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Research article

Developmental exposure to the Parkinson's disease-associated organochlorine pesticide dieldrin alters dopamine neurotransmission in α-synuclein pre-formed fibril (PFF)-injected mice

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Abstract

Parkinson's disease (PD) is the fastest-growing neurological disease worldwide, with increases outpacing aging and occurring most rapidly in recently industrialized areas, suggesting a role of environmental factors. Epidemiological, post-mortem, and mechanistic studies suggest that persistent organic pollutants, including the organochlorine pesticide dieldrin, increase PD risk. In mice, developmental dieldrin exposure causes male-specific exacerbation of neuronal susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and synucleinopathy. Specifically, in the α-synuclein (α-syn) pre-formed fibril (PFF) model, exposure leads to increased deficits in striatal dopamine (DA) turnover and motor deficits on the challenging beam. Here, we hypothesized that alterations in DA handling contribute to the observed changes and assessed vesicular monoamine transporter 2 (VMAT2) function and DA release in this dieldrin/PFF 2-hit model. Female C57BL/6 mice were exposed to 0.3 mg/kg dieldrin or vehicle every 3 days by feeding, starting at 8 weeks of age and continuing throughout breeding, gestation, and lactation. Male offspring from independent litters underwent unilateral, intrastriatal injections of α-syn PFFs at 12 weeks of age, and vesicular ^{3}H-DA uptake assays and fast-scan cyclic voltammetry were performed 4 months post-PFF injection. Dieldrin-induced an increase in DA release in striatal slices in PFF-injected animals, but no change in VMAT2 activity. These results suggest that developmental dieldrin exposure increases a compensatory response to synucleinopathy-triggered striatal DA loss. These findings are consistent with silent neurotoxicity, where developmental exposure to dieldrin primes the nigrostriatal striatal system to have an exacerbated response to synucleinopathy in the absence of observable changes in typical markers of nigrostriatal dysfunction and degeneration.

Keywords: Parkinson disease; alpha-synuclein; pesticides; dieldrin; dopamine; developmental neurotoxicity

Parkinson's disease (PD) is a multisystem disorder pathologically defined by the degeneration of dopaminergic neurons in the nigrostriatal pathway and the formation of α-synuclein (α-syn)-containing Lewy bodies. PD is the most common movement disorder, the second most common neurogenerative disease, and one of the fastest growing neurological diseases (de Lau and Breteler, 2006). From 1990 to 2016, the prevalence of PD has more than doubled globally (Dorsey *et al.*, 2018). In addition, a recent study suggests that PD incidence in the United States is 50% higher than previously estimated, with 90 000 diagnoses per year (Willis *et al.*, 2022). Of relevance to this work, the authors reported PD incidence rates higher in certain geographic areas including the "Rust Belt," a region with a history of heavy industrial manufacturing. This is consistent with epidemiological research that shows an association between increased risk of PD and environmental factors associated with industrialization, including heavy metals, solvents, and pesticide exposures (Ascherio *et al.*, 2006; Brown *et al.*, 2006; Cicchetti *et al.*, 2009; de Lau and Breteler, 2006; De Miranda *et al.*, 2022; Dorsey *et al.*, 2018; Elbaz *et al.*, 2009; Fleming, 2017; Freire and Koifman, 2012; Hatcher *et al.*, 2008; Moretto and Colosio, 2011; Semchuk *et al.*, 1992; Steenland *et al.*, 2006; Tanner and Aston, 2000; Tanner *et al.*, 2011; Wirdefeldt *et al.*, 2011). Multiple epidemiological studies have found elevated levels of organochlorines in general in serum and brain of PD subjects (Corrigan *et al.*, 1998, 2000; Elbaz *et al.*, 2009; Freire and Koifman, 2012; Steenland *et al.*, 2006). Of relevance here, one study reported a specific association

between dieldrin levels and PD risk with an odds ratio of 1.95 per interquartile range in never smokers, while other organochlorines did not show an association (Weisskopf *et al.*, 2010). In addition, when combined with post-mortem analysis and mechanistic studies, a potential role for dieldrin in PD emerges (Ascherio *et al.*, 2006; Brown *et al.*, 2006; Caudle *et al.*, 2012; Corrigan *et al.*, 1998, 2000; Elbaz *et al.*, 2009; Fleming *et al.*, 1994; Freire and Koifman, 2012; Hatcher *et al.*, 2007; Kanthasamy *et al.*, 2005; Le Couteur *et al.*, 1999; Moretto and Colosio, 2011; Priyadarshi *et al.*, 2000, 2001; Ritz and Yu, 2000; Semchuk *et al.*, 1991, 1992; Steenland *et al.*, 2006; Tanner and Aston, 2000; Tanner and Langston, 1990; Tanner *et al.*, 2011; Weisskopf *et al.*, 2010; Wirdefeldt *et al.*, 2011).

Because dieldrin was phased out in the 1970s and 1980s, the potential for new, acute exposure to dieldrin is low. However, the health effects of past exposures will continue for decades as the population currently diagnosed with PD and those that will develop PD in the next 20–30 years were likely exposed to dieldrin prior to its phase out during critical neurodevelopmental periods (de Jong *et al.*, 1997; Jorgenson, 2001; Kanthasamy *et al.*, 2005; Meijer *et al.*, 2001). Furthermore, well-established models of dieldrin exposure have demonstrated that dieldrin induces oxidative stress, is selectively toxic to dopaminergic cells, disrupts striatal dopamine (DA) activity, and may promote α-syn aggregation (Chun *et al.*, 2001; Hatcher *et al.*, 2007; Kanthasamy *et al.*, 2005; Kitazawa *et al.*, 2001, 2003; Moretto and Colosio, 2011; Richardson *et al.*, 2006; Sanchez-Ramos *et al.*, 1998). Thus, dieldrin serves as an important representative PD-related toxicant that has well-characterized animal exposure paradigms, and provide a roadmap for understanding how environmental exposures confer PD risk (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019).

Here, we utilize a mouse developmental dieldrin exposure model where exposure induces sex-specific stable alterations in the DA system that increase susceptibility to subsequent exposure to both α-synuclein (α-syn) pre-formed fibril (PFF)-induced synucleinopathy and MPTP in male, but not female, offspring, suggesting that this model is broadly applicable to investigating how this exposure affects PD risk and neuronal susceptibility (Figure 6) (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019; Luk *et al.*, 2012a,b; Richardson *et al.*, 2006) In this model, dams are fed dieldrin (0.3 mg/kg, every 3 days) throughout mating, gestation, and lactation and F1 pups are assessed for toxicity in PD models at 12 weeks of age (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019; Richardson *et al.*, 2006). This dose was chosen based on a previous dose response study and our results in the 2-hit dieldrin/PFF model (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019; Richardson *et al.*, 2006). Mice were exposed through oral ingestion by the dam because the most likely route of exposure to dieldrin in humans is through ingestion of contaminated foods (ATSDR, 2022). In this 2-hit model, we previously reported a male-specific dieldrin-associated exacerbation of synucleinopathy-induced increases in DA turnover at 6 months, but not at 2 months, as well as an exacerbation of motor deficits on challenging beam at 6 months (Gezer *et al.*, 2020) (Figure 7). We also reported no dieldrin effect on the number of α-syn aggregates 1 and 2 months after PFF injection or on the reductions in total striatal dopamine by high-performance liquid chromatography (HPLC) at 2- and 6-month post-PFF injection. We also demonstrated that synucleinopathy induced loss of DA neurons by TH and NeuN counts in the SN at 6 months is not exacerbated by dieldrin exposure. While we are unaware of specific epidemiological evidence of sex differences for the dieldrin-related increased in PD risk, this sex specificity of our observed phenotype is consistent with known sex differences in dopaminergic vulnerability to parkinsonian toxicants and our previously reported sex-specific epigenetic effects of developmental dieldrin exposure (Adamson *et al.*, 2022; Alves *et al.*, 2009; Baldereschi *et al.*, 2000; De Miranda *et al.*, 2019; Elbaz *et al.*, 2002; Georgiev *et al.*, 2017; Gillies *et al.*, 2014; Haaxma *et al.*, 2007; Kochmanski *et al.*, 2019; Taylor *et al.*, 2007; van den Eeden *et al.*, 2003; Weisskopf *et al.*, 2010; Wooten *et al.*, 2004).

Based on the observed exacerbation in PFF-induced increases in striatal DA turnover by dieldrin, we hypothesized here that dieldrin-induced alterations in DA packaging and synaptic vesicle function contribute to the exacerbated toxicity in PFF-injected animals. Proper packaging of DA into synaptic vesicles is critical for DA neurotransmission and neuronal health (Alter *et al.*, 2013). Because cytosolic DA is metabolized to 3,4-Dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and broken down to toxic products, disruption of DA handling and packaging can increase cytosolic DA and lead to oxidative stress and acceleration of the toxic interplay between dysregulated α-syn and DA (Alter *et al.*, 2013; Ben-Scachar *et al.*, 1995; Bezard and Gross, 1998; Caudle *et al.*, 2007; Gainetdinov *et al.*, 1999; Graham *et al.*, 1978; Guillot *et al.*, 2008; Hastings *et al.*, 1996; Iannitelli *et al.*, 2023; Lohr *et al.*, 2014; Meiser *et al.*, 2013; Miller *et al.*, 2011; Molina-Mateo *et al.*, 2017; Mor *et al.*, 2017; Onn *et al.*, 1986; Snyder *et al.*, 1990; Taylor *et al.*, 2009; Uhl, 1998; Zhang *et al.*, 1988; Zigmond, 1997; Zigmond *et al.*, 1984, 199, 1998). To test this, we assessed VMAT2 function by vesicular uptake assay and DA release and uptake by fast-scan cyclic voltammetry (FSCV) in the dieldrin/PFF 2-hit model 4 months post-PFF injection in male F1 offspring developmentally exposed to dieldrin. Testing at 4 months allowed us to capture changes in the striatal synapse prior to significant nigrostriatal degeneration. Assessing these endpoints at 6 months when degeneration of striatal terminals and nigral cell bodies is already pronounced would test mainly the effects of degeneration, rather than the functional changes that precede it.

Materials and methods

Animals

Male (11 weeks old) and female (7 weeks old) C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Animal husbandry and colony maintenance was completed as previously described (Kochmanski *et al.*, 2019; Gezer *et al.*, 2020). All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Michigan State University.

