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The effects of paraquat on regional brain neurotransmitter activity, hippocampal BDNF and behavioural function in female mice

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ABSTRACT

Accumulating evidence implicates pesticides such as paraquat in the development of Parkinson's disease (PD). Indeed, paraquat exposure is associated with an increased risk of PD and when administered to rodents the pesticide recapitulates many of the neuropathological and behavioural features of the disease. However, it is unclear whether any sexual dimorphism exists in the in vivo murine response to paraquat intoxication, since most studies have used exclusively males. Accordingly, we sought to determine the impact of repeated paraquat exposure on a range of neural and behavioural outcomes in female C57BL/6J mice. The present investigation revealed that the female mice were largely resistant to the paraquat-induced nigrostriatal dopamine changes and locomotor deficits that were reported previously in males. Similarly, in contrast to the reductions of hippocamapal brain-derived neurotrophic factor (BDNF) previously reported in paraquat treated male mice, the herbicide actually increased levels of the trophic factor in females. Yet, similar to our previous findings in males, paraquat increased norepinephrine utilization within the hippocampus and prefrontal cortex of the female mice. However, these changes did not translate into anxiety- or- depression-like behaviours in the open field test, as the females actually seemed to show enhanced exploration. Consistent with reports of a greater incidence of PD in males, these data suggest that female mice may be less susceptible than males to the nigrostriatal dopaminergic and motor effects of environmental toxins. The augmented hippocampal BDNF and noradrenergic changes observed could conceivably act to buffer female mice against some of the deleterious behavioural effects of parquat.

Parkinson's disease is a chronic, progressive disorder tied to the degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc) and the consequent loss of DA in the striatum. Dysregulation of the nigrostriatal DA system is primarily responsible for the hallmark motor symptoms of PD, including bradykinesia and cogwheel rigidity; however, the co-morbid psychological pathology experienced by many PD patients (e.g., anxiety and depression) is likely attributable to multi-neurotransmitter dysfunction in various emotional and cognitive brain regions, including the hippocampus, locus coeruleus and prefrontal cortex [16]. Although genetic vulnerability likely plays a role in disease provocation, mounting evidence implicates environmental contaminants, particularly pesticides such as rotenone and paraquat, as probable etiological factors in PD. Indeed, epidemiological studies reported an association between rural/occupational exposure to the commonly used herbicide, paraquat, and a heightened risk of developing PD [7,14]. Similarly, in rodents paraquat provoked nigrostriatal dopaminergic dysfunction (e.g., SNc DA neuron loss, changes in striatal DA) coupled with a neurobehavioural syndrome and neuroinflammatory/oxidative changes reminiscent of PD [4,5,16]. Recent studies have also suggested that reductions in trophic support (e.g., brain-derived neurotrophic factor (BDNF)) may facilitate the degenerative process of PD and its paraquat animal model [17,18].

While PD affects members of both sexes, incidence rates of the disease are substantially higher among males (~3:2, relative to females) [24]. Although it is uncertain precisely what accounts for this discrepancy, evidence suggests that female gonadal steroids, particularly estrogens, have potent anti-inflammatory and antioxidative CNS effects that are not necessarily mimicked by male sex hormones [3]. Also, it appears that PD risk is heightened in conditions causing or characterized by a premature reduction in endogenous estrogen (e.g., hysterectomy, oophorectomy, early menopause) [21] and that estrogen replacement therapy

Abbreviations: 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element binding protein; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HPLC, high performance liquid chromatography; MHPG, 3-methoxy-4-hydroexyphenylglycol; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NE, norepinephrine; PFC, prefrontal cortex; SNc, substantia nigra pars compacta; TH, tyrosine hydroxlase.

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may be effective in reducing parkinsonian symptoms among postmenopausal women (although conflicting data exist) [22]. It is therefore conceivable that differences in gonadal steroid hormones (and the anti-oxidative and anti-inflammatory responses they mediate) may be a key determinant of sexual dimorphism in PD.

Previous studies revealed that female rodents are less susceptible than males to the neurodegenerative and behavioural effects of the DA-targeting toxins, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) [9,19]. However, whether mice exhibit sexually dimorphic responses to the ecologically relevant toxin, paraquat remains unclear. In fact, surprisingly little is known concerning the neural and behavioural phenotypes of paraquat intoxication in female mice. Given the aforementioned link between pesticides and PD, such information could be relevant to our understanding of gender differences in PD. Accordingly, we assessed the impact of paraquat on a range of neural (central monoamine activity, striatal tyrosine hydroxlase (TH), hippocampal BDNF) and behavioural outcomes (locomotor activity, exploratory behaviour) in female mice.

Female C57/BL6 mice (random, undetermined estrous cycle stage), aged 8–10 weeks, were obtained from our in-house breeding colony (original breeders were purchased from The Jackson Laboratory, Bar Harbor, ME) and singly housed in standard (27 cm × 21 cm × 14 cm) polypropylene cages. The mice were maintained on a 12-h light/dark cycle with lights on at 0700 h. A diet of Ralston Purina (St. Louis, MO) mouse chow and water was provided *ad libitum*, and room temperature was maintained at approximately 21 °C. All experimental test paradigms were approved by the Carleton University Committee for Animal Care and adhered to guidelines set out by the Canadian Council for the Use and Care of Animals in Research.

