Paraquat Exposure Increases Oxidative Stress Within the Dorsal Striatum of Male Mice With a Genetic Deficiency in One-carbon Metabolism

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ABSTRACT

Paraquat is an herbicide that is commonly used worldwide. Exposure to paraquat results in Parkinson's disease (PD)-like symptoms including dopaminergic cell loss. Nutrition has also been linked in the pathogenesis of PD, such as reduced levels of folic acid, a B-vitamin, and component of one-carbon metabolism. Within one-carbon metabolism, methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. A polymorphism in MTHFR (677 C→T) has been reported in 5%–15% of North American and European human populations. The MTHFR polymorphism is also prevalent in PD patients. The goal of this study was to investigate the impact of paraquat-induced PD-like pathology in the context of reduced levels of MTHFR. Three-month-old male *Mthfr*^{+/-} mice, which model the MTHFR polymorphism observed in humans, were administered intraperitoneal injections of paraquat (10 mg/kg) or saline 6 times over 3 weeks. At the end of paraquat treatment, motor and memory function were assessed followed by collection of brain tissue for biochemical analysis. *Mthfr*^{+/-} mice treated with paraquat showed impaired motor function. There was increased microglial activation within the substantia nigra (SN) of *Mthfr*^{+/-} mice treated with paraquat. Additionally, all *Mthfr*^{+/-} mice that were treated with paraquat showed increased oxidative stress within the dorsal striatum, but not the SN. The present results show that paraquat exposure increases PD-like pathology in mice deficient in one-carbon metabolism.

Key words: paraquat; one-carbon metabolism; methyltetrahydrofolate reductase; Parkinson's disease; striatum; oxidative stress.

Paraquat, first marketed as an herbicide in the 1960s, remains in widespread use worldwide for weed control in a range of crops, including soybeans, tea, sugarcane, cotton, and many varieties of fruit (Bromilow, 2004; Vaccari et al., 2017). Although it has been banned in 32 countries and faces substantial restrictions in others, paraquat remains the herbicide of choice in many regions around the world. Indeed, epidemiological data have shown that exposure to paraquat has been linked to an increase in Parkinson's disease (PD) incidence worldwide in humans (Costello et al., 2009; Liou et al., 1997; Tanner et al., 2011). PD is characterized by a progressive loss of dopaminergic

(DA) neurons within the substantia nigra (SN) pars compacta (SNc) (Baba et al., 1998; Spillantini et al., 1998), resulting in deficits in motor functioning (Parkinson, 2002). A case-control study in California found an increased risk of developing PD for those exposed within 500 meters of the home to both paraquat and the fungicide maneb (OR = 1.75, 95% CI: 1.13–2.73) (Costello et al., 2009). Exposure at an earlier timepoints such as, childhood, adolescence, or young adulthood of individuals versus, to paraquat alone also increased risk of PD (OR = 2.27, 95% CI: 0.91–5.70). In a case-control study in Taiwan, the use of paraquat suggested an increased risk for the development of PD (OR

= 3.22; 95% CI: 2.41–4.31) (Liou *et al.*, 1997). This relationship also followed a dose-response relationship, with those who used paraquat for more than 20 years showing the greatest risk (OR = 6.44; 95% CI: 2.41–17.20).

In the brain, paraquat has been reported to cause damage through oxidative stress (Shimizu *et al.*, 2003) and increased inflammatory processes (Hirsch *et al.*, 2012). In animal models, paraquat permeates through the blood brain barrier and is found in subcortical areas, such as the SNc (Peng *et al.*, 2007). Paraquat also replicates key pathological features of the PD, including degeneration of DA neurons (Brooks *et al.*, 1999; Choi *et al.*, 2010; Jiao *et al.*, 2012; Mangano and Hayley, 2009; Mangano *et al.*, 2011, 2012; McCormack *et al.*, 2002) and Lewy body formation (Dauer and Przedborski, 2003).