Dieldrin exposure paradigm

Dosing was carried out as previously described (Kochmanski *et al.*, 2019; Gezer *et al.*, 2020). Adult C57BL/6 (8-week-old) female animals were treated throughout breeding, gestation, and lactation (Figure 1A). Mice were administered 0.3 mg/kg dieldrin (ChemService, CAS No. 60-57-1) dissolved in corn oil vehicle and mixed with peanut butter pellets every 3 days (Gezer *et al.*, 2020; Gonzales *et al.*, 2014; Kochmanski *et al.*, 2019). This dose was chosen based on a previous dose response study and our results in the 2-hit dieldrin/PFF model (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019; Richardson *et al.*, 2006). Mice were exposed through oral ingestion by the dam because the most likely route of exposure to dieldrin in humans was through ingestion of contaminated foods and ingestion of the resulting contaminated breast milk (ATSDR, 2022). Control mice received an equivalent amount of corn oil vehicle in peanut butter. Four weeks into female

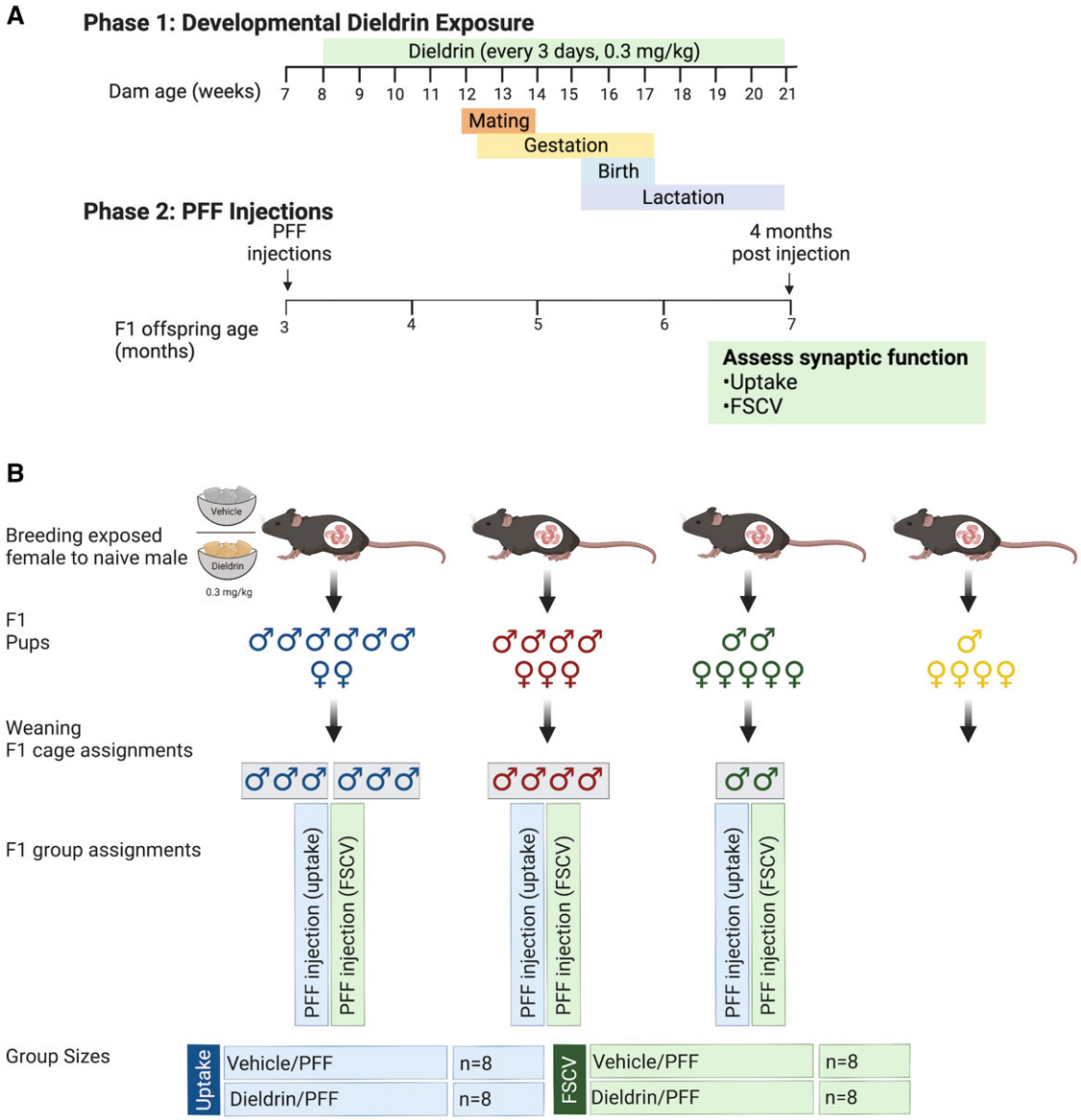

Figure 1. Experimental design including dosing schedule, weaning strategy, cage, and group assignments. A, Timeline of dieldrin-PFF 2-hit model: At 8 weeks of age, female C57BL/6 mice dieldrin exposure began via oral administration of 0.3 mg/kg dissolved in corn oil and injected into peanut butter pellets. At 12 weeks of age, mating began, and exposure continued through weaning of pups. F1 pups were weaned 3 weeks after birth and separated by litter and sex (2–4 animals per cage). At 3 months of age, male pups underwent intrastriatal injections of PFFs and were individually housed after surgery. B, Cage, group assignments, and group numbers: Male F1 offspring (F1) that underwent intrastriatal PFF-injections were assigned to endpoints such that every animal for each endpoint came from an independent litter. The Fourth F1 litter is an example of a litter excluded from endpoint assignments due to individual housing.

exposure, unexposed C57BL/6 males (8–12 weeks old) were introduced for breeding. Offspring were weaned at 3 weeks of age and separated by litter and by sex, with 2–4 animals per cage (Figure 1B). At 12 weeks of age, male offspring from independent litters were selected for PFF or monomer injection. This time point was chosen based on previous results demonstrating increased neuronal susceptibility to this age (Gezer *et al.*, 2020; Richardson *et al.*, 2006). This developmental dieldrin dosing paradigm has been previously used in our lab to study the role of epigenetics and the effects on synucleinopathy-induced toxicity (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019).

Preparation of α-synuclein PFFs and fibril size verification

Recombinant mouse α-syn monomers and PFFs were provided by the Luk lab, stored at −80°C, and prepared as previously described (Luk *et al.*, 2012a; Patterson *et al.*, 2019). Over 500 fibrils were measured to determine the average fibril length of 45.06 ± 14.7 nm (Figure 2). Fibril length was assessed before and after surgeries to ensure that fibrils did not reaggregate over the duration of the surgeries. All measurements were performed with ImageJ (Schneider *et al.*, 2012).

Intrastriatal injection of α-syn PFFs

At 12 weeks of age, animals received unilateral intrastriatal PFF injections according to their cage and group assignment (Figure 1B). Surgeries were performed as previously described (Gezer *et al.*, 2020; Luk *et al.*, 2012a). Mice received a total of 5 μg of PFFs (2.5 μl injection of 2 μg/μl PFFs) and received a single intrastriatal injection (anterior-posterior (AP) +0.2, medial-lateral (ML) +2.0, dorsal-ventral (DV) −2.6) with a flow rate of 0.5 μl/ml.

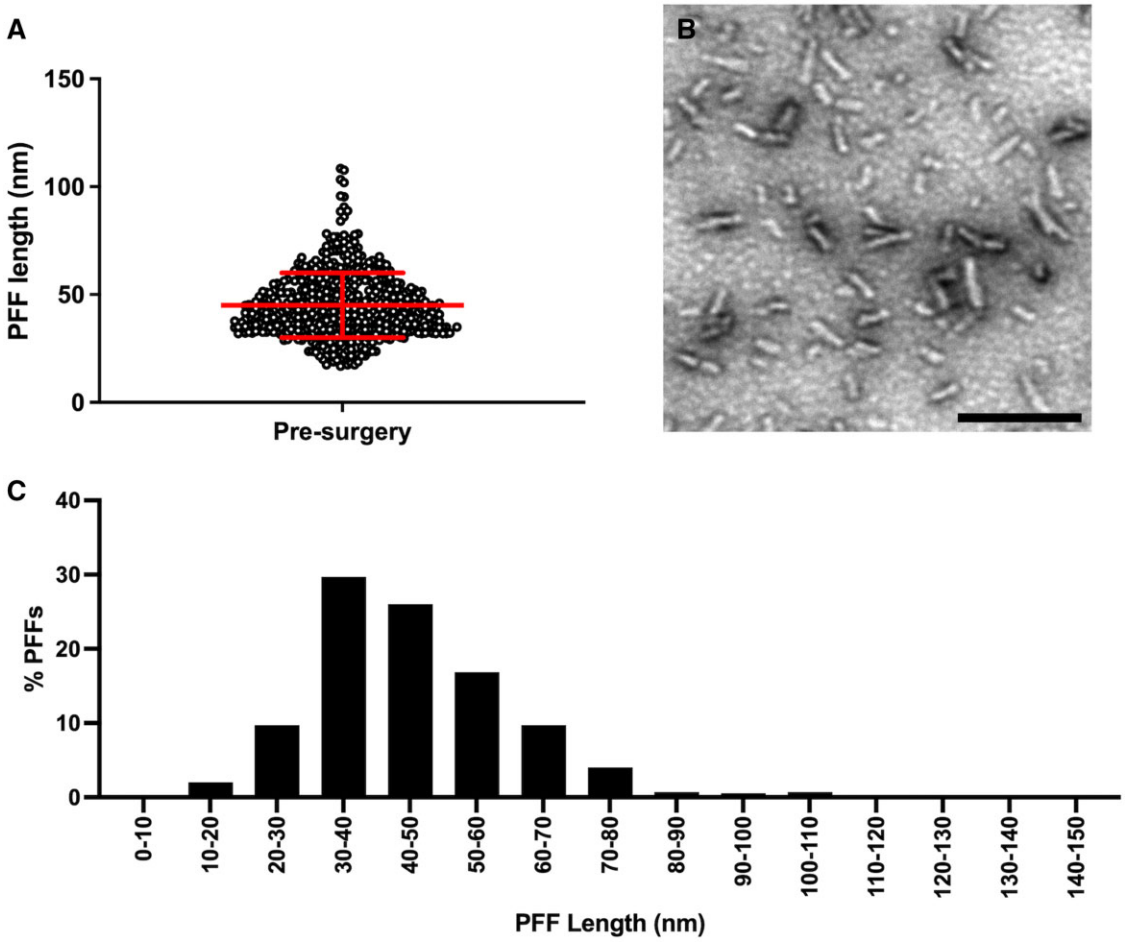

Figure 2. Verification of α-syn PFF size. A, PFF length distribution determined via TEM. Each point represents a measured fibril length, the error bars denote standard deviation. B, Representative TEM image of sonicated fibrils. C, Frequency distribution of PFF lengths post-sonication.

Post-surgery, mice received 1 mg/kg of sustained release buprenorphine by subcutaneous injection and were monitored closely until they recovered from anesthesia. In the 3 days following recovery, animals were monitored daily for adverse outcomes. A small subset of animals ($n = 2$ for FSCV and $n = 4$ for uptake) received α-syn monomer injections as a negative control to ensure that there were no effects of surgery itself. Animals were singly housed following surgeries for the duration of the experiment, consistent with our previous study (Gezer *et al.*, 2020).

Vesicular ^{3}H-dopamine uptake

Animals were killed by cervical dislocation and hemisected. Half of the brain from each group was homogenized for each statistical n, and vesicular DA uptake was performed as previously described (Bernstein *et al.*, 2012; Caudle *et al.*, 2007; Lohr *et al.*, 2014; Staal *et al.*, 2000). Data were normalized to protein level determined by BCA assay and expressed as pmol DA/mg protein/minute.

