Sex differences in motor behavior in the MPTP mouse model of Parkinson's disease

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ABSTRACT

Sex differences in Parkinson's disease (PD) have been reported in humans and rodent models, with a higher incidence in men and increased severity in male rodents. The current study examined sex differences and the effects of gonadal steroid hormones in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse model of PD. Male ($n = 51$) and female ($n = 50$) mice were gonadectomized and received physiologic replacement with testosterone or estrogen (Experiment 1), or no hormones (Experiment 2). Two weeks later, mice received either MPTP (10 mg/kg per day for 5 days) or saline. Higher doses killed female mice. Mice were tested one week after MPTP for motor performance using rotarod, pole and gait tests. In hormone-treated mice, males significantly outperformed females in all three tests ($p < 0.05$). Compared with females, males had a greater overall rotarod performance (ORP: 1317.1 ± 98.3 vs. 988.1 ± 95.6), descended a pole faster (7.1 ± 0.6 vs. 9.6 ± 0.7 s), and had longer stride lengths (hindlimb 7.3 ± 0.1 vs. 6.8 ± 0.1 cm). By contrast, ovariectomized female mice receiving saline outperformed castrated males on the rotarod (1296.6 ± 83.3 vs. 811.2 ± 113.7, $p < 0.05$) and descended a pole faster (9.7 ± 2.0 vs. 15.6 ± 1.9 s, $p < 0.05$). MPTP significantly impaired ORP ($p < 0.05$) in hormone-treated males (703.7 ± 65.5) and females (432.8 ± 88.6, $p < 0.05$). After MPTP, stride length was selectively decreased in males (hindlimb 6.6 ± 0.1 cm, $p < 0.05$), and pole test performance was unimpaired in either sex. After gonadectomy, MPTP did not decrease motor performance in males ($p > 0.05$) but significantly reduced ORP in females (975.9 ± 110.3 vs. saline females, $p < 0.05$). Our results show that small, chronic doses of MPTP produce subtle, sexually-dimorphic impairments in motor performance, but without a loss of tyrosine hydroxylase-positive neurons in the substantia nigra. In gonadectomized mice, this sex difference is reversed.

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor impairments that include resting tremor, rigidity, bradykinesia, and postural instability (reviewed in Jankovic, 2008). Sex differences have been reported for PD, and include a one- to two-fold higher incidence in men (reviewed in Shulman and Bhat, 2006). Sex differences are often driven by gonadal steroid hormones, acting both during development and in the adult. With respect to PD, estrogen is thought to exert neuroprotective effects which lessen both the incidence and phenotype in women (Benedetti et al., 2001; Dluzen, 2000; Haaxma et al., 2007; Shulman and Bhat, 2006; Tsang et al., 2001). In the current study, sex differences and the effects of gonadal steroid hormones were investigated using a mouse model of PD.

Rodent models of PD include the 6-hydroxydopamine (6-OHDA)-lesioned rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse (Bove et al., 2005; Jakowec and Petzinger, 2004; Jeon et al., 1995; Schober, 2004). Both models replicate key features of PD by lesioning dopaminergic neurons in the substantia nigra pars compacta (SNC) which project rostrally to the striatum. Thus, after either 6-OHDA or MPTP, striatal dopamine is depleted (Alvarez-Fischer et al., 2008; Jakowec et al., 2004). Furthermore, in both models, dopamine depletion is more severe in males (Miller et al., 1998; Murray et al., 2003; Tamas et al., 2006, Xu et al., 2006). With 6-OHDA, male rats also have an increased loss of dopaminergic neurons in SNC compared to females (Murray et al., 2003; Tamas et al., 2006) and have more severe behavioral deficits post-lesion, including decreased motor activity (Cass et al., 2005; Tamas et al., 2005).

The current study investigated sex differences in motor impairments after MPTP in C57BL/6 mice. 6-OHDA is commonly delivered by intracerebral injection to produce an acute, unilateral depletion of dopaminergic neurons in SNC of rats. In mice, MPTP can be delivered systemically to produce less severe, bilateral lesions to the nigrostriatal dopamine system. We used a subacute dose of MPTP to reveal

the potentially subtle sex differences in motor impairment between male and female mice. The behavioral tests measured distinct aspects of motor performance that parallel the motor deficits seen in human Parkinson's disease.

The goals of the current study were two-fold. Experiment 1 determined whether motor impairments after MPTP are sexually dimorphic. The results indicated that motor behavior was impaired in both male and female mice after MPTP, but that motor deficits were more severe in males. In addition, motor impairments in both sexes occurred without the loss of dopaminergic neurons in SNC. In Experiment 2, we determined whether gonadal hormones in adulthood underlie sex differences in motor impairments after MPTP. This was accomplished by comparing motor performance in gonadectomized male and female mice after treatment with either MPTP or saline.