Emerging research suggests that the lack of nutrients, such as folic acid, may contribute to the development of PD (de Lau *et al.*, 2006; dos Santos *et al.*, 2009; Seidl *et al.*, 2014; Shen, 2015). Folic acid is a component of one-carbon metabolism and a B-vitamin that has been well characterized with protective effects against neural tube defects (Botto *et al.*, 1999). Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme that participates in one-carbon metabolism. In all tissues including the brain, MTHFR catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methylTHF). The methyl group from 5-methylTHF is a substrate in the vitamin-B₁₂-dependent methylation of homocysteine to methionine. A common polymorphism in MTHFR (677C→T) has been described with a prevalence 5%–15% of North American and European populations (Frosst *et al.*, 1995; Schneider *et al.*, 1998). Interestingly, this polymorphism occurs at a greater frequency in PD patients compared with the general population (Gorgone *et al.*, 2012; Wu *et al.*, 2013; Yuan *et al.*, 2009; Zhu *et al.*, 2015); however, the mechanisms through which this occurs are not well characterized.

Studies using cell culture and rodent models have demonstrated a potential role for folic acid deficiency in neurodegeneration (Ho *et al.*, 2003) and development of PD (Duan *et al.*, 2002; Lee *et al.*, 2005). Hence, a genetic deficiency in one-carbon metabolism, caused by a polymorphism in the MTHFR enzyme may lead to exacerbated degeneration after paraquat exposure. Heterozygous (*Mthfr*^{+/-}) mice mimic individuals with the MTHFR 677TT genotype, with both mice and humans exhibiting reduced MTHFR enzymatic activity (Chen *et al.*, 2001; Frosst *et al.*, 1995). Therefore, the aim of this study was to investigate the impact of reduced MTHFR levels on neurodegeneration and oxidative stress, using the paraquat an environmental toxin mouse model of PD. This study will provide important insight on nutrigenetic interactions with environmental toxicant relevant for PD.

MATERIALS AND METHODS

Animals

All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care (CCAC). Two cohorts of male C57Bl/6 wild-type (*Mthfr*^{+/+}, *n* = 32) and heterozygous *Mthfr* heterozygote (*Mthfr*^{+/-}, *n* = 34) mice were housed in standard caging conditions with *ad libitum* standard mouse chow (Envigo) and water for duration of experiment. Animals were randomly assigned to treatment groups. Male mice were used because female animals have previously been reported to have less DA cell death and motor dysfunction induced by paraquat (Litteljohn *et al.*, 2011).

Experimental manipulations are outlined in Figure 1A. Briefly, 3-month-old mice received intraperitoneal (i.p.) injections of saline or paraquat (10 mg/kg) twice a week for 3 weeks for a total of 6 injections, as previously described (Mangano and Hayley 2009). Sickness behavior of mice was assessed prior to every paraquat injection using a 4-point scale (Sudom *et al.*, 2004). Briefly, a score of 1 = normal; 2 = slight signs of illness; 3 = obvious illness; and 4 = very sick animal. After the completion of 6 paraquat injections over 3 weeks, motor and memory function were assessed.

Once animals completed behavioral testing, they were euthanized with Euthasol and perfused; brain tissue was collected for immunohistochemistry analysis. Frozen brains were sectioned coronally at 40-μm thickness using a cryostat (ThermoScientific) for immunohistochemistry. Every third SNc section was collected for free-floating staining immunohistochemistry in a well plate and was stored in 0.01 M phosphate buffered saline with 0.05% sodium azide at 4°C. The remaining sections mounted on slides for immunofluorescence analysis and were stored at -20°C for immunofluorescence analysis.

The second cohort of mice was decapitated to obtain fresh brain tissue collection for Western blot analysis. Sequential coronal slices were collected using razor blades and a chilled stainless-steel microdissecting block with adjacent slots arranged 1.0 mm apart. As previously described SN and dorsal striatal nuclei were collected by micropunch (Mangano *et al.*, 2011). Tissue samples were stored at -80°C until protein isolation and Western blot experiments.