Fast-scan cyclic voltammetry

Animals were killed by cervical dislocation and brains were sectioned in oxygenated, 4°C artificial cerebrospinal fluid (aCSF) at 300 μm thick using a vibratome (Campden Instruments 5100mz-Plus) (Ferris *et al.*, 2014). FSCV was carried out in the lateral, dorsal striatal sections as previously described (Everett *et al.*, 2022; Ferris *et al.*, 2014; Lohr *et al.*, 2014; Ramsson, 2016; Ramsson *et al.*, 2015; Yorgason *et al.*, 2011).

Carbon fiber glass microelectrodes were constructed using a vacuum to pull carbon fiber through a glass capillary tube, pulled using a horizontal electrode puller, broken, and sealed with paraffin (Ramsson *et al.*, 2015). Microelectrodes were cycled for at least 15 min prior to recording at a frequency of 60 Hz, then cycled at 10 Hz until stable (Davis *et al.*, 2020; Ramsson, 2016; Ramsson *et al.*, 2015; Takmakov *et al.*, 2010). Carbon fiber microelectrodes were calibrated using a pipette-based calibration system by adding a dilute stock DA solution to a buffer and measuring the oxidation and/or reduction (Ramsson, 2016). All cycling and recordings occurred with a triangle waveform (−0.4 to 1.3 V to −0.4 V; 400 V/s 10 Hz) (Everett *et al.*, 2022; Kang *et al.*, 2021; Lohr *et al.*, 2014; Ramsson *et al.*, 2015). Dopamine release was elicited with a bipolar twisted electrode (PlasticsOne) and a 350 μA, 4 ms monophasic optically isolated stimulus pulse (Neurolog NL800). Data was collected and analyzed using Demon Voltammetry and Analysis Software (Wake Forest Innovations) (Yorgason *et al.*, 2011). A 5-recording survey of 2 different dorsal striatal release sites per hemisphere in 2 different slices was taken for each animal with a 5-min rest interval between each stimulation (Everett *et al.*, 2022; Lohr *et al.*, 2014). Peak Dopamine, upward velocity (DA release), downward velocity (uptake; a V_{max} estimate for DAT uptake), and tau (uptake; a K_m estimate for DAT uptake) were calculated for each recording (Figure 4A)

(Everett *et al.*, 2022). Ipsilateral values were normalized to contralateral values to account for animal-to-animal variability.

Immunohistochemistry

The rostral remainder of the brains used for FSCV were immersion fixed in 4% paraformaldehyde for 24 h and placed into 30% sucrose in PBS at 4°C for immunohistochemistry. Fixed brains were frozen on a sliding microtome and sliced at 40 μm coronally. Free-floating sections were stored in cryoprotectant (30% sucrose, 30% ethylene glycol, 0.05 M PBS) at −20°C. A 1:6 series of the entire rostral portion of the brain was used for staining and 2 nigral sections per animal were selected for imaging and quantification. Nonspecific staining was blocked with 10% normal goat serum, and sections were then incubated overnight in appropriate primary antibodies in TBS with 1% NGS/0.25% Triton X-100 followed by appropriate secondary antibodies for 2 hours (Table 1). Slides were cover-slipped with VECTASHIELD Vibrance Antifade Mounting Medium (VectaLabs) with DAPI and imaged on a Zeiss AxioScan 7 Digital Slide Scanning Microscope. Analysis of pSyn counts for immunohistochemistry was completed using the object colocalization module in the HALO Image Analysis Platform (Indica Labs). The SNpc was manually traced as the region of interest based on TH staining and pSyn positive objects within this region were identified on 2 sections per animal from the same level.

Data analysis and statistics

Statistical analysis and graphing were performed using GraphPad Prism 9. Vehicle-exposed animals injected with PFFs (Vehicle/PFF) and dieldrin-exposed animals injected with PFFs (Dieldrin/PFF) were compared with 2-tailed, unpaired-*t*-tests. All data are shown as mean ± SD and cutoff for statistical significance was $p < .05$. Monomer injected animals (Vehicle/Monomer, Dieldrin/Monomer) were used as controls to ensure there were not effects of surgery on its own, but these were not included in the statistical analysis, consistent with our preregistration and power analysis (Bernstein and Boyd, 2022).

Results

Confirmation of PFF-induced seeding of pSyn-positive aggregates

To confirm PFF-induced seeding, nigral slices were stained for TH and phosphorylated-synuclein (pSyn) from the remaining tissue of brains used for FSCV. We were unable to confirm seeding in animals used for uptake since the entire brain was used for that assay. We confirmed seeding in all animals used for FSCV and counted the number of pSyn-positive objects. At 4 months post-injection, as expected, we observed pSyn positive inclusions ipsilateral, but not contralateral, SN, in both dieldrin and vehicle/PFF groups (Figs. 3A and 3B). Consistent with previous results, dieldrin did not affect the number of pSyn-positive inclusions (Figure 3C) (Gezer *et al.*, 2020).

Table 1. Antibodies used for immunohistochemistry

Antibody	Host	Supplier	Dilution	RRID
Tyrosine Hydroxylase	Rabbit	Millipore AB152	1:4,000	AB_390204
α-synuclein (Phospho S129)/81A	Mouse	Abcam Ab184674	1:10,000	AB_2819037
Goat-anti-Mouse IgG2A Cross-Absorbed Alexa Fluor 555	Goat	Invitrogen A21137	1:500	AB_2535776
Goat-anti-Rabbit IgG (H+L) Cross-Absorbed Alexa Fluor 647	Goat	Invitrogen A21244	1:500	AB_2535812

Developmental dieldrin exposure increases DA release in PFF-injected animals

FSCV was performed in striatal slices to determine if developmental dieldrin exposure affects evoked DA release or uptake in PFF-injected animals 4 months after PFF injection (Figs. 4A–D). There was a significant increase in both peak DA concentration and upward velocity, a measure of DA release, in the dieldrin/PFF group compared to the vehicle/PFF group ($p = .0394$ and $p = .0434$, respectively) (Figs. 4E and 4F). However, there was no significant difference in DAT uptake as measure by tau or downward velocity, which are measures of DAT K_m and V_{max} ($p = .6435$ and .5303, respectively) (Figs. 4G and 4H). Calculated values are shown in Table 2. We verified that there was no difference in any metric on the contralateral side to confirm that dieldrin alone had no effect on DA release or uptake (Supplementary Figs. 1A–D). We also compared ipsilateral to contralateral metrics in the vehicle/PFF group to and observed no significant effect of PFFs alone (Supplementary Figs 1E and 1H). Monomer injected animals in both the vehicle and dieldrin exposed groups showed similar outcomes on all FSCV metrics.

Developmental dieldrin exposure does not alter VMAT2 uptake velocity in PFF-injected animals

To determine if dieldrin exposure alters VMAT2 function in PFF-injected animals, uptake assays were performed at 4 months post-PFF injection. Somewhat surprisingly, there was no difference in VMAT2-mediated uptake velocity between the vehicle/PFF and the dieldrin/PFF groups ipsilateral to injection site (Figure 5). As expected, there was no difference in uptake contralateral to the injection site, showing that dieldrin alone had no effect on uptake velocity (Supplementary Figure 2A). In addition, uptake was equivalent between the ipsilateral and contralateral sides within the vehicle/PFF group, demonstrating no significant effect of PFFs alone (Supplementary Figure 2B). Observed uptake velocity was consistent with previously published values for VMAT2 uptake velocity in WT C57BL/6 mice (Lohr *et al.*, 2014). Vehicle and dieldrin exposed animals injected with monomer showed similar VMAT2 uptake velocity in the hemisphere ipsilateral to the injection (vehicle/monomer: 7.020 ± 2.224 pmol/mg/min, $n = 4$; dieldrin/monomer: 5.460 ± 1.678 pmol/mg/min, $n = 4$).

Discussion

A model of environmental risk and silent neurotoxicity in PD

Based on the results reported here, we expand our model for how developmental dieldrin exposure leads to increased susceptibility to synucleinopathy-induced deficits in motor behavior (Figures 6 and 7) (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019; Richardson *et al.*, 2006). In this model, exposure to dieldrin occurs during prenatal and postnatal development. The half-life of dieldrin in mouse brain is less than a week, so no detectable dieldrin remains in the brain of F1 offspring by a few weeks after weaning (Hatcher *et al.*, 2007; Richardson *et al.*, 2006; World Health Organization & International Programme on Chemical Safety, 1989). When dieldrin is present in the developing brain, it is thought to act on developing DA neurons by inhibiting $GABA_A$

