

Research Report

Acute Effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or Paraquat on Core Temperature in C57BL/6J Mice

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Abstract.

Background: MPTP and paraquat are two compounds that have been used to model Parkinson's disease in mice. Previous studies in two non-traditional strains of mice have shown that a single dose of MPTP can induce changes in body temperature, while the effects of paraquat have not been examined. Examination of body temperature is important since small fluctuations in an animal's core temperature can significantly affect drug metabolism, and if significant enough can even culminate in an animal's death.

Objective: To determine how external heating can alter the survival of C57BL/6J mice following MPTP administration.

Methods: In this study, we examine the effects of MPTP (4 × 20 mg/kg, 2 hours apart) and paraquat (2 × 10 mg/kg/week for 3 weeks) on core temperature of C57BL/6J mice. Correlations of purine and catecholamine levels were also done in mice treated with MPTP.

Results: We find that MPTP induces a significant hypothermia in C57BL/6J mice that reduces their core temperature below the limit of fatal hypothermia. Unlike MPTP, paraquat did not induce a significant hypothermia. Placement of animals on heating pads significantly abrogates the loss of core temperature. In both heated and non-heated conditions, mice treated with MPTP showed a significant depletion of ATP within 2 hours of administration in both striatum and SN that started to recover 2 hours after MPTP administration was complete. Striatal DA and DOPAC are significantly reduced starting 4–6 hours after MPTP.

Conclusions: The fatal hypothermic effects of MPTP can be abrogated through use of external heating.

Keywords: Mouse strain, MPTP, paraquat, temperature, fatal hypothermic temperature

INTRODUCTION

Animal models of Parkinson's disease (PD) fall into two broad categories. These are: 1) models induced by exogenous administration of substances, i.e. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or paraquat, and 2) models generated through manipulation of the genome. In the former, substances are administered peripherally and through various mechanisms are metabolized until they effect a neurodegenerative action.

In terms of exogenous models, the most widely used compound for induction of dopamine insufficiency to mimic that seen in PD is the systemic administration of the tertiary amine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This agent was identified following the study of young adult heroin addicts who unknowingly injected the synthetic heroin, 1-methyl-4-phenyl-4-propionoxypiperidine (MPPP) that contained the contaminant, MPTP; after which they presented with symptoms indistinguishable from those of later stage PD patients [1, 2].

Another compound that has been used to induce experimental parkinsonism is 1,1'-dimethyl-4,4'-bipyridium dichloride (paraquat, PQ). PQ is a

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commonly used non-selective herbicide that has been epidemiological linked to Parkinson's disease [3, 4]. Experimentally, administration of PQ by systemic or direct injection into the CNS induces a relatively specific lesion in the SNpc that results in a small but significant dopaminergic neuron loss [5–9] (although note [10]). The relative small loss of DA neurons over a more protracted period of time appears to allow for plasticity in the system such that despite neuron loss in the SN, there is no apparent loss of dopamine in the striatum [11–13].

Paraquat can cross the blood brain barrier [14], but unlike MPTP, acts as a direct redox cycling agent that reduces molecular oxygen to its cationic radical that then further reduces molecular oxygen to its superoxide radical [15–17]. In addition to its direct effect on membrane phospholipids, PQ also actively transports into neurons via neutral amino acid transporters [18] where it damages mitochondria leading to secondary oxidative stress [19, 20] and eventual cell death through a BAK-dependent mechanism [21].

Since MPTP and PQ are generally administered via the periphery, either through direct injection or dietary means, one must also be aware of peripheral effects that could synergize with the CNS effects. Previous reports have shown that MPTP and/or PQ can induce cardiac [22, 23], lung [24] and kidney [25, 26] toxicity and alterations in muscle function [27]. In addition to these agent's abilities to damage organ systems, we, and others have observed that peripheral administration of these chemicals appears to induce changes in the animal's core body temperature [28–30].

Maintenance of body temperature is a combinatorial function of heat generation and heat loss; and small fluctuations in animal core temperature can have significant effects in physiological systems (cardiovascular, endocrine, nervous system) that can culminate in an animal's death [31]; each of which has been reported with MPTP [32] and paraquat [24].