Behavioral Tests

Rotarod. To assess coordination and balance function, animals were assessed using a standard accelerated rotarod (Omnitech Electronic Inc.). The rotarod consisted of a metal cylinder 3 cm in diameter and 6-cm wide, positioned at 30 cm above the ground using a Plexiglass container. Animals were tested as the rotarod accelerated (4–60 rpm) over three 8 min trials in a single day with an intertrial interval of 5 min and latency to fall was recorded (Jadavji *et al.*, 2017). The average latency to fall from all 3 trials was used for analysis.

Ladder beam. Skilled motor function was also assessed using the ladder beam. The apparatus consisting of 2 plexiglass walls and removable metal rungs was placed on top of 2 standard mouse housing cages (Farr *et al.*, 2006). The passage was large enough for a mouse to proceed forward but too narrow to allow it to turn around. Trials were recorded such that all 4 limbs could be viewed simultaneously. Recordings were analyzed frame by frame, with each step scored on quality of limb placement using a 7-point scoring scale as described previously (Farr *et al.*, 2006). The number of errors in each session was recorded for analysis of foot placement accuracy. An error was defined as a movement score for 0, 1, or 2 as previously described (Farr *et al.*, 2006). An error score was calculated based on the total number of errors and number of steps for each limb.

Spontaneous alternation y-maze. Short-term spatial memory was assessed using the spontaneous alternation y-maze. The maze was made of gray plastic fiber. Each mouse, naive to the maze, was placed at the end of 1 arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded. Arm entry was considered to be completed when the hind paws of the mouse had been completely placed in the arm; the number of entries per arm was recorded (Sarter *et al.*, 1988). Alternation was defined as successive

Figure 1. Impact of reduced MTHFR on paraquat mouse model. **A**, timeline of experimental manipulations. At 3-months of age *Mthfr*^{+/+} and *Mthfr*^{+/-} mice were administered 6 intraperitoneal injections of paraquat (10 mg/kg) or sterile saline. Animals were injected twice a week on Monday and Friday over 3 weeks. After which animals were tested on rotarod, ladder beam, and, spontaneous alternations, and 2-trial y-maze tasks. These tests were conducted over one week. At the completion of behavioral testing brain tissue and blood were collected from animals. **B**, survival curve of *Mthfr*^{+/+} and *Mthfr*^{+/-} mice receiving 3 injections of paraquat per week over 2 weeks from initial experiments. **C**, sickness scale scores of *Mthfr*^{+/+} and *Mthfr*^{+/-} mice after 6 injections (2 times/week) of paraquat over 3 weeks. ***p* < .01, Tukey's pairwise comparison between *Mthfr*^{+/-} saline and paraquat mice.

entries into all 3 arms, on overlapping triplet sets. The percentage of alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arms entered minus 2), multiplied by 100 (Maurice et al., 1994).

Two-trial y-maze. To assess short-term memory the 2-trial y-maze was used (Dellu et al., 2000). In the first trial, 1 arm was blocked, allowing the mouse to explore only 2 arms of the maze for 5 min. In the second trial, after a 60-min intertrial interval, the block to the third arm was removed and the mouse was able to explore all 3 arms for a period of 5 min. The amount of time spent in the novel arm was calculated within the first minute.

Tyrosine Hydroxylase Analysis

Substantia nigra pars compacta. For immunohistochemistry analysis of tyrosine hydroxylase (TH) free-floating sections of SNc were used as previously described (Mangano et al., 2011). Briefly, tissue was incubated in mouse TH primary antibody (1:2000; ImmunoStar) at room temperature with antibody overnight. The following day, tissue was incubated with the secondary antibody, antimouse sheep horseradish peroxidase (1:200; Sigma-Aldrich). TH staining in tissue was visualized with diaminobenzidine (Sigma).