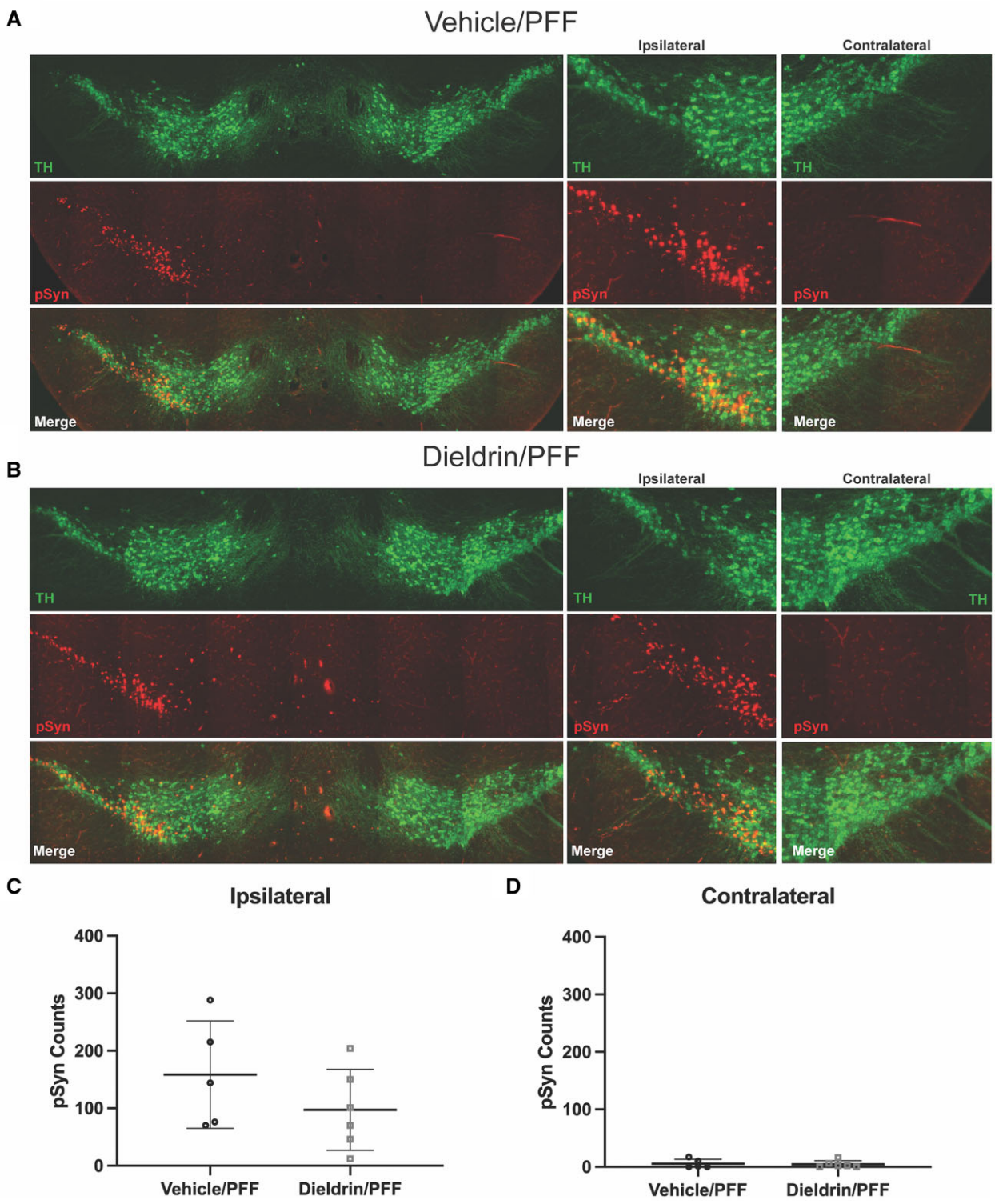

Figure 3. Confirmation of PFF-induced seeding in FSCV animals. A, Representative images from nigral tissue sections stained with TH (top panels) and pSyn (middle panels) from a vehicle/PFF (A) and dieldrin/PFF (B) animals 4 months post-PFF injection. C, pSyn counts in the SNpc show no effect of dieldrin on pSyn-positive objects in the SNpc ipsilateral to the PFF injection ($p = .2441$). D, As expected, there were no pSyn-positive objects contralateral to the injection in either group of animals. All data are shown as mean ± SD.

receptor-mediated chloride flux, resulting in increased neuronal activity (Lauder *et al.*, 1998; Liu *et al.*, 1997; Narahashi, 1996; Narahashi *et al.*, 1995; Okada *et al.*, 2004; Paladini and Tepper, 1999). Based on previous results, we propose that this net increase in neuronal activity modifies the dopamine system through persistent sex-specific changes in epigenetic mechanisms, leading to dysregulation of genes important for dopamine neuron development and maintenance in the substantia nigra and for the neuroinflammatory system in the striatum (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019). These changes alter the response of the nigrostriatal system to future insults via persistent alterations in striatal dopamine synapses that manifest

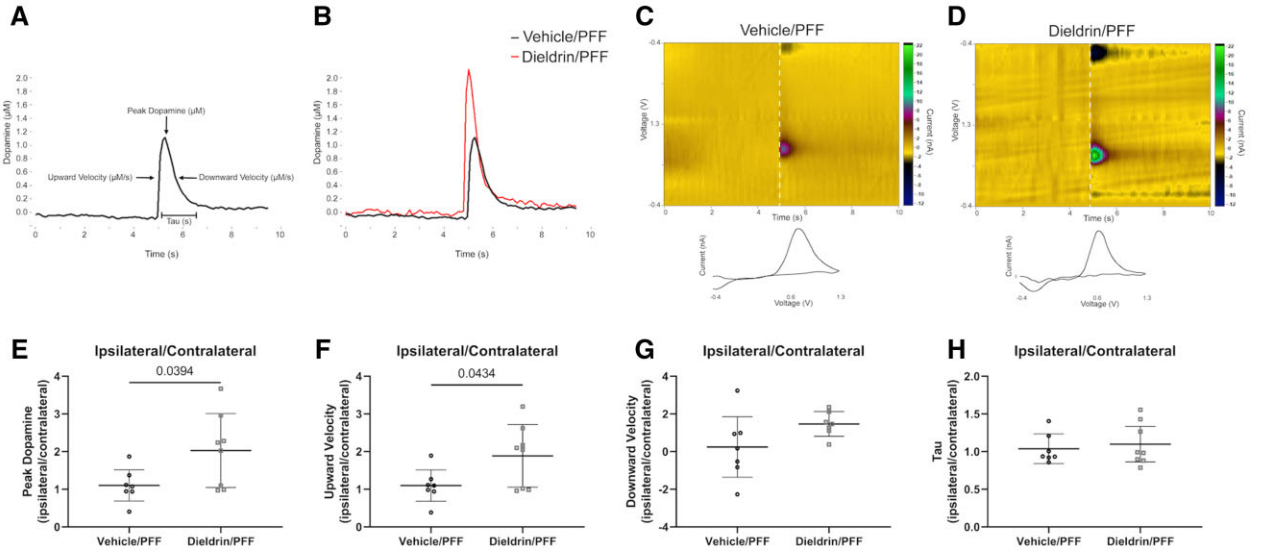

Figure 4. Dieldrin/PFFs increase peak dopamine and upward velocity in striatal tissues measured using FSCV. Four months post-PFF injection, animals were killed and FSCV was performed in dorsal striatum. A, Example dopamine versus time graph showing each quantified metric. B, Representative dopamine versus time graph for the groups vehicle/PFF (black) and dieldrin/PFF (red). C, D, Representative dopamine concentration vs time plot for (C) vehicle/PFF and (D) dieldrin/PFF following stimulation at $t = 5$ s. E–H, FSCV metrics represented as ipsilateral values normalized to contralateral values. E, Quantification of peak dopamine showed a significant dieldrin-related increase ($p = .0394$). F, Quantification of upward velocity showed a significant dieldrin-related increase ($p = .0434$). G, Quantification of downward velocity showed no significant effect of dieldrin ($p = .5303$). H, Quantification of tau showed no significant effect of dieldrin ($p = .6435$). Each individual data point represents a sum of 20 recordings per animal. All data shown as mean ± SD. A color version of this figure appears in the online version of this article.

Table 2. FSCV values (ipsilateral/contralateral) for vehicle/PFF and dieldrin/PFF groups (mean ± standard deviation)

Treatment	Peak dopamine (μM)	Upward velocity (μM/s)	Downward velocity (μM/s)	Tau (s)
Vehicle/PFF	1.102 ± 0.4470	1.097 ± 0.4483	0.2439 ± 1.736	1.038 ± 0.1970
Dieldrin/PFF	2.028 ± 0.9815	1.886 ± 0.8306	0.8454 ± 1.858	1.098 ± 0.2817

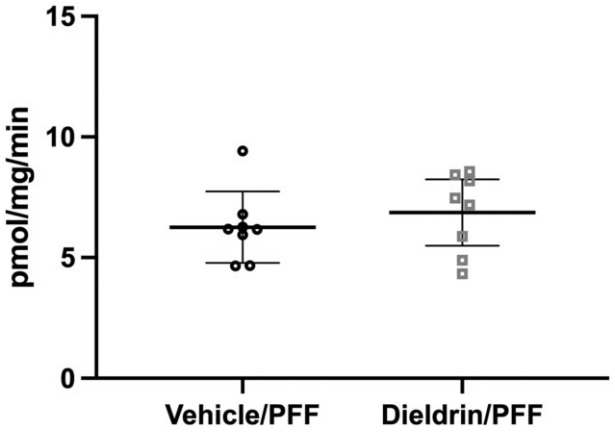

Figure 5. Dieldrin does not affect VMAT2 uptake velocity in PFF-injected male F1 offspring. There was no difference in uptake velocity ipsilateral to injection site 4 months post-PFF injection ($p = .4524$). All data shown as mean ± SD.

as an early increase in compensatory mechanisms triggered by synucleinopathy-induced striatal DA loss in adult male mice (Figure 6) (Gezer *et al.*, 2020).

Our results are also consistent with the idea of silent neurotoxicity, where the effects of early life exposures are unmasked by challenges later in life, the cumulative effects of exposures over the lifespan, or the effects of aging (Cory-Slechta *et al.*, 2005; Kraft *et al.*, 2016). In such a paradigm, developmental exposure to dieldrin primes the nigrostriatal striatal system in male offspring to have an exacerbated response to synucleinopathy induced by α-syn PFFs in the absence of observable changes in typical markers of nigrostriatal dysfunction and degeneration. In support of this, our previous studies identified persistent epigenetic and transcriptomic changes in genes related to DAergic differentiation and maintenance in the midbrain and altered expression of neuroinflammatory genes in the striatum at 12 weeks of age following developmental dieldrin exposure (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019). In a parallel study, we are also tracking the longitudinal patterns of dieldrin-induced epigenetic changes across the timeline of this entire 2-hit model from birth to 9 months of age to determine if dieldrin alters the trajectory of epigenetic changes across the lifespan.

Taken together, these results suggest that exploring dieldrin-induced changes that produce this high susceptibility state are critical to advancing our understanding of how exposures contribute to increased risk of PD and underscore the need to study PD-related exposures across the lifespan, particularly during sensitive periods of neurodevelopment.

Developmental dieldrin exposure alters the dopaminergic response to synucleinopathy-triggered dopamine deficits

Here, we demonstrate that developmental dieldrin exposure alters response to synucleinopathy and enhances DA release in PFF-injected male animals 4 months after PFF-injection (Figure 4). These results are consistent with our previous observation of an exacerbated increasein DA turnover at 6 months,

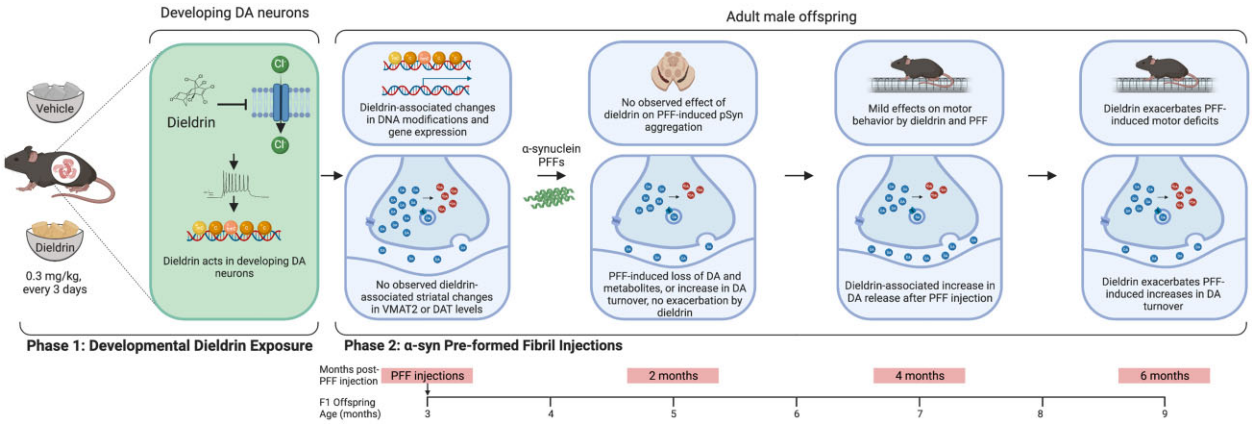

Figure 6. Overview of developmental dieldrin/PFF 2-hit model.