In this paper, we examine the effect of administering MPTP (acute administration of 4×20 mg/kg spaced two hours apart) and paraquat (2×10 mg/kg/week \times 3 weeks) on the core temperature in C57BL/6J mice. We find that acute MPTP treatment induces a rapid and lasting hypothermia that can be abrogated by increasing the environmental ambient temperature. The loss of body temperature correlates with a significant loss of ATP and dopamine in the striatum and ATP in the substantia nigra. Paraquat, like MPTP, also induces a transient hypothermia; however, unlike MPTP, animal's treated with paraquat return to normal temperature soon after administration.

MATERIALS AND METHODS

Ethics statement

All of the experimental procedures in the animals were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all protocols, were approved by the St Jude Children's Research Hospital IACUC (protocol 364). Experiments were carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiments.

Animals

16–20 week old male C57BL/6J mice (Jackson Labs, Bar Harbor, ME) mice were used in this study. Mice were housed 5 to a polycarbonate cage within the vivarium at St. Jude Children's Research Hospital and were maintained on a 12:12 hour light:dark cycle. The animals were provided with corn cob bedding, *ad libitum* food (LabDiet 5013, Purina) and water.

Measurement of cage temperature

To determine the temperature of the animal's environment, iButton[®] Temperature Data Loggers (QA Supplies, LLC, Norfolk, VA) were placed in each cage and temperature was determined at 1 hour intervals. Over the course of the experimental procedure, the ambient temperature of the cages in the holding room was 23.2°C ($73.8 \pm 0.10^{\circ}\text{F}$), never fluctuating more than $\pm 5\%$. Twenty-four hours prior to administration of MPTP or paraquat, mice were transferred from their holding room to caging maintained within a vented fume hood that had an empirically determined ambient temperature of $21.2 \pm 0.1^{\circ}\text{C}$ (70.2°F), which is within the optimal housing range for mice [33]. One half of the cages in the vented hood were maintained on heating pads set to "medium" which were empirically determined to be $35.1 \pm 1.0^{\circ}\text{C}$ ($95.2 \pm 2.0^{\circ}\text{F}$). When cages were placed on the heating pads the ambient cage temperature was empirically determined to be $24.6 \pm 0.3^{\circ}\text{C}$ ($76.3 \pm 0.6^{\circ}\text{F}$).

The other one-half of the mice were housed in cages seated on heating pads that were turned off. Mice were allowed 24 hours of acclimation in this new environment prior to administration of MPTP or paraquat.

MPTP treatment

C57BL/6J mice were administered either vehicle [0.9% sterile saline (Sal)] or MPTP (Catalog

M103, Sigma, St. Louis, MO) using the acute protocol (4×20 mg/kg MPTP-HCl ip injections at 2-h intervals, equivalent to 16.8 mg/kg free base) [34]. One-half of the mice injected with MPTP were maintained on heating pads and one-half of the mice were housed in cages seated on heating pads that were turned off. We have empirically observed that mice become hypokinetic and exhibit piloerection and wet dog shakes following administration of MPTP. Additionally, it has also been well documented that certain strains of mice, including C57BL/6J, can suffer from a non-predictable death (cause unknown, although speculated to be cardiac in nature) within 24 hours of its administration [35]. However, since there is no clear biomarker available that will allow one to determine if any particular mouse will succumb to MPTP toxicity, and almost all mice injected using the acute MPTP paradigm appear (at some time during the 8 hours of the injection period) moribund, an *a priori* determination of a humane endpoint during the injection protocol for euthanasia could not be made. However, prior to the start of the procedure, the protocol called for humane sacrifice within 24 hours of the final injection.

Paraquat treatment

1,1'-di methyl-4,4'-bipyridium dichloride (paraquat, PQ) (Catalog #36541 Sigma-St. Louis, MO) was dissolved in sterile saline to a final concentration of 20 mg/ml. Each animal was given a total of 60 mg/kg of PQ, using a dosage regimen of 10 mg/kg \times 2 per week for 3 weeks. One-half of the mice injected with PQ were maintained on heating pads and the other one-half of the mice were housed in cages seated on heating pads that were turned off. Like, MPTP, an *a priori* determination of a humane endpoint during the injection protocol for euthanasia could not be made. However, prior to the start of the procedure, the protocol called for humane sacrifice within 24 hours of the final injection.