2. Methods

2.1. Animals

Adult male and female C57BL/6 mice (8–12 weeks) were obtained from Charles River Laboratories (Wilmington, MA). Mice were group-housed on a 12:12 LD photoperiod with access to food and water *ad libitum*. Experimental procedures were approved by USC's Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (DHEW Publication 80–23, revised 1985, Office of Science and Health reports, DRR/NIH, Bethesda, MD 20205).

2.2. Gonadectomy

All mice were gonadectomized under Avertin anesthesia (250 mg/kg). Males were castrated via a mid-line scrotal incision, and females were ovariectomized via bilateral dorsal flank incisions. In Experiment 1, steroids were replaced chronically at physiologic levels by Silastic implant sc (Barkley and goldman, 1977; Jacob et al., 2001). Males received a 5 mm implant (o.d. 2.16 mm, i.d. 1.02 mm, Dow Corning, Midland, MI) filled with crystalline testosterone (Steraloids, Newport, RI). Females received a similar implant of estradiol (1:1 17β-estradiol:cholesterol). Hormone-responsive tissue weights were recorded for males (seminal vesicle: 297.3 ± 9.3 g) and females (uterus: 171.7 ± 4.8 g). These measurements were consistent with physiogic levels of steroids in both sexes (Froment et al., 2004; Grasso et al, 1998). Seminal vesicle weight in male mice was 297.3 ± 9.3 g and uterine weight in females was 171.7 ± 4.8 g. In Experiment 2, gonadectomized male and female mice without steroid replacement received blank implants. Mice were allowed to recover from surgery (up to 2 weeks) before behavioral acclimatization and MPTP treatment. This time-course is sufficient to demonstrate decreases in hormone-driven behaviors (e.g. mating) after gonadectomy (Hull et al., 2006; Pfaff et al., 2006).

2.3. MPTP lesion

Male and female mice differ in their systemic tolerance for MPTP, with females being more severely affected (Przedborski et al., 2001). Thus, pilot studies in gonadectomized, hormone-treated males and females established the appropriate dose and inter-injection interval for MPTP. MPTP (free-base, Sigma, St. Louis, MO) was dissolved in 0.9% saline and injected ip. Male and female mice received either (1) four injections of 20 mg/kg MPTP at 2 h intervals ($n = 15$ males, $n = 25$ females), (2) one injection of 30 mg/kg MPTP ($n = 5$ females), or (3) one injection of 10 mg/kg MPTP daily for 5 days ($n = 15$ males, $n = 15$ females). Males tolerated all doses and inter-injection intervals well. However, 80–90% of females died after MPTP at 4×20 mg/kg or 1×30 mg/kg (see Table 1). With 4×20 mg/kg MPTP, deaths occurred

Table 1
Mortality in male and female mice after MPTP.

MPTP dose	Survival rate (male mice)	Survival rate (female mice)
4×20 mg/kg MPTPq. 2 h	12 of 15 (80%)	3 of 25 (12%)
1×30 mg/kg MPTP daily for 5 days		1 of 5 (20%)
1×10 mg/kg MPTP daily for 5 days	15 of 15 (100%)	13 of 15 (87%)

after the second injection. Therefore, to investigate sex differences in motor and neuromorphologic responses to MPTP, male and female mice receiving 10 mg/kg MPTP per day for 5 days were used for Experiment 1. Control male and female mice ($n = 15$ each) received equivalent injections of 0.1 mL saline. Experiment 2 used the same regimen of MPTP or saline in gonadectomized males and females without hormone replacement ($n = 10$–11/group).

2.4. Behavioral tests

Rotarod, pole, and gait tests were used to measure locomotor ability, bradykinesia, and stride length, respectively. Initially, mice were acclimatized to the testing apparatus 1–2 days before lesion, and were tested for motor performance 1 week following the last injection of MPTP or saline. MPTP-induced loss of SNC dopaminergic neurons is complete within one week (Jackson-Lewis et al., 1995). Behavioral testing was conducted under red light conditions during the subjective dark phase.

2.4.1. Rotarod
The latency to fall off a rotating spindle (rat size, diameter 7.3 cm, Columbus Instruments, Columbus, OH) was measured according to the protocol of Rozas et al. (1998). Initially, mice were acclimatized to the rotarod for 10 min at 5 rpm. During acclimatization, mice that fell were replaced on the spindle. For behavioral testing, mice were tested at increasing speeds (12, 14, 16, 18, 20, 22, 24, and 26 rpm) and the latency to fall was recorded for each animal. The maximum duration at each speed was 150 s, with 150 s rest between trials. The overall rotarod performance (ORP) for each group was calculated by plotting the average latency to fall at each speed, and using the trapezoidal method of Rozas et al. (1997) to estimate the area under the curve.