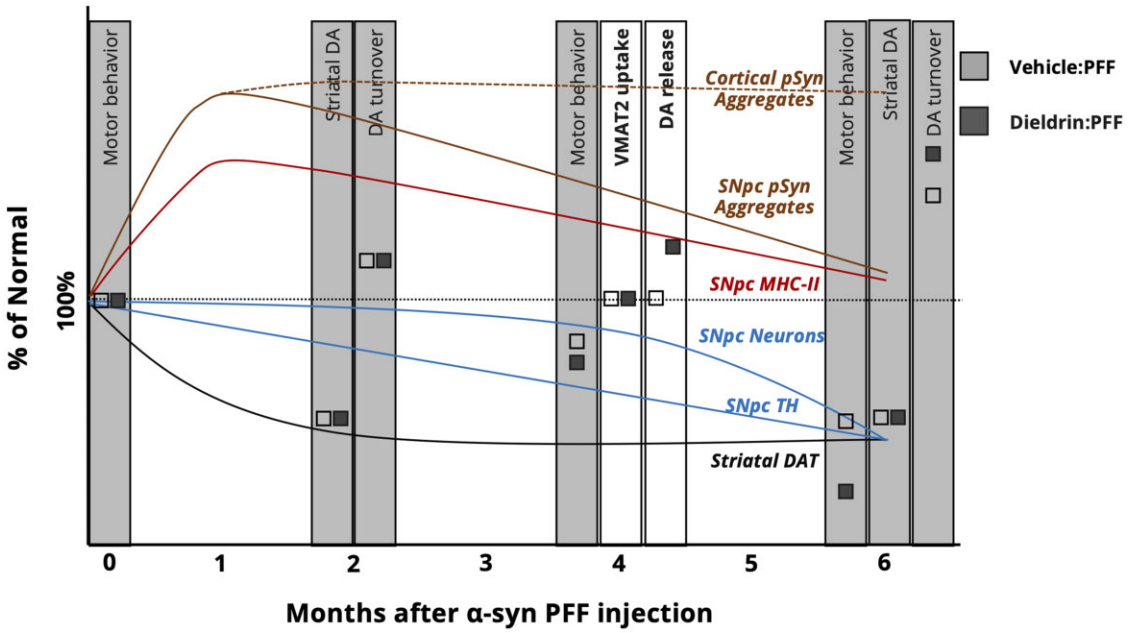

Figure 7. Summary of observed changes in the dieldrin PFF 2-hit model. Timelines show representative changes synuclein pathology, microglial activation, striatal loss, and nigral degeneration in the PFF model based on published literature, shown as the percent change in these markers compared to a saline/monomer injected mouse. Grey boxes indicate previous results from our lab in the dieldrin PFF 2-hit model. White boxes indicate FSCV and uptake results reported here at 4 months post-PFF injection. Light grey and dark grey squares represent results from vehicle: PFF and dieldrin: PFF animals, respectively.

summarized in 7 (Gezer *et al.*, 2020). If more DA is released at this 4 months, but DAT and VMAT2 uptake velocities are unchanged, this could lead to the increased DA turnover observed at 6 month post-PFF injection (Alter *et al.*, 2013). Importantly, our current data were collected 4 months post-PFF injection while our previous data showed increase striatal DA turnover at 6 months, suggesting that this enhanced DA release precedes effects on DA turnover and motor behavior (Gezer *et al.*, 2020). Of note, in our previous study, we reported that pSyn aggregation at 1 and 2 months, the loss of total striatal DA and its metabolites, DOPAC and HVA, at 2 and 6 months post-PFF injection and loss of nigral DA neurons at 6 months were not affected by dieldrin exposure (Gezer *et al.*, 2020) (Figure 7). Taken together, despite similar levels of synucleinopathy-induced pathology and total tissue DA levels in dieldrin and vehicle exposed animals, dieldrin exposed animals display an increase in evoked DA release at 4 months post-PFF injection.

While FSCV has been utilized in α-syn knockout and α-syn overexpressing models, to our knowledge, this is the first study to perform FSCV in either the dieldrin or α-syn PFF mouse model with intrastriatal injection (Somayaji *et al.*, 2020; Threlfell *et al.*, 2021; Yavich *et al.*, 2004). A previous paper performed FSCV in the mouse α-syn PFF model via intranigral injections at 2- and 5 months of age and reported decreased DA release in older animals only at 1- and 2 months post-injection (Sun *et al.*, 2021). These and other studies in different α-syn models indicate a critical role for a-syn in DA release, synaptic vesicle fusion, vesicle trafficking, and regulation of synaptic vesicle pool size (Abeliovich *et al.*, 2000; Bellani *et al.*, 2010; Cheng *et al.*, 2011; Dagra *et al.*, 2021; Ingelsson, 2016; Murphy *et al.*, 2000; Volles *et al.*, 2001; Xilouri *et al.*, 2013). Given that we did not observe any effect of dieldrin or PFF alone on DA release, dieldrin exposure appears to cause changes in the synaptic terminal that prime the nigrostriatal system for an exacerbated response to synucleinopathy

and striatal DA loss, resulting in early enhanced DA release and an eventual increase DA turnover (Supplementary Figure 1) (Gezer *et al.*, 2020; Kochmanski *et al.*, 2019). Early nigrostriatal compensatory changes are well-documented in human PD, multiple animal models of DA deficits, and more recently in a model of other monoaminergic (norepinephrine) loss (Bezard and Gross, 1998; Iannitelli *et al.*, 2023; Molina-Mateo *et al.*, 2017; Onn *et al.*, 1986; Snyder *et al.*, 1990; Zhang *et al.*, 1988; Zigmond, 1994, 1997; Zigmond *et al.*, 1984, 1998, 1989). Together, this suggests that dieldrin induces changes in the synaptic terminal that increase the compensatory response to early synucleinopathy-induced striatal DA loss that contributes to greater long-term increases in DA turnover due to increases in cytosolic DA, the resulting oxidative stress, and acceleration of the toxic interplay between dysregulated α-syn and DA (Bezard and Gross, 1998; Iannitelli *et al.*, 2023; Molina-Mateo *et al.*, 2017; Onn *et al.*, 1986; Snyder *et al.*, 1990; Zhang *et al.*, 1988; Zigmond, 1997; Zigmond *et al.*, 1984, 1989, 1998). Such a relationship between DA and α-syn is well-established and interfering with either can lead to a cycle of neurotoxicity where DA and α-syn interact and exacerbate the toxic effects of one another (Nemani *et al.*, 2010; Roy, 2017; Peng *et al.*, 2005; Perez *et al.*, 2002; Tehranian *et al.*, 2006; Venda *et al.*, 2010; Yavich *et al.*, 2004).

Developmental dieldrin exposure does not affect DAT- or VMAT2-mediated uptake in PFF-injected animals

We expected to see a relative increase in DAT function compared to VMAT2 function 4 months after PFF injection that was greater in animals developmentally exposed to dieldrin that would lead to increased cytosolic DA and DA turnover and explain the dieldrin-induced exacerbation of synucleinopathy-induced changes in DA turnover (Gezer *et al.*, 2020; Richardson *et al.*, 2006). However, we did not observe any dieldrin related effect on VMAT2 uptake in PFF-injected animals (Figure 5). It is possible that there is an effect on VMAT2 uptake velocity in the intact system that was not observed here due to methodology. Specifically, isolating synaptic vesicles for this assay removes them from their biological context and measures persistent changes in function (Caudle *et al.*, 2007; Lohr *et al.*, 2014, 2015, 2016). In addition, this assay involves homogenizing the entire hemisphere of the brain, so effects may be diluted by DAergic vesicles from areas of the brain not affected in our PFF model, including unaffected terminals within the striatum. Thus, this assay may not have the sensitivity to detect a small change in this small subpopulation of terminals. Unfortunately, technical limitations preclude us from performing this assay on unilateral striata from mouse brain.

It is also possible to increase the relative levels of DAT to VMAT2 function by affecting DAT function without alerting VMAT2 function. Multiple prior studies show that both dieldrin and PFFs can impact DAT expression and function (Hatcher *et al.*, 2007; Luk *et al.*, 2012a; Richardson *et al.*, 2006; Sossi *et al.*, 2022). Specifically, developmental dieldrin exposure was previously reported to lead to increases in striatal DAT levels at 12 weeks of age and changes in the DAT/VMAT2 ratio, but we did not observe the same effect in our previous study (Gezer *et al.*, 2020; Richardson *et al.*, 2006). In the mouse PFF model, striatal DAT protein expression at 6 months post-PFF injection in C57BL/6 mice was observed, but not at 1- and 3 months (Luk *et al.*, 2012a). Thus, it is possible that dieldrin-induced increases in striatal DAT function lead to a less severe loss of DAT following PFF-induced synucleinopathy and a relative increase of DAT to VMAT2 function. However, we observed no change in DAT uptake in this study as measured by Tau and downward velocity, measures of DAT K_m and V_{max}, from FSCV data (Figure 4). Thus, it is possible that if more DA is released, but neither DAT- nor VMAT2-mediated uptake velocity is changed, the observed increase in turnover is due to both intracellular breakdown and extracellular metabolism of DA. Despite these caveats regarding VMAT2 and DAT uptake, this new data is consistent with our previous results in this 2-hit model showing no increase in α-syn pathology but an enhanced response to synucleinopathy in dieldrin exposed F1 male offspring (Gezer *et al.*, 2020).

Synucleinopathy alone does not affect DA release 4 months after PFF injection

Contrary to our hypothesis, we did not observe a synucleinopathy-induced decrease in DA release despite approximately 45% loss of total striatal DA levels 2 months post-PFF (Gezer *et al.*, 2020; Luk *et al.*, 2012a). This discrepancy is likely due to methodological differences and the underlying biology of DA neurons. HPLC measures total tissue DA levels from tissue punches, while FSCV measures extracellular DA only from the area immediately surrounding the electrode. Biologically, while there is a significant loss of total tissue DA at 2 months post-PFF injection, reductions in DA release at these synapses may be delayed relative to this loss. Most striatal DA synapses are silent, and the majority of synaptic vesicles are located within the reserve pool rather than the readily releasable pool (Goldstein, 2012, 2013, 2021; Sulzer *et al.*, 2016; Trudeau *et al.*, 2014). Additionally, within the striatum of PFF-injected animals, we expect only a third to a half of terminals to be affected. Together, this leaves a pool of both surviving neurons and vesicles within affected neurons to maintain DA release. Consistent with this, we previously observed only mild PFF-associated effects on motor behavior at 4 months post-PFF injection, suggesting that DA release is maintained even with 45% loss of total striatal DA at 2 months post-PFF (Gezer *et al.*, 2020). In line with this, it is generally accepted that DA-related symptoms in human PD do not present until at least 30% of dopaminergic neurons in the nigrostriatal pathway are lost, suggesting that remaining neurons release sufficient quantities of DA despite loss of total DA (Cheng *et al.*, 2010).

Supplementary data

Supplementary data are available at *Toxicological Sciences* online.