Numbers of DA cells were quantified as previously described (Mangano et al., 2011). Briefly, the SNc was outlined under 2.5× magnification. TH positive (TH⁺) neurons were counted using 60× oil immersion objective from multiple Bregma levels (−2.70 to −3.80) for each animal (Bobyne et al., 2012). The SNc was sampled in a systematic random fashion according to the optical fractionator method outlined by MicroBrightField Inc. Cells were quantified in 3D counting frames using a counting grid size of 90 × 90 μm in a counting frame size of 60 × 60 μm with a 15-μm dissector height and 3-μm upper and lower guard zones. The TH⁺ cells were included if their nuclei were clearly discernable and had no obvious damage within the SNc. We analyzed

stained brain tissue from 6 to 7 animals per group. Sections from each Bregma level were then compared across treatment groups to determine if any differences existed due to genotype or injection type. The number of TH⁺ cells was averaged across sections to obtain one value per animal.

Striatum. Immunofluorescence analysis of TH fibers in the striatum was previously described (Ojha et al., 2015). Briefly, striatal containing tissue was incubated with TH primary antibody (1:100; ImmunoStar) over night at 4°C in 0.5% Triton-X. Primary antibody staining was visualized with antimouse Alexa 555 (Cell Signalling).

Optical density of TH fibers within the striatum from both hemispheres was measured using ImageJ (NIH) as previously described (Ojha et al., 2015). A minimum of 2 sections were measured per animal, each hemisphere was analyzed, and an average was generated for each animal.

Immunofluorescence Staining

Primary antibodies for antirabbit ionized calcium-binding adapter molecule 1 (Iba1) (1:100, AbCam) and antirat cluster of differentiation 68 (CD68) (1:500, BioRad), markers of microglial activation, were diluted in 0.5% Triton-X and used to assess microglial activity. Primary antibody staining was visualized using antirat Alexa Fluor 594 and antirabbit Alex Fluor 488 (Cell Signalling). Sections were then incubated with 4', 6-diamidino-2-phenylindole (1:10 000) to stain for nuclei. Staining was visualized at 20× magnification from multiple Bregma levels (−2.70 to −3.80). Microglial activation was determined by counting the number of cells when Iba1 was colocalized with CD68 using the open-source software package Fiji (Schindelin et al., 2012). Cells demonstrating visual evidence of both antibodies were counted as positive cells. Both hemispheres of 2–3 brain sections were analyzed per animal. Average numbers of positive cells were then determined for each animal.

Western blot

The dorsal striatum (approximately 20mg) or SN (approximately 10mg) was used to extract protein in RIPA buffer containing phosphatase and proteinase inhibitors (Cell Signalling). Proteins were separated by SDS-PAGE and transferred to nitrocellulose membranes. Primary antibodies used include antirabbit superoxide dismutase 2 (SOD2) (1:500; Life Technologies), antirabbit Gp91^{phox}(Gp91) (1:5000; AbCam), and antirabbit glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:10 000; Cell Signalling) overnight. Primary antibodies were visualized with infrared dye goat antirabbit IgG (1:10 000, 800 Channel; LiCor Biosciences). Imaging was performed using Near Infrared detection (Li-Cor Biosciences). Quantification of bands was determined using densitometry via ImageJ (National Institutes of Health) and normalized to GAPDH.

Statistical Analysis

All data analysis was carried out by 2 investigators blinded to experimental groups. Statistical analysis was completed using GraphPad Prism (version 6.0). The Gehan-Breslow-Wilcoxon test was used to compare survival curves of *Mthfr*^{+/+} and *Mthfr*^{+/-}. Two-way analysis of variance was used to compare genotype (*Mthfr*^{+/+} and *Mthfr*^{+/-}) and treatment (saline and paraquat) groups. Significant findings were followed up using Tukey's pairwise comparisons. In all analyses, a *p*-value of $\leq .05$ was considered significant. Data are presented using mean $\pm$ SEM.