Measurement of core temperature

Seven days prior to treatment with MPTP or paraquat, mice were anesthetized with inhaled isoflurane. Once deep tendon reflexes were absent, a 2 cm² area of fur was removed from the intrascapular region and the exposed skin was cleaned with alternating iodine and 70% ethanol. The skin was then incised and a 10 g syringe preloaded with a temperature transponder with a programmable Radio Frequency Identification (RFID) signal (IPTT-300, BioMedic

Data Systems, Inc., Seaford, DE) was placed subcutaneously and the transponder was expelled. Once the transponder was in place, the needle was removed and the skin was closed using a drop of n-butyl cyanoacrylate glue (Vet Bond[®], Fisher Scientific). Once the glue had set, the mouse was allowed to recover from the isoflurane anesthesia and placed into their home cage.

Core temperatures of the C57BL/6J mice were measured using a hand held reader (DAS-7066/7r, BioMedic Data Systems, Inc., Seaford, DE). For the MPTP study, animals were checked and temperatures were taken at baseline and 15, 30, and 60 and 120 minutes after each MPTP injection with an additional measurement of 180 and 240 minutes following the final (4th) injection (total of 600 minutes). For paraquat, animals were checked and temperatures were taken at baseline, 15, 30, 60, 120 and 24 hours after each injection through out the entire 3-week injection period.

Determination of purine and catecholamine levels in striatum and substantia nigra

At Time 0, 15 minutes after each injection of MPTP and 2 hours following the last injection of MPTP, C57BL/6J mice were rapidly decapitated. Brains were rapidly removed and placed in a brain matrix (Model BS-AL-5000C, Braintree Scientific, Braintree, MA) and sliced into 2 mm thick sections and placed on an ice-cooled plate. Striatum and substantia nigra were dissected using the following coordinates: SN (Bregma: -2.00- -4.00), striatum (Bregma: +0.00- +2.00 mm) [36]. HPLC-ECD was used to quantitate the levels of purines (adenosine (ADO), ATP, ADP and AMP) and catecholamines (dopamine (DA) and its metabolites (HVA and DOPAC), as previous described [37, 38]. Following chromatography, the signal from the electrochemical detector was recorded using a model SS420x integration device (Scientific Software Inc) and the retention time of each of the analytes were empirically determined. For both purines and catecholamines, a 4-point concentration curve (12.5, 25, 50 and 100 ng/ml) of external standards (Sigma) was generated and concentrations in tissues were quantified by comparing the peak areas of the sample chromatograms with the external standard chromatograms.

Statistics

Differences in mean core temperatures, as well as purine and catecholamine concentrations were analyzed by ANOVA using PRIZM software (GraphPad

Software). If overall significance was found, individual comparisons were performed using Neumann-Keuls *post-hoc* comparisons.

RESULTS

Effect of external heat on animal environment

The ambient cage temperature in the procedure room at SJCRH that were not provided access to an external heat source was empirically determined to be $20.1 \pm 0.3^\circ\text{C}$ ($68.2 \pm 0.6^\circ\text{F}$). The ambient cage temperature in the procedure room at SJCRH that were provided access to an external heat source ($35.1 \pm 1.0^\circ\text{C}$, $95.2 \pm 2.0^\circ\text{F}$) was empirically determined to be $24.6 \pm 0.3^\circ\text{C}$ ($76.3 \pm 0.6^\circ\text{F}$).

Effect of MPTP on core temperature

10 male C57BL/6J mice were administered 4 ip injections of MPTP (20 mg/kg) spaced 2 hours apart, while 10 C57BL/6J male C57BL/6J control animals were placed on heating pads and injected ip with 0.1 ml sterile 0.9% saline. Two temperature conditions were examined: those housed on heating pads (heat) and those housed at ambient temperature (no heat). Both groups had similar core temperatures at the start of the experiment (C57BL/6J with heat = $38.5 \pm 0.1^\circ\text{C}$ ($101.2 \pm 0.29^\circ\text{F}$); C57BL/6J without heat = $38.3 \pm 0.1^\circ\text{C}$ ($100.8 \pm 0.21^\circ\text{F}$), and (Table 1 and Fig. 1).

C57BL/6J mice housed in non-heated conditions ($n = 5$) that were administered an ip injection of MPTP showed a rapid 4.5% (35.7°C , 96.3°F) drop in core temperature that progressed to a loss of 10% (32.8°C , 90.9°F) at the conclusion of the first 2 hour inter-injection period. Following the second injection-at the 120-minute mark- the core temperature continued to fall, so that at the end of the 2nd inter-injection period, the core temperature of the C57BL/6J mice without parental heat support fell another 7.0% (29.2°C , 84.6°F). After the third injection-at the 360 minute mark- the core temperature continued to fall another 5.0% (26.9°C , 80.4°F), while 4 hours after the 4th injection, the core temperature of these C57BL6J mice fell another 10% (20.4°C , 68.8°F) for a total loss of 31% over the whole injection period. Twenty-four hours following the start of the injection, all of the mice (5 out of 5) that did not have additional access to an external heat source had died, likely due to the fact that they had surpassed the point of Fatal Hypothermic Temperature [31].