2.4.2. Pole test
Bradykinesia is measured by the time to a) turn head-down and b) completely descend a metal pole wrapped in cloth tape, according to Matsuura et al. (1997). Mice were acclimatized to the pole (1 cm diameter, 50 cm height) over 5 trials, each separated by 60 s. The first trial was limited to 300 s and subsequent trials were limited to 120 s. Behavioral testing was identical to acclimatization, with the exception that all five trials were capped at 120 s. Mice that failed to turn or fell off the pole were assigned a time of 120 s. The three best scores were averaged for each mouse. There was no sex difference and no effect of MPTP on the time to turn head-down; these data are not reported.

2.4.3. Gait test
Stride length was measured according to the methods of Fernagut et al. (2002). Briefly, mice were placed on an illuminated runway (4.5 cm wide, 42 cm long, with walls 12 cm high) and were allowed to run toward a dark goal box ($20 \times 17 \times 10$ cm). Mice were acclimatized over two trials. Gait was measured in a single trial. To differentiate between forelimb and hindlimb strides, front and back paws were painted with red and black ink, respectively (Winsor and Newton, London, England) as mice ran down the paper-lined runway. Stride length was measured as the distance between successive paw prints. The average of three strides was taken for each animal. Because forelimb and hindlimb stride lengths were similar and demonstrated an equivalent sex difference and effect of MPTP, data are reported for hindlimb stride length only.

2.5. Perfusion and immunocytochemistry

In gonadectomized mice with hormone replacement (Experiment 1), we determined whether MPTP reduced the number of TH-positive neurons in SNC in males and females. TH is the rate-limiting enzyme in dopamine biosynthesis and is used as a marker for dopamine-producing neurons. Because we found no sex differences and no effect of MPTP, TH neurons were not counted for gonadectomized mice in Experiment 2.

Mice were deeply anesthetized with sodium pentobarbital (150 mg/kg BW) and perfused intracardially with 150 mL of 0.1 M sodium phosphate buffer (PB, pH = 7.4) containing 0.9% NaCl and 0.1% NaNO$_3$. The brains were removed, post-fixed in the perfusion fixative overnight and cryoprotected for 5 days at 4 °C with 20% sucrose in PB. Brains were rapidly frozen and sectioned coronally at 30 um through the rostro-caudal extent of SNC according to the stereotaxic atlas of the mouse brain (Paxinos and Franklin, 2001). Sections were stored in PB with 0.01% sodium azide at 4 °C until processed for TH immunocytochemistry.

Every sixth section was stained for TH. Sections from mice in different groups of the same experiment were stained at the same time. Sections were incubated overnight at room temperature (RT) in polyclonal rabbit anti-TH antibody (1:5000; Chemicon, Temecula, CA) with 4% normal donkey serum and 0.3% Triton X-100 in PB. The following day, sections were incubated in biotinylated donkey anti-rabbit secondary antibody (1:200; Jackson Immunoresearch, West Grove, PA) and the avidin–biotin–horseradish peroxidase complex (Vector Elite Kit; Vector Laboratories, Burlingame, CA), each for 1 h at RT with extensive washes in between. TH-labeled cells were visualized using NiCl-enhanced 3′3-diaminobenzidine tetrahy-drochloride with 0.25% hydrogen peroxide. Sections were mounted onto gelatin-coated slides, air-dried, and counterstained for Nissl to identify neuronal nuclei. Afterwards, the slides were dehydrated in alcohols, cleared in xylenes and coverslipped with permount.

Unbiased stereological counts of TH neurons in SNC were performed on 7–9 animals randomly selected from each experimental group. TH-positive, Nissl-stained cells were counted bilaterally on coded slides by an observer blind to the treatment group using an Olympus BX-50 microscope (Olympus Optical, Tokyo, Japan) with motorized stage and Retiga-cooled CCD camera (Q-imaging, Burnaby, British Columbia, Canada) according to the methods of Petzinger et al. (2007). SNC was outlined in each section at low magnification (10×) using the third cranial nerve and cerebral peduncle as landmarks. A grid was placed over the outlined SNC and counting frames were established at grid intersections. Counting was performed at 80× using the optical fractionator technique assisted by the computer program BioQuant NovaPrime (BioQuant Image Analysis, Nashville, TN). TH-positive cells were counted if they had a visible nucleolus and were located within the counting frame. The total number of TH-positive neurons in SNC was calculated based on the method of Gundersen and Jensen (1987).