RESULTS

The Impact of Paraquat on *Mthfr*^{+/-} Mice

Initially we started with a 3 injection of paraquat per week as done previously (Mangano and Hayley, 2009), however, the mortality rate of *Mthfr*^{+/-} mice was enhanced by week 2 of this administration regimen (Figure 1B, $p > .05$). Since the viability of *Mthfr*^{+/-} mice was negatively impacted by the 3 injections per week, we revised our experimental design and reduced the number of injections to 2 times per week for 3 weeks as outlined in the experimental timeline in Figure 1A. We observed that there was a treatment difference in the sickness score after 6 injections of paraquat (Figure 1C; $F_{[1, 60]} = 10.34$, $p < .01$). *Mthfr*^{+/-} mice had mildly increased sickness score ($p < .01$) compared with *Mthfr*^{+/-} saline animals. There was no difference in body weight between groups during and after paraquat administration (data not shown).

Impaired Coordination in Paraquat Treated *Mthfr*^{+/-} Mice

After the sixth paraquat injection, we tested animals on the accelerating rotarod. We averaged 3 trials and observed a treatment effect (Figure 2A; $F_{[1, 26]} = 6.62$, $p < .05$). *Mthfr*^{+/-} mice treated with paraquat fell off the accelerating rotarod sooner than *Mthfr*^{+/+} saline mice ($p < .05$). We also observed a trend for increased number of errors in *Mthfr*^{+/-} mice treated with paraquat while crossing the ladder beam (Figure 2B; $F_{[1, 20]} = 3.68$, $p = .06$).

Short-Term Spatial Memory

There was no treatment effect on the number of alternations or entries made using the spontaneous y-maze (data not shown). However, using the 2-trial y-maze, we observed a nonsignificant trend for *Mthfr*^{+/-} mice to spend less time in the novel arm of the y-maze (Figure 2C, genotype effect $F_{[1, 19]} = 3.16$, $p = .09$).

Figure 2. Behavioral analysis in *Mthfr*^{+/+} and *Mthfr*^{+/-} mice treated with paraquat. A, time to fall off accelerating rotarod. B, the percent errors made while crossing the ladder beam. C, time spent in the novel arm of the y-maze. Bars represent mean $\pm$ SEM of 8 animals per group. * $p < .05$, Tukey's pairwise comparison between *Mthfr*^{+/-} paraquat and *Mthfr*^{+/+} saline animals.

DA Cell Body Loss Induced by Paraquat Is Not Exacerbated by Reduced Levels of MTHFR

To assess DA degeneration TH⁺ cells were counted representative images of staining are shown in Figure 3A. Within the SNC there was no difference between groups in the number of TH⁺ cells (Figure 3B, $p > .05$). Additionally, we did not observe any differences between groups when TH fibers were assessed in the striatum between groups (Figure 3C, $p > .05$).

Increased Neuroinflammation Within the SN of Paraquat Mice With Reduced Levels of MTHFR

Given that neuroinflammation is an important component of PD pathogenesis (Hirsch et al., 2012; Lecours et al., 2018); we assessed microglial activation using the markers, Iba1, and CD68. Consistent with an enhanced activation state of

Figure 3. TH quantification of DA neurons in *Mthfr*^{+/+} and *Mthfr*^{+/-} mice treated with paraquat. A, representative images of TH-positive cells within the SNc. B, quantification of TH cells within the SNc and C, TH fibers within the striatum. Bars represent mean $\pm$ SEM of 7 to 8 animals per group.

Figure 4. Neuroinflammation in *Mthfr*^{+/+} and *Mthfr*^{+/-} mice treated with paraquat. A, Representative images of Iba1 colocalized with CD68 and quantification with the SN, and striatum. B, Quantification of co-localized Iba1 and CD68 in SN, C and striatum. D, Bars represent mean $\pm$ SEM of 4–6 animals per group. ** $p < .01$, Tukey's pairwise comparison between *Mthfr*^{+/+} paraquat and *Mthfr*^{+/+} or *Mthfr*^{+/-} saline animals.

microglia, the representative images shown in Figure 4A clearly indicate increased levels of both Iba1 and CD68 within the SN (Figure 4C; $F_{[1, 10]} = 7.76$, $p < .05$). Specifically, *Mthfr*^{+/-} mice treated with paraquat had more neuroinflammatory markers colocalized within the SN compared with *Mthfr*^{+/+} (Figure 4C, $p < .01$) and *Mthfr*^{+/-} ($p < .01$) treated with saline. There was no difference observed in these microglial markers in the striatum (representative images Figure 4B and quantification Figure 4D, $p > .05$).