A second group of 5 male C57BL/6J mice were housed on heating pads and given the same dosing regimen of MPTP. These mice showed a rapid 2.8% drop in core temperature (36.9°C , 98.4°F) that progressed to a loss of 5.5% (35.3°C , 95.5°F) at the conclusion of the first 2-hour inter-injection period. Following the second injection-at the 120 minute mark- the core temperature continued a gradual fall, so that at the end of the 2nd inter-injection period, the core temperature of the C57BL/6J mice that had parental heat support fell another 10.8% (29.6°C , 85.3°F). After the third injection, the behavior of the core temperatures changed. Instead of continuing to fall, the core temperature of the mice rose by 3% (31.0°C , 87.8°F), while 4 hours after the 4th injection, the core temperature continued to recovered (32.2°C , 90.0°F) so that the total loss of body temperature was only 17% over the whole injection period and by 6 hours the total loss was only 1.1%. Unlike the mice that did not have access to an external heat source, only 1 out of 5 of these animals died.

To determine the effect of animal handling and ip injections on body temperature with ($n = 5$) and without ($n = 5$) supplementary heat, C57BL/6J mice were administered 4 ip injections of 0.9% saline using the same average injection volume as the MPTP-treated mice (0.1 ml/injection). As can be seen in Fig. 1 and Table 1, the core temperature variance of these mice

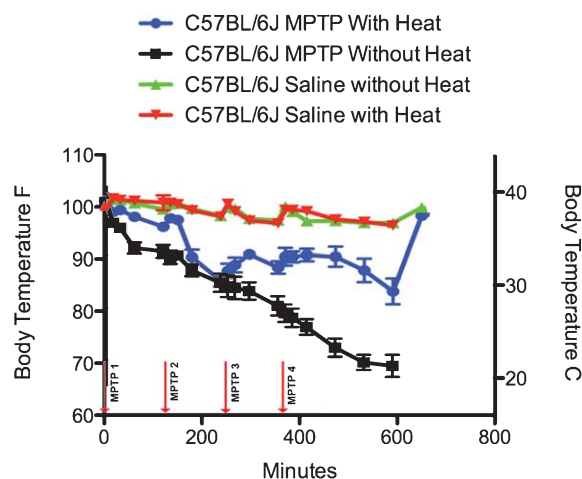


Fig. 1. Effect of MPTP on Core Temperature in C57BL/6J mice. In C57BL/6J mice administered acute MPTP-HCl (4×20 mg/kg, each injection spaced 2 hours apart) without heat support (black lines), there is a rapid hypothermic effect that is progressive throughout the entire measurement period of 600 minutes. When heat support is provided, MPTP is still hypothermic, although the effect is significantly modified (blue lines). Administration of saline with supplemental heat (red) or without supplemental heat (green) has no effect on the core temperature of C57BL/6J mice.

Table 1
Effect of Heat Supplementation on Core Temperature (°C) following MPTP in the Male C57BL/6J Mouse