Author contributions

S.L.B.: Investigation, Software, Formal Analysis, Writing—Original Draft, Writing—Review & Editing, Visualization, Project Administration; N.C.K.: Methodology, Investigation, Project Administration; J.R.P.: Investigation, Validation, Writing—Review & Editing; A.C.S.: Investigation; S.A.Z.: Investigation; M.R.K.: Investigation; J.J.N.: Investigation; K.C.L.: Resources; E.S.R.: Conceptualization, Investigation, Supervision, Writing—Review & Editing; C.E.S.: Conceptualization, Investigation, Supervision, Writing—Review & Editing; A.I.B.: Conceptualization, Supervision, Data Curation, Writing—Review & Editing, Funding Acquisition.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Data availability

This study was preregistered with Open Science Framework at https://doi.org/10.17605/osf.io/qv4ya (Bernstein and Boyd, 2022).

All data acquired and analyzed for this study are available in the Dryad Data Repository https://doi.org/10.5061/dryad.qz612jmmq (Bernstein and Boyd, 2023).

References

Abeliovich, A., Schmitz, Y., Fariñas, I., Choi-Lundberg, D., Ho, W. H., Castillo, P. E., Shinsky, N., Verdugo, J. M., Armanini, M., Ryan, A., *et al.* (2000). Mice lacking a-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* **25**, 239–252.

Adamson, A., Buck, S. A., Freyberg, Z., and De Miranda, B. R. (2022). Sex differences in dopaminergic vulnerability to environmental toxicants—implications for Parkinson's disease. *Curr. Environ. Health Rep.* **9**, 563–573.

Agency for Toxic Substances and Disease Registry (ATSDR) (2022). *Toxicological Profile for Aldrin and Dieldrin*. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Alter, S. P., Lenzi, G. M., Bernstein, A. I., and Miller, G. W. (2013). Vesicular integrity in parkinson's disease. *Curr. Neurol. Neurosci. Rep.* **13**, 362.

Alves, G., Norwegian ParkWest study group, Müller, B., Herlofson, K., HogenEsch, I., Telstad, W., Aarsland, D., Tysnes, O. B., and Larsen, J. P. (2009). Incidence of parkinson's disease in Norway: The Norwegian ParkWest study. *J. Neurol. Neurosurg. Psychiatry.* **80**, 851–857.

Ascherio, A., Chen, H., Weisskopf, M. G., O'Reilly, E., McCullough, M. L., Calle, E. E., Schwarzschild, M. A., and Thun, M. J. (2006). Pesticide exposure and risk for Parkinson's disease. *Ann. Neurol.* **60**, 197–203.

Baldereschi, M., Di Carlo, A., Rocca, W. A., Vanni, P., Maggi, S., Perissinotto, E., Grigoletto, F., Amaducci, L., and Inzitari, D. (2000). Parkinson's disease and parkinsonism in a longitudinal study. *Neurology* **55**, 1358–1363.

Bellani, S., Sousa, V. L., Ronzitti, G., Valtorta, F., Meldolesi, J., and Chieregatti, E. (2010). The regulation of synaptic function by α-synuclein. *Commun. Integr. Biol.* **3**, 106–109.

Ben-Shachar, D., Zuk, R., and Glinka, Y. (1995). Dopamine neurotoxicity: Inhibition of mitochondrial respiration. *J. Neurochem.* **64**, 718–723.

Bernstein, A. I., and Boyd, S. (2022). Striatal synaptic function in the dieldrin-PFF two-hit model. Open Science Framework. doi:10.17605/OSF.IO/QV4YA.

Bernstein, A. I., and Boyd, S. L. (2023). Data for: Parkinson's disease-associated organochlorine pesticide dieldrin alters dopamine neurotransmission in α-synuclein pre-formed fibril (PFF)-injected mice. doi:10.5061/dryad.qz612jmmq.

Bernstein, A. I., Stout, K. A., and Miller, G. W. (2012). A fluorescent-based assay for live cell, spatially resolved assessment of vesicular monoamine transporter 2-mediated neurotransmitter transport. *J. Neurosci. Methods.* **209**, 357–366.

Bezard, E., and Gross, C. E. (1998). Compensatory mechanisms in experimental and human parkinsonism: Towards a dynamic approach. *Prog. Neurobiol.* **55**, 93–116.

Brown, T. P., Rumsby, P. C., Capleton, A. C., Rushton, L., and Levy, L. S. (2006). Pesticides and parkinson's disease—Is there a link? *Environ. Health Perspect.* **114**, 156–164.

Caudle, W. M., Guillot, T. S., Lazo, C. R., and Miller, G. W. (2012). Industrial toxicants and Parkinson's disease. *Neurotoxicology* **33**, 178–188.

Caudle, W. M., Richardson, J. R., Wang, M. Z., Taylor, T. N., Guillot, T. S., McCormack, A. L., Colebrooke, R. E., Di Monte, D. A., Emson, P. C., and Miller, G. W. (2007). Reduced vesicular storage of dopamine causes progressive nigrostriatal neurodegeneration. *J. Neurosci.* **27**, 8138–8148.

Cheng, H. C., Ulane, C. M., and Burke, R. E. (2010). Clinical progression in Parkinson disease and the neurobiology of axons. *Ann. Neurol.* **67**, 715–725.

Cheng, F., Vivacqua, G., and Yu, S. (2011). The role of alpha-synuclein in neurotransmission and synaptic plasticity. *J. Chem. Neuroanat.* **42**, 242–248.

Chun, H. S., Gibson, G. E., DeGiorgio, L. A., Zhang, H., Kidd, V. J., and Son, J. H. (2001). Dopaminergic cell death induced by MPP(+), oxidant and specific neurotoxicants shares the common molecular mechanism. *J. Neurochem.* **76**, 1010–1021.

Cicchetti, F., Drouin-Ouellet, J., and Gross, R. E. (2009). Environmental toxins and Parkinson's disease: What have we learned from pesticide-induced animal models? *Trends Pharmacol. Sci.* **30**, 475–483.

Corrigan, F. M., Murray, L., Wyatt, C. L., and Shore, R. F. (1998). Diorthosubstituted polychlorinated biphenyls in caudate nucleus in Parkinson's disease. *Exp. Neurol.* **150**, 339–342.

Corrigan, F. M., Wienburg, C. L., Shore, R. F., Daniel, S. E., and Mann, D. (2000). Organochlorine insecticides in substantia nigra in Parkinson's disease. *J. Toxicol. Environ. Health. A.* **59**, 229–234.

Cory-Slechta, D. A., Thiruchelvam, M., Richfield, E. K., Barlow, B. K., and Brooks, A. I. (2005). Developmental pesticide exposures and the Parkinson's disease phenotype. *Birth Defects Res. A Clin. Mol. Teratol.* **73**, 136–139.

Le Couteur, D., McLean, A., Taylor, M., Woodham, B., and Board, P. (1999). Pesticides and Parkinson's disease. *Biomed. Pharmacother.* **53**, 122–130.

Dagra, A., Miller, D. R., Lin, M., Gopinath, A., Shaerzadeh, F., Harris, S., Sorrentino, Z. A., Støier, J. F., Velasco, S., Azar, J., *et al.* (2021). α-Synuclein-induced dysregulation of neuronal activity contributes to murine dopamine neuron vulnerability. *NPJ Parkinsons. Dis.* **7**, 76.

Davis, S. E., Korich, A. L., and Ramsson, E. S. (2020). Enhancement of fast scan cyclic voltammetry detection of dopamine with tryptophan-modified electrodes. *PLoS One.* **15**, e0235407.

de Jong Geert, H., Swaen, G.M.M., Slangen Joseph, J.M., Slangen, J.J., and de Jong, G. (1997). Mortality of workers exposed to dieldrin and aldrin: A retrospective cohort study. *Occup. Environ. Med.* **54**, 702–707.

de Lau, L. M., and Breteler, M. M. (2006). Epidemiology of Parkinson's disease. *Lancet. Neurol.* **5**, 525–535.

De Miranda, B. R., Fazzari, M., Rocha, E. M., Castro, S., and Greenamyre, J. T. (2019). Sex differences in rotenone sensitivity reflect the male-to-female ratio in human Parkinson's disease incidence. *Toxicol. Sci.* **170**, 133–143.

De Miranda, B. R., Goldman, S. M., Miller, G. W., Greenamyre, J. T., and Dorsey, E. R. (2022). Preventing Parkinson's disease: An environmental agenda. *J. Parkinsons. Dis.* **12**, 45–68.

Dorsey, E. R., Elbaz, A., Nichols, E., Abbasi, N., Abd-Allah, F., Abdelalim, A., Adsuar, J. C., Ansha, M. G., Brayne, C., Choi, J.-Y. J., *et al.* (2018). Global, regional, and national burden of Parkinson's

disease, 1990–2016: A systematic analysis for the global burden of disease study 2016. *Lancet Neurol* **17**, 939–953.

Elbaz, A., Bower, J. H., Maraganore, D. M., Mcdonnell, S. K., Peterson, B. J., Ahlskog, J. E., Schaid, D. J., and Rocca, W. A. (2002). Risk tables for parkinsonism and Parkinson's disease. *J. Clin. Epidemiol.* **55**, 25–31.

Elbaz, A., Clavel, J., Rathouz, P. J., Moisan, F., Galanaud, J. P., Delemotte, B., Alpérovitch, A., and Tzourio, C. (2009). Professional exposure to pesticides and Parkinson disease. *Ann. Neurol.* **66**, 494–504.

Everett, A. C., Graul, B. E., Ronström, J. W., Robinson, J. K., Watts, D. B., España, R. A., Siciliano, C. A., and Yorgason, J. T. (2022). Effectiveness and relationship between biased and unbiased measures of dopamine release and clearance. *ACS Chem. Neurosci.* **13**, 1534–1548.

Ferris, M. J., España, R. A., Locke, J. L., Konstantopoulos, J. K., Rose, J. H., Chen, R., and Jones, S. R. (2014). Dopamine transporters govern diurnal variation in extracellular dopamine tone. *Proc. Natl. Acad. Sci. U.S.A.* **111**, E2751–E2759.

Fleming, S. M. (2017). Mechanisms of gene-environment interactions in Parkinson's disease. *Curr. Environ. Health Rep.* **4**, 192–199.

Fleming, L., Mann, J. B., Bean, J., Briggle, T., and Sanchez-Ramos, J. R. (1994). Parkinson's disease and brain levels of organochlorine pesticides. *Ann. Neurol.* **36**, 100–103.

Freire, C., and Koifman, S. (2012). Pesticide exposure and Parkinson's disease: Epidemiological evidence of association. *Neurotoxicology* **33**, 947–971.

Gainetdinov, R. R., Levey, A. I., Caron, M. G., Miller, G. W., Gainetdinov, R. R., Levey, A. I., and Caron, M. G. (1999). Dopamine transporters and neuronal injury. *Trends Pharmacol. Sci.* **20**, 424–429.

Georgiev, D., Hamberg, K., Hariz, M., Forsgren, L., and Hariz, G.-M. (2017). Gender differences in Parkinson's disease: A clinical perspective. *Acta Neurol. Scand.* **136**, 570–584.