Markers of Oxidative Stress Increased in the Dorsal Striatum of *Mthfr*^{+/-} Mice after Paraquat Administration

Paraquat (Jenner, 2003) has been reported to increase levels of oxidative stress in the brain. In the present study, we measured protein levels of Gp91, the catalytic subunit of NADPH oxidase. In the SN we observed no changes between groups (Figure 5A, $p > .05$), but there was a change within the dorsal striatum (Figure 5B, $F_{[1, 12]} = 5.89$, $p < .05$). Specifically, *Mthfr*^{+/-} mice treated with paraquat had increased levels compared with *Mthfr*^{+/+} saline treated animals. To confirm the Gp91 results, we also measured SOD2, an enzyme involved in scavenging reactive oxygen species (Barone et al., 2015). There were again no changes within the SN (Figure 5C, $p > .05$). But within the dorsal striatum SOD2 was increased as a result of genotype (Figure 5D,

$F_{[1, 14]} = 6.71$, $p < .05$). *Mthfr*^{+/-} mice treated with paraquat had increased levels when compared with *Mthfr*^{+/+} saline animals ($p < .05$).

DISCUSSION

Substantial evidence indicates that paraquat can trigger the degeneration of DA neurons and manifestation of PD-like pathology (Brooks et al., 1999; Choi et al., 2010; Jiao et al., 2012; Mangano and Hayley, 2009; Mangano et al., 2011, 2012; McCormack et al., 2002), but the role of one-carbon metabolism has yet to be assessed. MTHFR is an enzyme that participates in one-carbon metabolism. The polymorphism in MTHFR (677C $\rightarrow$ T) has been observed at greater frequencies in PD patients than the general population in some studies (De Lau et al., 2005; Fong et al., 2011; Gorgone et al., 2012). With an increase in the global aging population, and predicted increases in PD (Bach et al., 2011), there is a critical need to understand how a MTHFR-deficiency affects PD pathology. Using an environmental toxicant-induced model of PD and a mouse model that mimics the MTHFR polymorphism (Chen et al., 2001), our study has demonstrated that a MTHFR-deficiency does in fact make the brain, specifically the dorsal striatum, more vulnerable to paraquat-induced damage through inflammatory and oxidative stress. Previous work has

Figure 5. Oxidative stress response after exposure to paraquat in the brain tissue of *Mthfr*^{+/+} and *Mthfr*^{+/-} mice. Gp91^{phox}(Gp91) levels within the SN (A), and dorsal striatum (B), SOD2 levels within SN (C), and dorsal striatum, (D) bars represent mean $\pm$ SEM of 5–6 animals per group. The panels below the graphs depict a representative Western blot. **p* < .05 Tukey's pairwise comparison between *Mthfr*^{+/-} paraquat and *Mthfr*^{+/+} saline animals.

reported that the negative effects of reduced MTHFR levels can be diminished through adequate folic acid levels (Anderson et al., 2013; Ashfield-Watt et al., 2002). In our study, *Mthfr*^{+/-} mice were maintained on a standard mouse chow diet that provided them with enough folic acid and other B-vitamins, yet we still observed functional and biochemical changes suggesting that there is an interaction between MTHFR and paraquat. In the SN, the *Mthfr*^{+/-} paraquat treated mice also displayed increased microglial CD68 and Iba-1 staining, indicative of a reactive state. Curiously however, we did not observe any DA cell loss within the SNc within the timeframe of our study.