Injection time	Cumulative minutes	C57BL/6J+MPTP+No Heat	C57BL/6J+MPTP+Heat	C57BL/6J+saline+No Heat	C57BL/6J+saline+Heat	Statistics
Baseline (0 minutes)	0	38.3 ± 0.1	38.5 ± 0.2	37.9 ± 0.2	37.2 ± 0.2	NS
Injection 1–15 minutes	15	35.7 ± 0.3	36.9 ± 0.2	38.2 ± 0.2	38.3 ± 0.2	1, 2, 3, 4, 5
Injection 1–30 minutes	30	35.2 ± 0.3	37.1 ± 0.2	38.2 ± 0.1	38.2 ± 0.1	1, 2, 3
Injection 1–60 minutes	60	33.1 ± 0.6	36.4 ± 0.3	37.8 ± 0.2	38.1 ± 0.3	1, 2, 3
Injection 1–120 minutes	120	32.7 ± 0.7	35.3 ± 0.5	37.2 ± 0.1	37.9 ± 0.3	1, 2, 3
Injection 2–15 minutes	135	32.1 ± 0.7	36.2 ± 0.4	37.7 ± 0.2	37.7 ± 0.4	1, 2, 3
Injection 2–30 minutes	150	32.3 ± 0.5	36.1 ± 0.3	37.8 ± 0.2	37.1 ± 0.3	1, 2, 3
Injection 2–60 minutes	180	30.7 ± 0.6	32.1 ± 0.8	37.2 ± 0.2	36.4 ± 0.3	2, 3, 4, 5
Injection 2–120 minutes	240	29.4 ± 1.0	29.6 ± 0.6	36.5 ± 0.2	37.8 ± 0.2	2, 3, 4, 5
Injection 3–15 minutes	255	29.0 ± 1.2	30.7 ± 0.8	37.4 ± 0.2	37.0 ± 0.2	2, 3, 4, 5
Injection 3–30 minutes	270	28.8 ± 1.2	31.3 ± 0.8	37.3 ± 0.1	36.0 ± 0.5	1, 2, 4, 5
Injection 3–60 minutes	300	28.5 ± 0.9	32.4 ± 0.5	36.2 ± 0.3	35.7 ± 0.3	1, 2, 3, 4, 5
Injection 3–120 minutes	360	26.9 ± 1.0	31.0 ± 0.7	36.0 ± 0.2	37.1 ± 0.2	1, 2, 3, 4, 5
Injection 4–15 minutes	375	26.1 ± 0.9	32.2 ± 0.9	37.5 ± 0.2	37.1 ± 0.3	1, 2, 3, 4, 5
Injection 4–30 minutes	390	25.6 ± 1.0	32.1 ± 0.6	36.9 ± 0.1	37.0 ± 0.3	1, 2, 3, 4, 5
Injection 4–60 minutes	420	24.6 ± 0.8	32.3 ± 0.6	35.9 ± 0.1	36.1 ± 0.1	1, 2, 3, 4, 5
Injection 4–120 minutes	480	22.4 ± 1.0	32.2 ± 1.0	35.9 ± 0.1	35.8 ± 0.2	1, 2, 3
Injection 4–540 minutes	540	21.1 ± 0.8	30.7 ± 1.3	35.7 ± 0.4	35.5 ± 0.0	1, 2, 3
Injection 4–600 minutes	600	20.5 ± 1.0	38.5 ± 1.4	35.7 ± 0.1	37.5 ± 0.3	2, 3, 4, 5

1: $p \leq 0.05$, C57BL/6J MPTP+no heat vs C57BL/6J MPTP+Heat. 2: $p \leq 0.05$, C57BL/6J MPTP+no heat vs C57BL/6J saline+no heat. 3: $p \leq 0.05$, C57BL/6J+MPTP+no heat vs C57BL/6J Saline+Heat. 4: $p \leq 0.05$, C57BL/6J MPTP Heat vs C57BL/6J saline+no heat. 5: $p \leq 0.05$, C57BL/6J MPTP Heat vs C57 Saline Heat. 6: $p \leq 0.05$, C57BL/6J saline+no heat vs C57BL/6J Saline+Heat.

never varied more than 4% and at the end of the period had risen on average only 0.5%. Twenty-four hours after administration of ip saline, none of these male C57BL/6J mice had died.

Effect of paraquat on core temperature

10 male C57BL/6J mice were administered a total of 6 ip injections of paraquat (10 mg/kg) over a period of 3 weeks, with 2 injections/week spaced 2 days apart. A second group of 10 male C57BL/6J control animals were injected ip with 0.1 ml sterile 0.9% saline using the same injection calendar. Two temperature conditions were examined: those housed on heating pads (heat) and those housed at ambient temperature (no heat). Each groups had similar core temperatures at the start of the experiment (C57BL/6J with heat = 99.7 ± 0.3 F ($n = 5$); C57BL/6J without heat = 100.5 ± 0.2 F ($n = 5$), C57BL/6J+PQ+No Heat = 100.4 ± 0.2 F ($n = 5$), and C57BL/6J + PQ + Heat = 99.6 ± 0.3 F ($n = 5$)) (Table 2 and Fig. 2).