2.6. Statistics

All data are reported as Mean ± SEM. For both experiments, group differences were analyzed by a factorial analysis of variance (ANOVA). Post-hoc comparisons using the Fisher's LSD test were conducted when statistically significant differences ($p < 0.05$) were found.

3. Results

3.1. Experiment 1: gonadectomized mice with hormone replacement

In MPTP-treated mice, 100% of males survived the daily 10 mg/kg injection schedule, compared with 87% of females ($p < 0.05$). At the time of sacrifice, saline-treated males weighed 26.7 ± 0.3 g while control females weighed 24.2 ± 0.2 g ($p < 0.05$). Body weight in MPTP-treated mice increased over the course of the experiment. Male mice weighed 24.3 ± 0.3 g prior to MPTP treatment and 26.0 ± 0.3 g at the time of sacrifice ($p < 0.05$). In females, MPTP caused a non-significant increase in body weight (23.2 ± 0.3 g vs. 23.6 ± 0.7 g, $p > 0.05$). Among MPTP-treated animals, males outweighed females at the time of sacrifice (26.0 ± 0.3 vs. 23.6 ± 0.7 g).

Saline-treated mice exhibited sex differences in motor performance measured by the rotarod, pole and gait tests. For all tests, males outperformed females. On the rotarod, ORP was greater in male mice (1317.0 ± 98.3) than in females (988.1 ± 95.6, $p < 0.05$, Fig. 1A). At low speeds (12 rpm), male and female mice had similar latencies to fall (134.3 ± 6.0 vs. 138.1 ± 8.7 s, $p > 0.05$). However, sex differences emerged at higher speeds. At 26 rpm, males stayed on the rotarod 3 times longer than females (49.6 ± 13.1 vs. 14.9 ± 2.9 s, $p < 0.05$). On the pole test, male mice descended the pole faster than females (7.1 ± 0.5 vs. 9.6 ± 0.7 s, $p < 0.05$, Fig. 2). On the gait test, males had a longer stride length (hindlimb: 7.3 ± 0.1 cm) than females (6.8 ± 0.1 cm, $p < 0.05$, Fig. 3).

MPTP lesion produced a comparable deficit in rotarod performance in both male and female mice. In males, MPTP reduced ORP by 47% to 703.7 ± 65.5 ($p < 0.05$ vs. unlesioned males, Fig. 1A). In MPTP-lesioned female mice, ORP was reduced 56% to 432.8 ± 88.6 ($p < 0.05$ vs. unlesioned females). MPTP treatment also altered the relative performance on the rotarod in males and females (Fig. 1B). At low speeds, MPTP-lesioned males remained on the rotarod longer than females (99.5 ± 14.8 vs. 59.8 ± 8.8 s, $p < 0.05$). However, at 26 rpm, the latency to fall for males (14.6 ± 4.4 s) and females (4.5 ± 1.8 s) was not significantly different. On the pole test, MPTP did not impair performance in either sex (Fig. 2). The time to descend the pole was 7.0 ± 0.5 s in males and 10.3 ± 1.0 s in females ($p > 0.05$ vs. same-sex conspecifics, Fig. 2). Finally, gait was slightly, but selectively reduced in male mice after MPTP. Hindlimb stride length was 6.9 ± 0.2 cm in MPTP-lesioned males ($p < 0.05$ vs. saline-treated males, Fig. 3). By contrast, MPTP-lesioned female mice had comparable stride lengths (6.8 ± 0.2 cm) compared to unlesioned females ($p > 0.05$, Fig. 3).

Cell counts of TH-positive, Nissl-stained neurons in SNC are shown in Fig. 4. Unlesioned male and female mice had similar numbers of TH-positive neurons in SNC (11,174.1 ± 620.0 vs. 9927.6 ± 978.9, $p > 0.05$, Fig. 4). MPTP lesioning did not deplete TH neurons in males (11,192.6 ± 1295.4) or in females (11,246.1 ± 1064.9), and there were no sex differences (Fig. 4).

3.2. Experiment 2: gonadectomized mice without hormone replacement

The 10 mg/kg daily dose of MPTP was well-tolerated by gonadec-tomized male and female mice, with 100% of mice surviving. Body weights of saline-injected gonadectomized male and female mice were not different at the time of sacrifice (25.1 ± 0.4 vs. 24.8 ± 0.3 g, $p > 0.05$). As in Experiment 1, MPTP treatment increased body weight. Male mice weighed 24.1 ± 0.6 g prior to MPTP and 24.9 ± 0.4 g at the time of sacrifice ($p < 0.05$). Female mice in the MPTP group weighed 21.1 ± 0.2 g prior to MPTP and 24.8 ± 0.3 g at the time of sacrifice ($p < 0.05$). There were no differences in body weight between MPTP-treated male and female mice at the time of sacrifice.