Gezer, A. O., Kochmanski, J., VanOeveren, S. E., Cole-Strauss, A., Kemp, C. J., Patterson, J. R., Miller, K. M., Kuhn, N. C., Herman, D. E., McIntire, A., *et al.* (2020). Developmental exposure to the organochlorine pesticide dieldrin causes male-specific exacerbation of α-synuclein-preformed fibril-induced toxicity and motor deficits. *Neurobiol. Dis.* **141**, 104947.

Gillies, G. E., Pienaar, I. S., Vohra, S., and Qamhawi, Z. (2014). Sex differences in Parkinson's disease. *Front. Neuroendocrinol.* **35**, 370–384.

Goldstein, D. S. (2012). Stress, allostatic load, catecholamines, and other neurotransmitters in neurodegenerative diseases. *Cell. Mol. Neurobiol.* **32**, 661–666.

Goldstein, D. S. (2013). Biomarkers, mechanisms, and potential prevention of catecholamine neuron loss in arkinson disease. *Adv. Pharmacol.* **68**, 235–272.

Goldstein, D. S. (2021). The catecholaldehyde hypothesis for the pathogenesis of catecholaminergic neurodegeneration: What we know and what we do not know. *IJMS.* **22**, 5999.

Gonzales, C., Zaleska, M. M., Riddell, D. R., Atchison, K. P., Robshaw, A., Zhou, H., and Sukoff Rizzo, S. J. (2014). Alternative method of oral administration by peanut butter pellet formulation results in target engagement of BACE1 and attenuation of gavage-induced stress responses in mice. *Pharmacol. Biochem. Behav.* **126**, 28–35.

Graham, D., Tiffany, S., Bell, W., and Gutknecht, W. (1978). Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine, and related compounds toward C1300 neuroblastoma cells in vitro. *Mol Pharmacol* **14**, 644–653.

Guillot, T. S., Shepherd, K. R., Richardson, J. R., Wang, M. Z., Li, Y., Emson, P. C., and Miller, G. W. (2008). Reduced vesicular storage of dopamine exacerbates methamphetamine-induced neurodegeneration and astrogliosis. *J. Neurochem.* **106**, 2205–2217.

Haaxma, C. A., Bloem, B. R., Borm, G. F., Oyen, W. J. G., Leenders, K. L., Eshuis, S., Booij, J., Dluzen, D. E., and Horstink, M. W. I. M. (2007). Gender differences in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry.* **78**, 819–824.

Hastings, T. G., Lewis, D. A., and Zigmond, M. J. (1996). Role of oxidation in the neurotoxic effects of intrastriatal dopamine injections. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1956–1961.

Hatcher, J. M., Pennell, K. D., and Miller, G. W. (2008). Parkinson's disease and pesticides: A toxicological perspective. *Trends Pharmacol. Sci.* **29**, 322–329.

Hatcher, J. M., Richardson, J. R., Guillot, T. S., McCormack, A. L., Di Monte, D. A., Jones, D. P., Pennell, K. D., and Miller, G. W. (2007). Dieldrin exposure induces oxidative damage in the mouse nigrostriatal dopamine system. *Exp. Neurol.* **204**, 619–630.

Iannitelli, A. F., Kelberman, M. A., Lustberg, D. J., Korukonda, A., McCann, K. E., Mulvey, B., Segal, A., Liles, L. C., Sloan, S. A., Dougherty, J. D., *et al.* (2023). The neurotoxin DSP-4 dysregulates the locus Coeruleus-Norepinephrine system and recapitulates molecular and behavioral aspects of prodromal neurodegenerative disease. *eNeuro* **10**, ENEURO.0483-22.2022. doi:10.1523/ENEURO.0483-22.2022.

Ingelsson, M. (2016). Alpha-synuclein oligomers-neurotoxic molecules in Parkinson's disease and other lewy body disorders. *Front. Neurosci.* **10**, 408–410.

Jorgenson, J. L. (2001). Aldrin and dieldrin: A review of research on their production, environmental deposition and fate, bioaccumulation, toxicology, and epidemiology in the United States. *Environ. Health Perspect.* **109(Suppl 1)**, 113–139.

Kang, Y., Goyal, A., Hwang, S., Park, C., Cho, H. U., Shin, H., Park, J., Bennet, K. E., Lee, K. H., Oh, Y., *et al.* (2021). Enhanced dopamine sensitivity using steered fast-scan cyclic voltammetry. *ACS Omega.* **6**, 33599–33606.

Kanthasamy, A. G., Kitazawa, M., Kanthasamy, A., and Anantharam, V. (2005). Dieldrin-induced neurotoxicity: Relevance to Parkinson's disease pathogenesis. *Neurotoxicology* **26**, 701–719.

Kitazawa, M., Anantharam, V., and Kanthasamy, A. G. (2001). Dieldrin-induced oxidative stress and neurochemical changes contribute to apoptopic cell death in dopaminergic cells. *Free Radic. Biol. Med.* **31**, 1473–1485.

Kitazawa, M., Anantharam, V., and Kanthasamy, A. G. (2003). Dieldrin induces apoptosis by promoting caspase-3-dependent proteolytic cleavage of protein kinase Cδ in dopaminergic cells: Relevance to oxidative stress and dopaminergic degeneration. *Neuroscience* **119**, 945–964.

Kochmanski, J., VanOeveren, S. E., Patterson, J. R., and Bernstein, A. I. (2019). Developmental dieldrin exposure alters DNA methylation at genes related to dopaminergic neuron development and Parkinson's disease in mouse midbrain. *Toxicol. Sci.* **169**, 593–607.

Kraft, A. D., Aschner, M., Cory-Slechta, D. A., Bilbo, S. D., Caudle, W. M., and Makris, S. L. (2016). Unmasking silent neurotoxicity following developmental exposure to environmental toxicants. *Neurotoxicol. Teratol.* **55**, 38–44.

Lauder, J., Jiu, J., Devaud, L., and Morrow, A. (1998). GABA as a trophic factor for developing monoamine neurons. *Perspect Dev Neurobiol* **5**, 247–259.

Liu, J., Morrow, A. L., Devaud, L., Grayson, D. R., and Lauder, J. M. (1997). GABA a receptors mediate trophic effects of GABA on embryonic brainstem monoamine neurons in vitro. *J. Neurosci.* **17**, 2420–2428.

Lohr, K. M., Chen, M., Hoffman, C. A., McDaniel, M. J., Stout, K. A., Dunn, A. R., Wang, M., Bernstein, A. I., and Miller, G. W. (2016). Vesicular monoamine transporter 2 (VMAT2) level regulates MPTP vulnerability and clearance of excess dopamine in mouse striatal terminals. *Toxicol. Sci.* **153**, 79–88.

Lohr, K. M., Stout, K. A., Dunn, A. R., Wang, M., Salahpour, A., Guillot, T. S., and Miller, G. W. (2015). Increased vesicular monoamine transporter 2 (VMAT2; Slc18a2) protects against methamphetamine toxicity. *ACS Chem. Neurosci.* **6**, 790–799.

Lohr, K. M., Bernstein, A. I., Stout, K. A., Dunn, A. R., Lazo, C. R., Alter, S. P., Wang, M., Li, Y., Fan, X., Hess, E. J., *et al.* (2014). Increased vesicular monoamine transporter enhances dopamine release and opposes Parkinson disease-related neurodegeneration in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 9977–9982.

Luk, K. C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J. Q., and Lee, V. M.-Y. (2012a). Pathological α-Synuclein transmission initiates Parkinson-like neurodegeneration in non-transgenic mice. *Science* **338**, 949–953.

Luk, K. C., Kehm, V. M., Zhang, B., O'Brien, P., Trojanowski, J. Q., and Lee, V. M. Y. (2012b). Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. *J. Exp. Med.* **209**, 975–986.

Meijer, S. N., Halsall, C. J., Harner, T., Peters, A. J., Ockenden, W. A., Johnston, A. E., and Jones, K. C. (2001). Organochlorine pesticide residues in archived UK soil. *Environ. Sci. Technol.* **35**, 1989–1995.

Meiser, J., Weindl, D., and Hiller, K. (2013). Complexity of dopamine metabolism. *Cell Commun. Signal.* **11**, 34–18.

Miller, G. W., Taylor, T. N., and Caudle, W. M. (2011). VMAT2-deficient mice display nigral and extranigral pathology and motor and nonmotor symptoms of Parkinson's disease. *Parkinsons. Dis.* **2011**, 124165.

Molina-Mateo, D., Fuenzalida-Uribe, N., Hidalgo, S., Molina-Fernández, C., Abarca, J., Zárate, R. V., Escandón, M., Figueroa, R., Tevy, M. F., and Campusano, J. M. (2017). Characterization of a presymptomatic stage in a Drosophila Parkinson's disease model: Unveiling dopaminergic compensatory mechanisms. *Biochim. Biophys. Acta. Mol. Basis Dis.* **1863**, 2882–2890.

Mor, D. E., Tsika, E., Mazzulli, J. R., Gould, N. S., Kim, H., Daniels, M. J., Doshi, S., Gupta, P., Grossman, J. L., Tan, V. X., *et al.* (2017). Dopamine induces soluble α-synuclein oligomers and nigrostriatal degeneration. *Nat. Neurosci.* **20**, 1560–1568.

Moretto, A., and Colosio, C. (2011). Biochemical and toxicological evidence of neurological effects of pesticides: The example of Parkinson's disease. *Neurotoxicology* **32**, 383–391.

Murphy, D. D., Rueter, S. M., Trojanowski, J. Q., and M-Y Lee, V. (2000). Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J. Neurosci.* **20**, 3214–3220.

Narahashi, T. (1996). Neuronal ion channels as the target sites of insecticides. *Pharmacol. Toxicol.* **79**, 1–14.

Narahashi, T., Carter, D., Frey, J., Ginsburg, K., Hamilton, B., Nagata, K., Roy, M., Song, J., and Tatebayashi, H. (1995). Sodium channels and GABAA receptor-channel complex as targets of environmental toxicants. *Toxicol. Lett.* **82-83**, 239–245.

Nemani, V. M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M. K., Chaudhry, F. A., Nicoll, R. A., and Edwards, R. H. (2010). Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* **65**, 66–79.

Okada, H., Matsushita, N., Kobayashi, K., and Kobayashi, K. (2004). Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons. *J. Neurochem.* **89**, 7–14.

Onn, S.-P., Berger, T. W., Stricker, E. M., and Zigmond, M. J. (1986). Effects of intraventricular 6-hydroxydopamine on the dopaminergic innervation of striatum: Histochemical and neurochemical analysis. *Brain Res.* **376**, 8–19.

Paladini, C., and Tepper, J. (1999). GABA(A) and GABA(B) antagonists differentially affect the firing pattern of substantia nigra dopaminergic neurons in vivo. *Synpase* **32**, 165–176.

Patterson, J. R., Polinski, N. K., Duffy, M. F., Kemp, C. J., Luk, K. C., Volpicelli-Daley, L. A., Kanaan, N. M., and Sortwell, C. E. (2019). Generation of alpha-synuclein preformed fibrils from monomers and use in vivo. *J Vis Exp* **2019**, 1–10.

Peng, X. M., Tehranian, R., Dietrich, P., Stefanis, L., and Perez, R. G. (2005). α-synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *J. Cell Sci.* **118**, 3523–3530.