Unlike C57BL/6J male mice administered MPTP, the first 4 injections of paraquat (weeks 1 and 2) did not induced hypothermia, but rather there seemed to be a small 1–2°C increase in core temperature. However, as can be seen in Table 2 and Fig. 2, this transient hyperthermia was observed in both mice that received PQ or saline; suggesting that this change was due to

stress induced by the injection procedure and not the PQ itself. In the third week of injections, we did note that mice receiving PQ had a reduced core temperature compared to those receiving saline. Interestingly, animals given PQ without external heat support had a higher core body temperature 24 hours after PQ (Despite the lack of a significant change in body core temperature, we did observe that mice that were housed in cages with an external heat source did have lower core temperatures than those housed in cages without an external heat source (Fig. 2, red lines compared to green lines).

Effect of MPTP on purine levels in the striatum and substantia nigra

Given that we observed a significant decrease in core temperature in C57BL/6J mice following MPTP, we examined the concentration of purines, including adenosine (ADO), adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in two regions of the brain (striatum and substantia nigra) that are known to be affected by this toxin.

Following MPTP, there was no change in adenosine, ADP or AMP in the striatum in animals with and without external heat (Fig. 3). We did find a significant drop in striatal ATP 15 minutes after the 2nd injection of MPTP (135 minutes after the 1st injection) that lasted

Table 2
Effect of Heat Supplementation on Core Temperature (°C) following Paraquat in the C57BL/6J Mouse

	C57BL/6J+ PQ+No Heat	C57BL/6J+ PQ+Heat	C57BL/6J+ saline+Heat	C57BL/6J+ saline+No Heat	Statistics
Baseline (injection 1)	38.0 ± 0.1	37.4 ± 0.2	37.6 ± 0.3	38.5 ± 0.3	NS
15 minutes	37.7 ± 0.2	37.9 ± 0.1	38.0 ± 0.4	38.7 ± 0.3	NS
30 minutes	37.1 ± 0.2	37.1 ± 0.1	37.9 ± 0.3	38.3 ± 0.2	3,5
60 minutes	37.4 ± 0.3	37.4 ± 0.1	37.5 ± 0.3	37.5 ± 0.3	NS
120 minutes	37.1 ± 0.4	36.4 ± 0.3	37.0 ± 0.6	37.8 ± 0.3	NS
24 hours	37.3 ± 0.1	37.1 ± 0.2	36.3 ± 0.4	38.3 ± 0.2	2,3,5,6
Baseline (injection 2)	36.7 ± 0.3	36.7 ± 0.4	36.6 ± 0.2	38.6 ± 0.3	3,6
15 minutes	38.1 ± 0.2	37.8 ± 0.1	37.7 ± 0.3	38.7 ± 0.3	NS
30 minutes	37.8 ± 0.2	37.3 ± 0.1	37.4 ± 0.1	38.4 ± 0.3	3,6
60 minutes	37.2 ± 0.2	36.6 ± 0.1	36.9 ± 0.2	37.9 ± 0.4	5
120 minutes	36.3 ± 0.2	35.6 ± 0.2	37.1 ± 0.2	37.8 ± 0.3	3,4,5
24 hours	36.7 ± 0.2	35.9 ± 0.2	36.6 ± 0.3	38.0 ± 0.2	3,5,6
Baseline (injection 3)	36.7 ± 0.3	37.1 ± 0.4	36.3 ± 0.4	36.8 ± 0.4	NS
15 minutes	37.9 ± 0.3	38.2 ± 0.1	38.4 ± 0.2	38.6 ± 0.2	NS
30 minutes	37.8 ± 0.3	37.3 ± 0.1	38.2 ± 0.1	38.5 ± 0.3	5
60 minutes	37.1 ± 0.3	36.8 ± 0.1	36.8 ± 0.1	37.7 ± 0.3	NS
120 minutes	37.0 ± 0.2	36.0 ± 0.1	36.0 ± 0.1	37.3 ± 0.2	1,2,5,6
24 hours	36.1 ± 0.8	37.2 ± 0.2	37.0 ± 0.2	37.9 ± 0.1	NS
Baseline (injection 4)	37.0 ± 0.2	37.1 ± 0.2	36.6 ± 0.2	37.6 ± 0.3	NS
15 minutes	38.2 ± 0.4	38.2 ± 0.1	37.8 ± 0.2	38.8 ± 0.2	6
30 minutes	38.0 ± 0.2	37.5 ± 0.3	37.2 ± 0.1	38.4 ± 0.2	5,6
60 minutes	37.6 ± 0.2	36.8 ± 0.1	36.9 ± 0.3	37.4 ± 0.2	NS
120 minutes	37.0 ± 0.6	36.2 ± 0.4	36.1 ± 0.2	37.2 ± 0.2	NS
24 hours	36.4 ± 0.2	36.0 ± 0.5	35.3 ± 0.2	37.1 ± 0.3	6
Baseline (injection 5)	37.5 ± 0.3	36.6 ± 0.9	36.3 ± 0.4	37.3 ± 0.4	NS
15 minutes	37.9 ± 0.6	36.6 ± 0.9	37.8 ± 0.2	38.8 ± 0.2	NS
30 minutes	37.9 ± 0.6	36.4 ± 0.7	37.2 ± 0.1	38.1 ± 0.2	NS
60 minutes	37.2 ± 0.4	36.0 ± 0.6	36.3 ± 0.2	37.5 ± 0.2	NS
120 minutes	36.9 ± 0.2	35.7 ± 0.3	36.1 ± 0.2	36.6 ± 0.2	1
24 hours	37.2 ± 0.6	35.3 ± 1.0	35.0 ± 0.1	36.8 ± 0.2	NS
Baseline (injection 6)	36.8 ± 0.4	35.5 ± 1.6	36.1 ± 0.1	37.5 ± 0.2	
15 minutes	38.0 ± 0.4	35.3 ± 1.8	37.8 ± 0.1	38.9 ± 0.2	
30 minutes	37.9 ± 0.3	35.2 ± 1.3	37.5 ± 0.3	38.5 ± 0.2	
60 minutes	37.6 ± 0.3	35.7 ± 1.5	36.7 ± 0.3	37.5 ± 0.3	
120 minutes	37.7 ± 0.4	35.7 ± 0.9	36.0 ± 0.3	37.5 ± 0.3	
24 hours	37.0 ± 0.0	33.9 ± 2.4	35.3 ± 0.2	36.8 ± 0.2	