Saline-treated ovariectomized female mice outperformed castrat-ed males on the rotarod and pole tests. On the rotarod, ORP was significantly greater in gonadectomized female mice (1296.6 ± 83.3) than in gonadectomized males (811.2 ± 113.7, $p < 0.05$, Fig. 1C). In saline-injected mice, sex differences were most apparent at 16–20 rpm, where ovariectomized females stayed on the rotarod longer than orchidectomized males (Fig. 1D). On the pole test, ovariecto-mized females took significantly less time to completely descend the pole (9.7 ± 2.0 s) than castrated males (15.6 ± 1.9 s, $p < 0.05$, Fig. 2). Finally, gonadectomized male and female mice did not differ in measures of stride length (Fig. 3). Hindlimb stride length in
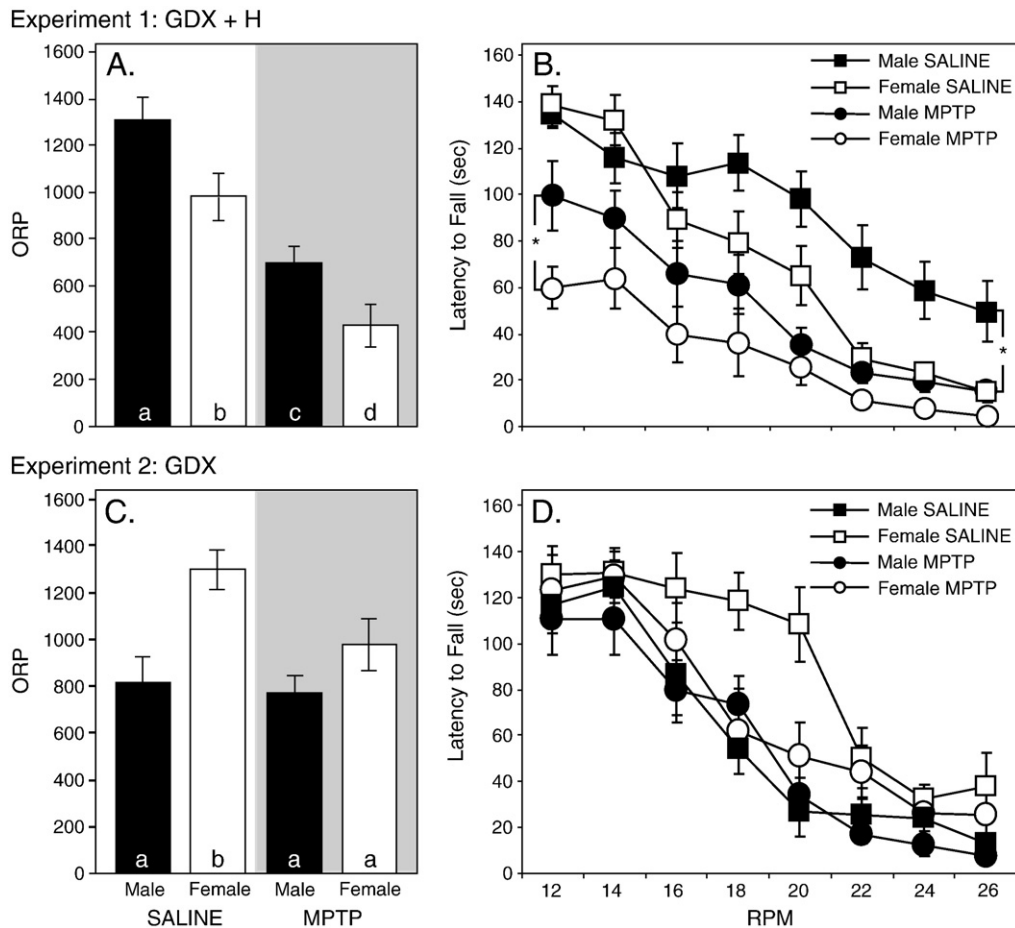
Fig. 1. Rotarod performance in gonadectomized male (black) and female (white) mice ($n = 13$–15 per group). Overall rotarod performance (ORP) for saline- (clear) and MPTP-treated (shaded) for GDX + H mice (A) and GDX mice (C). Latency to fall off the rotarod at 12–26 rpm for saline- (squares) and MPTP-treated (circles) for GDX + H mice (B) and GDX mice (D). Bars with different letter subscripts are significantly different. Asterisks represent sex differences ($p < 0.05$) within the same treatment group (MPTP or Saline). Abbreviations: GDX, gonadectomy; GDX + H, gonadectomy with hormone replacement.

ovariectomized females was 7.0 ± 0.1 cm compared with 6.7 ± 0.2 cm in castrated male mice ($p > 0.05$, Fig. 3).

