Reviews Disease Primers*, *3*(1). https://doi.org/10.1038/nrdp.2017.13

Ray, D. E., & Fry, J. R. (2006). A reassessment of the neurotoxicity of pyrethroid insecticides. *Pharmacology & Therapeutics*, *111*(1), 174–193. https://doi.org/10.1016/j.pharmthera.2005.10.003

Rehman, H., Aziz, A. T., Saggu, S., Abbas, Z. K., Mohan, A., & Ansari, A. A. (2014). Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies*, *2*(6), 60–70.

Richardson, J. R., Taylor, M. M., Shalat, S. L., Guillot, T. S., III, Caudle, W. M., Hossain, M. M., Mathews, T. A., Jones, S. R., Cory-Slechta, D. A., & Miller, G. W. (2015). Developmental pesticide exposure reproduces features of attention deficit hyperactivity disorder. *The FASEB Journal*, *29*(5), 1960–1972. https://doi.org/10.1096/fj.14-260901

Riederer, A. M., Bartell, S. M., Barr, D. B., & Ryan, P. B. (2008). Diet and nondiet predictors of urinary 3-phenoxybenzoic acid in NHANES 1999–2002. *Environmental Health Perspectives*, *116*(8), 1015–1022. https://doi.org/10.1289/ehp.11082

Rodríguez, J. L., Ares, I., Castellano, V., Martínez, M., Martínez-Larrañaga, M. R., Anadón, A., & Martínez, M. A. (2016). Effects of exposure to pyrethroid cyfluthrin on serotonin and dopamine levels in brain regions of male rats. *Environmental Research*, *146*, 388–394. https://doi.org/10.1016/j.envres.2016.01.023

Saillenfait, A.-M., Ndiaye, D., & Sabaté, J.-P. (2015). Pyrethroids: Exposure and health effects – An update. *International Journal of Hygiene and Environmental Health*, *218*(3), 281–292. https://doi.org/10.1016/j.ijheh.2015.01.002

Sawaguchi, T., & Goldman-Rakic, P. S. (1991). D1 dopamine receptors in prefrontal cortex: Involvement in working memory. *Science*, *251*(4996), 947–950. https://doi.org/10.1126/science.1825731

Schapira, A. H. V., Chaudhuri, K. R., & Jenner, P. (2017). Non-motor features of Parkinson disease. *Nature Reviews Neuroscience*, *18*(7), 435–450. https://doi.org/10.1038/nrn.2017.62

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, *9*(7), 676–682. https://doi.org/10.1038/nmeth.2019

Seeman, P. (2013). Schizophrenia and dopamine receptors. *European Neuropsychopharmacology*, *23*(9), 999–1009. https://doi.org/10.1016/j.euroneuro.2013.06.005

Seeman, P., & Niznik, H. B. (1990). Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *The FASEB Journal*, *4*(10), 2737–2744. https://doi.org/10.1096/fasebj.4.10.2197154

Sereda, S., Bouwman, H., & Meinhardt, H. R. (2005). Pyrethroid and DDT residues in human breast milk from Kwazulu-Natal, South Africa. *Epidemiology*, *16*(5), S157. https://doi.org/10.1097/00001648-200509000-00403

Simaremare, S. R. S., Hung, C.-C., Hsieh, C.-J., & Yiin, L.-M. (2019). Relationship between organophosphate and pyrethroid insecticides in blood and their metabolites in urine: A pilot study. *International Journal of Environmental Research and Public Health*, *17*(1), 34. https://doi.org/10.3390/ijerph17010034

Singh, A., Mudawal, A., Shukla, R. K., Yadav, S., Khanna, V. K., Sethumadhavan, R., & Parmar, D. (2014). Effect of gestational exposure of cypermethrin on postnatal development of brain cytochrome P450 2D1 and 3A1 and neurotransmitter receptors. *Molecular Neurobiology*, *52*(1), 741–756. https://doi.org/10.1007/s12035-014-8903-6

Stephenson, J. (2000). Exposure to home pesticides linked to parkinson disease. *JAMA*, *283*(23), 3055–3056. https://doi.org/10.1001/jama.283.23.3055

Stojanovic, T., Orlova, M., Sialana, F. J., Höger, H., Stuchlik, S., Milenkovic, I., Aradska, J., & Lubec, G. (2017). Validation of dopamine receptor DRD1 and DRD2 antibodies using receptor deficient mice. *Amino Acids*, *49*, 1101–1109. https://doi.org/10.1007/s00726-017-2408-3