Perez, R. G., Waymire, J. C., Lin, E., Liu, J. J., Guo, F., and Zigmond, M. J. (2002). A role for-Synuclein in the regulation of dopamine biosynthesis. *J. Neurosci.* **22**, 3090–3099.

Priyadarshi, A., Khuder, S. A., Schaub, E. A., and Priyadarshi, S. S. (2001). Environmental risk factors and parkinson's disease: A metaanalysis. *Environ. Res.* **86**, 122–127.

Priyadarshi, A., Khuder, S., Schaub, E., and Shirvastava, S. (2000). A meta-analysis of Parkinson's disease and exposure to pesticides. *Neurotoxicology* **21**, 435–440.

Ramsson, E. S. (2016). A pipette-based calibration system for fast-scan cyclic voltammetry with fast response times. *Biotechniques.* **61**, 269–271.

Ramsson, E. S., Cholger, D., Dionise, A., Poirier, N., Andrus, A., and Curtiss, R. (2015). Characterization of fast-scan cyclic voltammetric electrodes using paraffin as an effective sealant with in vitro and in vivo applications. *PLoS One.* **10**, e0141340.

Richardson, J. R., Caudle, W. M., Wang, M., Dean, E. D., Pennell, K. D., Miller, G. W., Richardson, J. R., Caudle, W. M., Wang, M., and Dean, E. D. (2006). Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. *FASEB J.* **20**, 1695–1697.

Ritz, B., and Yu, F. (2000). Parkinson's disease mortality and pesticide exposure in California 1984–1994. *Int. J. Epidemiol.* **29**, 323–329.

Roy, S. (2017). Synuclein and dopamine: The bonnie and clyde of parkinson's disease. *Nat. Neurosci.* **20**, 1514–1515.

Sanchez-Ramos, J., Facca, A., Basit, A., and Song, S. (1998). Toxicity of dieldrin for dopaminergic neurons in mesencephalic cultures. *Exp. Neurol.* **150**, 263–271.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH image to ImageJ: 25 years of image analysis HHS public access. *Nat. Methods.* **9**, 671–675.

Semchuk, K. M., Love, E. J., and Lee, R. G. (1992). Parkinson's disease and exposure to agricultural work and pesticide chemicals. *Neurology* **42**, 1328–1335.

Semchuk, K. M., Love, E. J., and Lee, R. G. (1991). Parkinson's disease and exposure to rural environmental factors: A population based Case-Control study. *Can. J. Neurol. Sci.* **18**, 279–286.

Snyder, GL., Keller, R., and Igmond, M. (1990). Dopamine efflux from striatal slices after intracerebral 6-hydroxydopamine: Evidence for compensatory hyperactivity of residual terminals. *J Pharmacol Exp Ther* **253**, 867–876.

Somayaji, M., Cataldi, S., Choi, J., Edwards, R. H., Mosharov, E. V., and Sulzer, D. (2020). A dual role for α-synuclein in facilitation and depression of dopamine release from substantia nigra neurons in vivo. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 32701–32710.

Sossi, V., Patterson, J. R., McCormick, S., Kemp, C. J., Miller, K. M., Stoll, A. C., Kuhn, N., Kubik, M., Kochmanski, J., Luk, K. C., *et al.* (2022). Dopaminergic positron emission tomography imaging in

the Alpha-Synuclein preformed fibril model reveals similarities to early Parkinson's disease. *Mov. Disord.* **37**, 1739–1748.

Staal, R. G. W., Hogan, K. A., Liang, C. L., German, D. C., and Sonsalla, P. K. (2000). In vitro studies of striatal vesicles containing the vesicular monoamine transporter (VMAT2): Rat versus mouse differences in sequestration of 1-methyl-4-phenylpyridinium. *J. Pharmacol. Exp. Ther.* **293**, 329–335.

Steenland, K., Hein, M. J., Cassinelli, R. T., Prince, M. M., Nilsen, N. B., Whelan, E. A., Waters, M. A., Ruder, A. M., and Schnorr, T. M. (2006). Polychlorinated biphenyls and neurodegenerative disease mortality in an occupational cohort. *Epidemiology* **17**, 8–13.

Sun, F., Salinas, A. G., Filser, S., Blumenstock, S., Medina-Luque, J., Herms, J., and Sgobio, C. (2022). Impact of α-synuclein spreading on the nigrostriatal dopaminergic pathway depends on the onset of the pathology. *Brain Pathol.* **32**, e13036.

Sulzer, D., Cragg, S. J., and Rice, M. E. (2016). Striatal dopamine neurotransmission: Regulation of release and uptake. *Basal Ganglia.* **6**, 123–148.

Takmakov, P., Zachek, M. K., Keithley, R. B., Walsh, P. L., Donley, C., McCarty, G. S., and Wightman, R. M. (2010). Carbon microelectrodes with a renewable surface. *Anal. Chem.* **82**, 2020–2028.

Tanner, C. M., and Aston, D. A. (2000). Epidemiology of parkinson's disease and akinetic syndromes. *Curr. Opin. Neurol.* **13**, 427–430.

Tanner, C. M., and Langston, J. W. (1990). Do environmental toxins cause Parkinson's disease? A critical review. *Neurology* **40**, suppl 17.

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., *et al.* (2011). Rotenone, paraquat, and Parkinson's disease. *Environ. Health Perspect.* **119**, 866–872.

Taylor, T. N., Caudle, W. M., Shepherd, K. R., Noorian, A. R., Jackson, C. R., Iuvone, P. M., Weinshenker, D., Greene, J. G., and Miller, G. W. (2009). Nonmotor symptoms of Parkinson's disease revealed in an animal model with reduced monoamine storage capacity. *J. Neurosci.* **29**, 8103–8113.

Taylor, K. S. M., Cook, J. A., and Counsell, C. E. (2007). Heterogeneity in male to female risk for Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry.* **78**, 905–906.

Tehranian, R., Montoya, S. E., Van Laar, A. D., Hastings, T. G., and Perez, R. G. (2006). Alpha-synuclein inhibits aromatic amino acid decarboxylase activity in dopaminergic cells. *J. Neurochem.* **99**, 1188–1196.

Threlfell, S., Mohammadi, A. S., Ryan, B. J., Connor-Robson, N., Platt, N. J., Anand, R., Serres, F., Sharp, T., Bengoa-Vergniory, N., Wade-Martins, R., *et al.* (2021). Striatal dopamine transporter function is facilitated by converging biology of α-Synuclein and cholesterol. *Front. Cell. Neurosci.* **15**, 658244.

Trudeau, L. E., Hnasko, T. S., Wallén-Mackenzie, Å., Morales, M., Rayport, S., and Sulzer, D. (2014). The multilingual nature of dopamine neurons. *Prog. Brain Res.* **211**, 141–164.

Uhl, G. R. (1998). Hypothesis: The role of dopaminergic transporters inselective vulnerability of cells in parkinson's disease. *Ann. Neurol.* **43**, 555–560.

van den Eeden, S. K., Tanner, C. M., Bernstein, A. L., Fross, R. D., Leimpeter, A., Bloch, D. A., and Nelson, L. M. (2003). Incidence of parkinson's disease: Variation by age, gender, and race/ethnicity. *Am. J. Epidemiol.* **157**, 1015–1022.

Venda, L. L., Cragg, S. J., Buchman, V. L., and Wade-Martins, R. (2010). α-Synuclein and dopamine at the crossroads of parkinson's disease. *Trends Neurosci.* **33**, 559–568.

Volles, M. J., Lee, S. J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., and Lansbury, P. T. (2001). Vesicle permeabilization by protofibrillar α-synuclein: Implications for the pathogenesis and treatment of Parkinson's disease. *Biochemistry* **40**, 7812–7819.

Weisskopf, M. G., Knekt, P., O'Reilly, E. J., Lyytinen, J., Reunanen, A., Laden, F., Altshul, L., and Ascherio, A. (2010). Persistent organochlorine pesticides in serum and risk of Parkinson disease. *Neurology* **74**, 1055–1061.

Willis, A. W. Roberts, E., Beck, J. C., Fiske, B., Ross, W., Savica, R., van den Eeden, S. K., Tanner, C. M., Marras, C., and Alcalay, R.; Parkinson's Foundation P4 Group (2022). Incidence of Parkinson disease in North America. *NPJ Parkinsons. Dis.* **8**, 170.

Wirdefeldt, K., Adami, H. O., Cole, P., Trichopoulos, D., and Mandel, J. (2011). Epidemiology and etiology of parkinson's disease: A review of the evidence. *Eur. J. Epidemiol.* **26(Suppl 1)**, S1–S58.

Wooten, G. F., Currie, L. J., Bovbjerg, V. E., Lee, J. K., and Patrie, J. (2004). Are men at greater risk for Parkinson's disease than women? *J. Neurol. Neurosurg. Psychiatry.* **75**, 637–639.

World Health Organization & International Programme on Chemical Safety (1989). *Aldrin and Dieldrin: Health and Safety Guide*. World Health Organization. https://apps.who.int/iris/handle/10665/39358.

Xilouri, M., Brekk, O. R., and Stefanis, L. (2013). α-Synuclein and protein degradation systems: A reciprocal relationship. *Mol. Neurobiol.* **47**, 537–551.

Yavich, L., Tanila, H., Vepsäläinen, S., and Jäkälä, P. (2004). Role of α-synuclein in presynaptic dopamine recruitment. *J. Neurosci.* **24**, 11165–11170.

Yorgason, J. T., España, R. A., and Jones, S. R. (2011). Demon voltammetry and analysis software: Analysis of cocaine-induced alterations in dopamine signaling using multiple kinetic measures. *J. Neurosci. Methods.* **202**, 158–164.

Zhang, W. Q., Tilson, H. A., Nanry, K. P., Hudson, P. M., Hong, J. S., and Stachowiak, M. K. (1988). Increased dopamine release from striata of rats after unilateral nigrostriatal bundle damage. *Brain Res.* **461**, 335–342.

Zigmond, M. (1994). Chemical transmission in the brain: Homeostatic regulation and its functional implications homeostasis of neuronal function. *Prog. Brain Res.* **100**, 115–122.

Zigmond, M. (1997). Do compensatory processes underlie the preclinical phase of neurodegenerative disease? Insights from an animal model of parkinsonism. *Neurobiol. Dis.* **4**, 247–253.

Zigmond, M. J., Berger, T. W., Grace, A. A., and Stricker, E. M. (1989). Compensatory responses to nigrostriatal bundle injury. Studies WITH 6-hydroxydopamine in an animal model of parkinsonism. *Mol. Chem. Neuropathol.* **10**, 185–200.

Zigmond, M., Castro, S., Keefe, K., Abercrombie, E., and Sved, A. (1998). Role of excitatory amino acids in the regulation of dopamine synthesis and release in the neostriatum. *Amino Acids.* **14**, 57–62.

Zigmond, M. J., Acheson, A. L., Stachowiak, M. K., and Stricker, E. M. (1984). Neurochemical compensation after nigrostriatal bundle injury in an animal model of preclinical parkinsonism. *Arch. Neurol.* **41**, 856–861.