After MPTP, rotarod performance was reduced in gonadectomized female mice. MPTP-injected female mice had an average ORP of 975.9 ± 110.3 ($p < 0.05$ vs. saline-injected females, Fig. 1C). ORP was similar in MPTP-treated males (771.8 ± 70.6), but was not different from saline-treated males ($p > 0.05$, Fig. 1C).

Performance on the pole and gait tests was unaffected by MPTP treatment. MPTP did not increase the time to descend the pole in female (12.0 ± 2.4 s) or male (17.5 ± 0.5) mice ($p > 0.05$ vs. same-sex conspecifics, Fig. 2). However, the sex difference persisted after MPTP, with female mice descending the pole faster than males ($p < 0.05$, Fig. 2). Finally, stride length was similar in saline- vs. MPTP-treated mice. Hindlimb stride length was 6.9 ± 0.1 cm in MPTP-treated females and 6.5 ± 0.1 cm in MPTP-treated males ($p > 0.05$ vs. same-sex conspecifics, Fig. 3).

4. Discussion

The current study investigated sex differences and the effects of gonadal steroids in the MPTP mouse model of Parkinson's disease using a subacute MPTP treatment (10 mg/kg MPTP per day for five days). Higher doses of MPTP killed female mice. In gonadectomized mice with physiologic hormone replacement, we observed a baseline sex difference in motor performance with male mice outperforming females on the rotarod, pole, and gait tests. MPTP lesioning reduced rotarod performance in both male and female mice but gait was

selectively impaired only in males. In SNC, the total number of dopaminergic neurons was similar in all groups. To determine if these sex differences persist in the absence of gonadal steroids, we then tested MPTP lesions in gonadectomized mice. Ovariectomized, saline-treated females outperformed castrated males on the rotarod and pole tests. After MPTP, rotarod performance was reduced in ovariectomized females; castrated males did not show further motor deficits post-MPTP. Taken together, our results show that small, chronic doses of MPTP attenuate locomotor ability without depleting SNC neurons and reveal a sex-selective motor impairment in males. Gonadectomy reversed this sex difference.

Interestingly, the largest MPTP-induced sex difference was in mortality. At 10 mg/kg for 5 days, more females than males died after MPTP. In fact, in our pilot studies using larger doses of MPTP, 80–90% of female mice died. In contrast, male mice have received substantially higher doses of MPTP without mention of mortality (Fredriksson and Archer, 1994; Schmidt and Ferger, 2001). Death following MPTP is likely due to the systemic actions of MPTP on the cardiovascular system (Jackson-Lewis and Przedborski, 2007), but it is unknown why females are particularly susceptible. Based on our pilot studies the lethal threshold for female mice is approximately 30 mg/kg MPTP. A single injection of 30 mg/kg MPTP killed 80% of female mice within 2 h. However, when females were given 20 mg/kg injections at two-h intervals, the majority of females died shortly after the second injection.

Although female mortality was the primary reason for selecting the low MPTP dose and prolonged inter-injection interval, subacute administration of MPTP at 10 mg/kg revealed modest impairments in
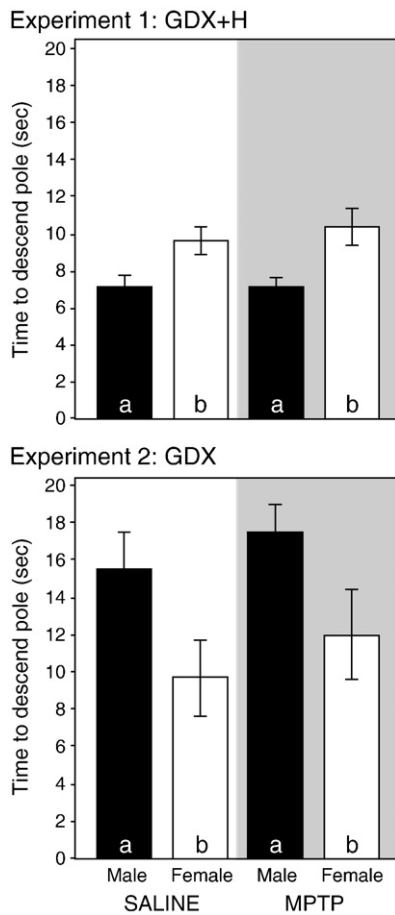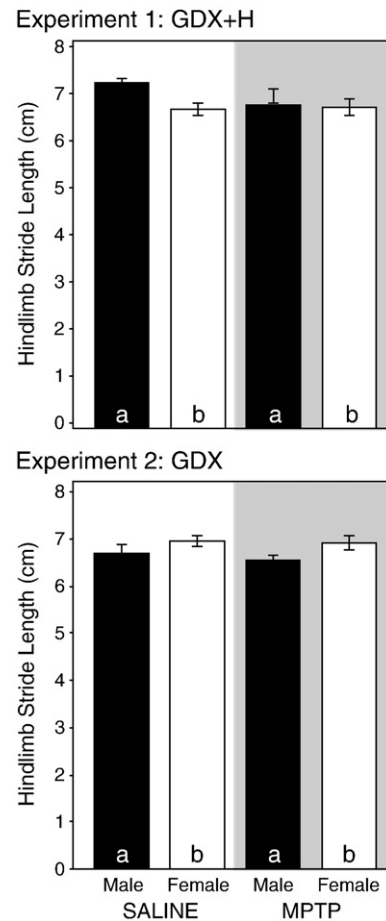
Fig. 2. Pole test performance in male (black bars) and female (white bars) mice ($n = 13$–15 per group). Time to completely descend the pole for saline- (clear) and MPTP-treated (shaded) GDX + H mice (Top) and GDX mice (Bottom). Bars with different letter subscripts are significantly different. Abbreviations: GDX, gonadectomy; GDX + H, gonadectomy with hormone replacement.