1: $p \leq 0.05$, C57BL/6J PQ+no heat vs C57BL/6J PQ+Heat. 2: $p \leq 0.05$, C57BL/6J PQ+no heat vs C57BL/6J saline+no heat. 3: $p \leq 0.05$, C57BL/6J+PQ+no heat vs C57BL/6J Saline+Heat. 4: $p \leq 0.05$, C57BL/6J PQ Heat vs C57BL/6J saline+no heat. 5: $p \leq 0.05$, C57BL/6J PQ Heat vs C57 Saline Heat. 6: $p \leq 0.05$, C57BL/6J saline+no heat vs C57BL/6J Saline+Heat.

until 2 hours after the final injection of MPTP, when ATP levels start to recover. In the SN, we observed a similar pattern of purine effect (no loss of ADO or ADP, significant loss of ATP, Fig. 4).

Examination of acute MPTP effects on dopamine, DOPAC and HVA in striatum revealed a significant loss of DA, 15 minutes after the 2nd injection (135 minutes after MPTP was first administered) in animals that had external heat while animals not exposed to an external heat source first showed significant changes after the 3rd injection (195 minutes after the initial injection of MPTP) (Fig. 5). A similar pattern of change was observed in the measurement of DOPAC, while significant effects on HVA were only noted after the 4th injection of MPTP. (Fig. 5). Although changes in DA were noted as early as 15 minutes after the second

injection of MPTP, changes in DA turnover, calculated as (DOPAC+HVA)/DA were only observed in animals provided an external heat source starting 15 minutes after the 4th injection of MPTP (Fig. 5).

DISCUSSION

In this study we examined the effect of two chemicals, MPTP and paraquat, that have been used to induce experimental parkinsonism on core body temperature in C57BL/6J mice. We find that ip injection of MPTP has a rapid and significant hypothermic effect on body temperature that ultimately can prove fatal if no external heat source is supplied to mitigate this change. Unlike MPTP, we do not observe these large