Animals received intraperitoneal (i.p.) treatment with paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride; Sigma–Aldrich, USA), at a dose of 10 mg/kg, or an equivalent volume of saline (Sigma–Aldrich). Injections were administered 3 times a week for 3 consecutive weeks; during which time repeated assessments of home-cage locomotor activity (1 d-post 3rd, 6th and 9th paraquat/saline injection) and open-field exploration (1 d-post 2nd and 8th injection) were made among a subset of the mice (n = 8–10), as described previously [15]. Notably, in male mice, an identical paraquat dose regimen has been associated with a moderate but statistically significant loss of SNc DA neurons [18] and changes in nigrostriatal and limbic neurotransmission, together with several motor and co-morbid psychological symptoms [15]. Two hours following their final treatment injection, animals were rapidly decapitated and relevant brain regions collected by micropunch dissection for later processing by HPLC or western immunoblot. All experimental manipulations were conducted between 0800 and 1200 h to minimize variability owing to diurnal variations.

Following the method of Litteljohn et al. [15], HPLC (n = 8–10) was used to determine levels of DA and its primary metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in SNc, striatal and prefrontal cortex (PFC) tissue punches. Likewise, levels of norepinephrine (NE) and serotonin (5-HT), and their respective metabolites, 3-methoxy-4-hydroexyphenylglycol (MHPG) and 5-hydroxyindole acetic acid (5-HIAA), were assessed in PFC and hippocampal samples.

Striatal tyrosine hydroxylase (TH) content, as well as hippocampal and PFC protein expression of brain-derived neurotrophic factor (BDNF) and phosphorylated cAMP response element binding protein (p-CREB) was quantified by western immunoblot (n = 3 for p-CREB and TH and n = 7 for BDNF; each sample lane represented a pool of 2 mice; thus, a total of 6–14 mice were used for the samples run in the blots). The immunoblotting procedure used in the present study has recently been described elsewhere [17]. Primary antibodies were applied for 1.5-h at room temperature and include: anti-TH (1:1000, 22941, Immunostar), anti-BDNF (1:500, sc-546, Santa Cruz), and anti-p-CREB (1:500, sc-101663, Santa Cruz). Band density was quantified using AlphaEaseFC v.3.1.2 densitometry software (Alpha Innotech) and normalized against β-actin. These values were then averaged and the standard error of the mean determined for each treatment group.

All data were analysed by two-tailed Student's t test and expressed as mean ± SEM; the level of significance was set at $p < 0.05$.

As shown in Fig. 1, paraquat provoked very few changes in nigrostriatal DA neurotransmission and motor functioning in the female mice. Specifically, DA levels within both the SNc (Fig. 1A) and striatum (Fig. 1C) were unaffected by the paraquat treatment. Although levels of the primary DA metabolite, DOPAC, were significantly reduced within the SNc of paraquat-treated females relative to saline-treated controls (t = 3.00, df = 17, $p < 0.01$) (Fig. 1B), striatal DOPAC concentration remained unchanged (Fig. 1D). Similarly, western immunoblot analysis failed to detect a significant difference in striatal TH protein expression between paraquat-treated and saline-injected mice (Fig. 1E). Consistent with these findings, the pesticide had no effect on home-cage locomotor activity in the female mice at any of the time points assayed (Fig. 1F).

Similar to what we previously observed in male mice [15], paraquat influenced noradrenergic transmission within the hippocampus and PFC. In this regard, while the pesticide did not significantly alter levels of NE in the hippocampus or PFC among female mice (Fig. 2A and C), accumulation of MHPG was enhanced within both of these brain regions (ts = 6.03 and 6.60, respectively, df = 17, $p < 0.001$) (Fig. 2B and D). In contrast, levels of 5-HT and its metabolite, 5-HIAA, were not affected by paraquat in either of these limbic brain regions; and, likewise, the pesticide had no influence on DA within the PFC (Table 1). Additionally, female mice treated with paraquat appeared to exhibit increased exploration of a novel open field arena, although the results just missed statistical significance. As shown in Fig. 2E, the frequency of entry into the centre zone of the arena trended towards being higher among paraquat-treated females, relative to saline-treated control animals (t = 2.02, df = 19, p = 0.057).

Alterations of neuroplasticity within the hippocampus and PFC have been implicated in a range of neuropsychiatric conditions, including anxiety and depression [1]; and such co-morbid psychological pathology is fairly common in PD, particularly among female patients [20]. Thus, it was of interest to determine the female-specific effects of paraquat on hippocampal and PFC protein expression of the pro-neuroplastic trophic factor, BDNF, as well as its main transcription factor, CREB. As shown in Fig. 3, paraquat significantly increased BDNF levels within the hippocampus of the female mice (t = 2.70, df = 12, $p < 0.05$). There was no significant difference, however, in the immunoblot detected levels of hippocampal CREB between the treatment groups, and western blotting likewise failed to detect changes in the PFC expression patterns of either of the proteins assayed ($p > 0.30$, data not shown).

Though not without exception, previous studies have demonstrated that chronic, systemic exposure of male mice to paraquat in moderate doses is capable of reproducing a PD-like neurobehavioural syndrome coupled with nigrostriatal dopaminergic pathology and altered neurochemical activity in brainstem (locus coeruleus) and limbic regions (hippocampus, PFC) [16]. However, because most in vivo paraquat studies have used exclusively males, the question remains whether mice exhibit sexually dimorphic responses to paraquat in a manner similar to what has previously been reported for the more well established but less ecologically valid MPTP and 6-OHDA models of PD [3].

The present investigation revealed that female C57/BL6 mice were resistant to many of the nigrostriatal dopaminergic changes