Fig. 3. Stride length in male (black bars) and female (white bars) mice ($n = 13$–15 per group). Hindlimb stride lengths for saline- (clear) and MPTP-treated (shaded) GDX + H mice (top) and GDX mice (bottom). Bars with different letter subscripts are significantly different. Abbreviations: GDX, gonadectomy; GDX + H, gonadectomy with hormone replacement.

motor behavior that were sexually dimorphic. By contrast, larger doses of MPTP in males produce considerable motor deficits (reviewed in Sedelis et al., 2001) which may mask potential sex differences. These large doses of MPTP are used to replicate late-stage Parkinsonism (Schober, 2004). In PD patients, symptom severity is sexually dimorphic, with less severe motor impairment in women (Haaxma et al., 2007; Lyons et al., 1998; Shulman and Bhat, 2006). However, by end-stage PD, sex differences are no longer mentioned. Presumably, sex differences in motor behavior that are present in earlier stages of PD disappear as the disease progresses and as patients become more profoundly disabled.

As a model of early PD, our data demonstrate a substantial deficit in motor performance (50% decline in ORP) without destruction of dopaminergic neurons in SNC. This is likely due to the loss of dopaminergic terminals in the striatum and the subsequent reduction of striatal dopamine (Lau and Meredith, 2003; Petroske et al., 2001). In the current study, 10 mg/kg MPTP did not significantly deplete TH-positive neurons in SNC. This is consistent with Petroske et al. (2001) who delivered daily injections of 25 mg/kg MPTP without inducing dopaminergic cell death in SNC. However, unlike Petroske et al. (2001), we observed significant motor deficits on the rotarod in both male and female mice. While Petroske et al. (2001) tested mice on a small-diameter rotarod spindle, we followed the protocol of Rozas et al. (1998), and used a larger, rat-sized spindle (7.3 cm diameter). This spindle distinguishes locomotor ability from balance and/or coordination, and is more effective than smaller spindles at detecting motor deficits after nigrostriatal lesions (Duan and Mattson, 1999; Rozas et al., 1998).

Our data also demonstrate that multiple behavioral tests are necessary to show deficits in motor performance and, more specifically, to reveal subtle sex differences in behavioral impairment after MPTP. Unfortunately, there is little consensus in published literature on motor deficits following MPTP treatment (reviewed in Sedelis et al., 2001). This reflects the tremendous variability in the dose and timing of MPTP administration and in the selection of behavioral assessments. In our study, 10 mg/kg MPTP reduced ORP in hormone-treated males and females, but there was no effect on pole test performance in hormone-treated or in gonadectomized mice. The absence of brady-kinesia in the pole test may be related to the low dose of MPTP. Bradykinesia in rodents has been primarily revealed after substantial lesions and destruction of SNC cells and/or dopaminergic terminals in the striatum (Diguet et al., 2005; Ogawa et al., 1985). Given that our dose and timing of MPTP were relatively modest, it is not surprising that mice did not show deficits on the pole test. By contrast, measures of stride length proved to be the most sensitive for detecting sex differences after MPTP in hormone-treated mice. Male mice had a selective reduction in stride length after MPTP. Other studies have produced comparable reductions in gait using male mice, with larger doses of MPTP (Amende et al., 2005; Mohanasundari et al., 2006). The larger body weight of males may account for the longer stride length in vehicle-injected male mice, as shown previously (Heglund et al., 1974; Koopmans et al., 2007; Parker and Clarke, 1990). However, this cannot explain why MPTP decreased stride length only in males.