Surmeier, D. J., Eberwine, J., Wilson, C. J., Cao, Y., Stefani, A., & Kitai, S. T. (1992). Dopamine receptor subtypes colocalize in rat striatonigral neurons. *Proceedings of the National Academy of Sciences*, *89*(21), 10178–10182. https://doi.org/10.1073/pnas.89.21.10178

Tabrez, S., R. Jabir, N., Shakil, S., Greig, N. H., Alam, Q., Abuzenadah, A. M., Damanhouri, G. A., & Kamal, M. A. (2012). A synopsis on the role of tyrosine hydroxylase in Parkinson's disease. *CNS Neurological Disorders - Drug Targets*, *11*(4), 395–409. https://doi.org/10.2174/187152712800792785

Tagliaferro, P., & Burke, R. E. (2016). Retrograde axonal degeneration in Parkinson disease. *Journal of Parkinson's Disease*, *6*(1), 1–15. https://doi.org/10.3233/jpd-150769

Tang, T.-S., Chen, X., Liu, J., & Bezprozvanny, I. (2007). Dopaminergic signaling and striatal neurodegeneration in Huntington's disease. *The Journal of Neuroscience*, *27*(30), 7899–7910. https://doi.org/10.1523/jneurosci.1396-07.2007

Thanvi, B. R., & Lo, T. C. N. (2004). Long term motor complications of levodopa: Clinical features, mechanisms, and management strategies. *Postgraduate Medical Journal*, *80*(946), 452–458. https://doi.org/10.1136/pgmj.2003.013912

Todd, G. D. (2003). *Toxicological Profile for Pyrethrins and Pyrethroids* (pp. 13–17). Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/toxprofiles/tp155.pdf

Tudi, M., Li, H., Li, H., Wang, L., Lyu, J., Yang, L., Tong, S., Yu, Q. J., Ruan, H. D., Atabila, A., Phung, D. T., Sadler, R., & Connell, D. (2022). Exposure routes and health risks associated with pesticide application. *Toxics*, *10*(6), 335. https://doi.org/10.3390/toxics10060335

Viel, J.-F., Warembourg, C., Le Maner-Idrissi, G., Lacroix, A., Limon, G., Rouget, F., Monfort, C., Durand, G., Cordier, S., & Chevrier, C. (2015). Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE mother–child cohort. *Environment International*, *82*, 69–75. https://doi.org/10.1016/j.envint.2015.05.009

Wakeling, E. N., Neal, A. P., & Atchison, W. D. (2012). Pyrethroids and their effects on ion channels. In *Pesticides - Advances in Chemical and Botanical Pesticides*. http://dx.doi.org/10.5772/50330

Wang, X. H., Souders, C. L., II, Xavier, P., Li, X. Y., Yan, B., & Martyniuk, C. J. (2020). The pyrethroid esfenvalerate induces hypoactivity and decreases dopamine transporter expression in embryonic/larval zebrafish (Danio rerio). *Chemosphere*, *243*, 125416. https://doi.org/10.1016/j.chemosphere.2019.125416

WHO. (2017). *Deltamethrin Long-Lasting (Coated onto Filaments) Insecticidal Net. (s)α-Cyano-3-Phenoxybenzyl (1r,3r)-3-(2,2dibromovinyl)-2,2-Dimethylcyclopropane Carboxylate*. World Health Organization. https://extranet.who.int/pqweb/sites/default/files/vcp-documents/WHOVC-SP_Deltamethrin_Incorporated%20LN_2021.pdf

WHO. (2022a). *Parkinson disease*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/parkinson-disease

WHO. (2022b). *Pesticide residues in food*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/pesticide-residues-in-food

Williamson, E. G., Long, S. F., Kallman, M. J., & Wilson, M. C. (1989). A comparative analysis of the acute toxicity of technical-grade pyrethroid insecticides and their commercial formulations. *Ecotoxicology and Environmental Safety*, *18*(1), 27–34. https://doi.org/10.1016/0147-6513(89)90089-4

Wilson, A. L., Courtenay, O., Kelly-Hope, L. A., Scott, T. W., Takken, W., Torr, S. J., & Lindsay, S. W. (2020). The importance of vector control for the control and elimination of vector-borne diseases. *PLOS Neglected Tropical Diseases*, *14*(1), e0007831. https://doi.org/10.1371/journal.pntd.0007831

Xu, M., Moratalla, R., Gold, L. H., Hiroi, N., Koob, G. F., Graybiel, A. M., & Tonegawa, S. (1994). Dopamine D1 receptor mutant mice are deficient in striatal expression of

dynorphin and in dopamine-mediated behavioral responses. *Cell*, *79*(4), 729–742. https://doi.org/10.1016/0092-8674(94)90557-6