The absence of dopamine degeneration assessed by TH staining in this study could be a result of the reduced schedule of paraquat administration, owing to enhanced peripheral toxicity of the MTHFR heterozygote mice. Previous studies using paraquat animals that show DA cell loss have generally used 9 injections of paraquat over 3 weeks (Kang et al., 2010; Littelljohn et al., 2009; Mangano and Hayley, 2009; McCormack et al., 2002). In this study, mice were administered 6 injections over the 3 weeks. Conceivably, the reduced schedule might be sufficient to induce pathology but not severe enough to reach a threshold required to cause DA degeneration (Littelljohn et al., 2009; McCormack et al., 2002). In fact, the lesion caused by paraquat alone is typical rather modest (approximately 30% loss of SN neurons). Often paraquat is combined with another insults, such as lipopolysaccharide to maximize neuronal degeneration

(Mangano and Hayley, 2009). That said, we hypothesized that *Mthfr*^{+/-} mice would be more impacted by the paraquat. Indeed, we observed increased vulnerability for oxidative stress and microglial markers, but not so with regards to actual neuronal loss.

It is important to note that the SN of *Mthfr*^{+/-} mice administered paraquat was not completely spared from damage. Inflammation is a component of PD (Hirsch et al., 2012; Lecours et al., 2018) and activated microglia are present within the SN of PD patients (Block et al., 2007). When paraquat is administered to animals increased inflammation has also been reported (Mangano and Hayley, 2009; Mangano et al., 2012). In our study, within the SN of *Mthfr*^{+/-} mice treated with paraquat, we observed increased levels of microglial activation. Indeed, paraquat administration did affect the SN in the *Mthfr*^{+/-} mice in terms of increased microglial activation. The increased microglial activation within the SN may be a precursor to DA degeneration, as well as the motor impairments observed in *Mthfr*^{+/-} mice administered paraquat (Block et al., 2007; Purisai et al., 2007; Wu et al., 2005). Interestingly, astrocyte activation may also contribute to the inflammatory process within the SN (Niranjan, 2014).

Consistent with clinical PD cases (Kish et al., 1988; Tatsch et al., 1997), we found that the dorsal striatum was especially impacted in *Mthfr*^{+/-} mice treated with paraquat. In this study, we show consistent data that the dorsal striatum is negatively

impacted in *Mthfr*^{+/-} mice treated with paraquat. The increased oxidative stress may lead to the impairments in balance, coordination, and skilled motor function since the dorsal striatum is involved in these motor behaviors (DeLong et al., 1986). Paraquat increases oxidative stress (Shimizu et al., 2003) and in our study higher levels of oxidative stress were localized to the dorsal striatum and not the SN. In human PD, some studies have reported that the dopamine neuronal damage begins within dorsal striatum prior to the SNc (Fearnley and Lees, 1991). According to our data, a combination of a genetic deficiency in one-carbon metabolism and paraquat damages the dorsal striatum in early stages of the disease. Over time the damage to the dorsal striatum results in the fiber denervation that may eventually culminates in DA loss within the SNc. In the cell, MTHFR generates methyl groups through S-adenosylmethionine. Previous work has shown that the striatum is implicated in methylation changes in response to Levodopa, a common treatment for PD (Miller et al., 1997). It could be proposed that changes in one-carbon metabolism may be targeting the striatum that then results in PD pathology.

Much attention has focused upon how alteration in metabolism is linked to PD pathology. Mitochondrial dysfunction in particular, along with increased oxidative stress, and altered redox metabolism have all been reported to be involved in PD (Anandhan et al., 2017). Other toxicants, such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) results in PD-like pathology and serve as models to study disease pathology. In addition, the MPTP model also promotes changes in metabolism (Campello et al., 2013). To our knowledge, however, this is the first study investigating the how a toxin interacts with MTHFR-deficiency. The data presented in this study have facilitated further insight into how deficiencies in one-carbon metabolism affect vulnerability to PD. Based on these findings, reducing oxidative stress within the brain may be future interventions that could reduce the severity of PD-related pathology.

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DECLARATION OF CONFLICTING INTERESTS

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