After establishing a sex difference in motor impairment after MPTP, our next goal was to determine whether gonadal steroids could
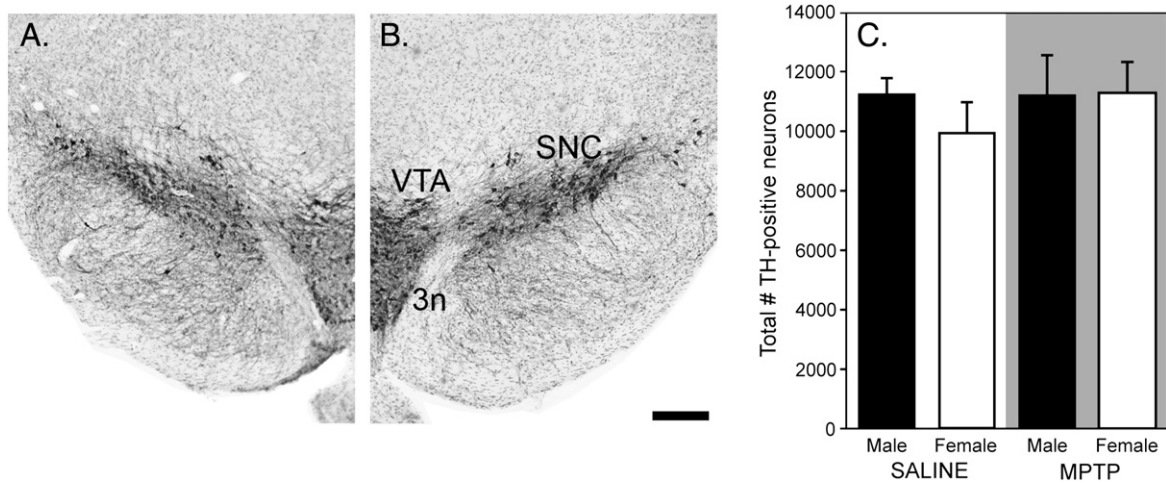
Fig. 4. Photomicrographs of TH-positive neurons in SNC of saline- (A) and MPTP-treated (B) mice. (C) Mean ± SEM number of TH-labeled neurons in male (black bars) and female (white bars) mice ($n = 7$–9/group) in SNC. There were no significant differences between groups. Scale bar = 200um. Abbreviations: 3n, third cranial nerve; SNC, substantia nigra pars compacta; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

account for these sex differences. Instead, castrated male mice performed more poorly on motor tests than ovariectomized females. After MPTP, motor impairments were observed in ovariectomized females, but not in castrated males. This is a complete reversal of our findings in hormone-treated mice, where males outperformed females. Perhaps the poor motor performance of castrated males is not surprising, given testosterone's anabolic effects to increase muscle mass and improve motor performance (American College of Sports Medicine, 2006). The implication is that castration has a more profound effect in males, compared with low-dose MPTP. Nonetheless, we cannot directly compare the results of Experiments 1 and 2, since the studies were not conducted at the same time.

A substantial body of evidence suggests that estrogen is neuroprotective in brain, including in the nigrostriatal system (Dluzen, 2000; Garcia-Segura et al., 2001). The neuroprotective effects of estrogen would be expected to minimize MPTP-induced neuronal damage in females. Instead, female mice in the present study had comparable decreases in motor performance after MPTP, regardless of hormone status. It is important to note that our study is not an appropriate test of estrogen neuroprotection. First, the present study investigated behavioral endpoints, rather than neurologic outcomes. Motor behavior is expressed through the interactions of different physiologic systems (neurologic, cardiovascular, musculoskeletal), all of which respond to gonadal steroid hormones. Furthermore, it is difficult to demonstrate neuroprotection using a dose of MPTP which does not kill cells. It is more appropriate to test the neuroprotective effects of estrogen using higher doses of MPTP in males, as previously reported (Dluzen, 2000).

Ultimately, gonadal steroid hormones are also relevant to understanding sex differences in PD. Symptoms of PD emerge at approximately 60 years of age (Lees et al., 2009). By this age, most women are post-menopausal and testosterone levels are declining in men. Our results suggest that hypogonadism might have minimal effect on motor performance in females but could drastically reduce motor performance in males. In men with PD, the loss of SNC dopamine neurons superimposed over the decline of endogenous testosterone has the potential to increase symptom severity.

Acknowledgements

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