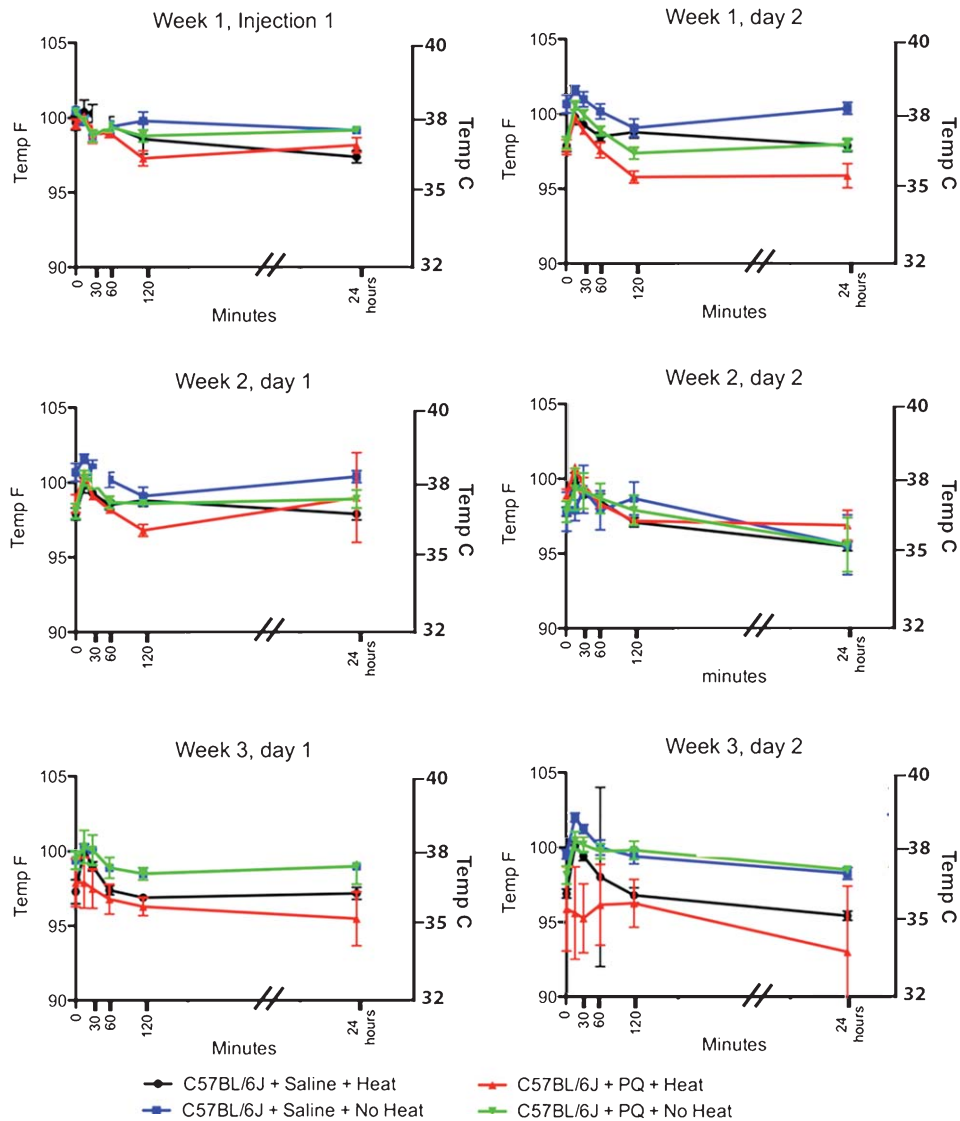


Fig. 2. Effect of Paraquat on Core Temperature in C57BL/6J mice. Paraquat (2 injections of 10 mg/kg PQ/week spaced 48 hours apart for 3 weeks) administered to C57BL/6J mice has no significant effect on the core temperature of mice throughout the entire injection period. One does note, however, a slight hyperthermia that is observed 15 minutes after administration of paraquat (increase of 3%) starting after the 2nd paraquat injection that recedes within 30 minutes of administration.

hypothermic effects following ip administration of paraquat.

Previous studies that have examined body temperature following one injection of high doses of MPTP have shown this compound induces transient hyperthermia followed by a profound hypothermia in both MPTP-sensitive ddY [39] and MPTP-resistant CD-1 mice, while C57BL/6N mice only showed a hypothermic response [28, 40]. A similar response was seen following administration of MPP+, with the caveat that this also induced hyperthermia in C57BL/6N mice [28, 40]. This suggests changes in body temperature

are independent of the central dopaminergic effects of MPTP since MPP+, the neurotoxic metabolite of MPTP cannot cross the blood-brain-barrier [41]. In this study, we examined the core temperature of another strain of mouse (C57BL/6J) widely used in MPTP and other models of Parkinson's disease [42]. Since the neurotoxic effects of this compound are only seen with repeated administration, we determined body temperature for 10 hours, a period that spans the entire MPTP administration period in our "acute" administration protocol [43]. In this study, we find that repeated injection of MPTP induces a 31% loss of

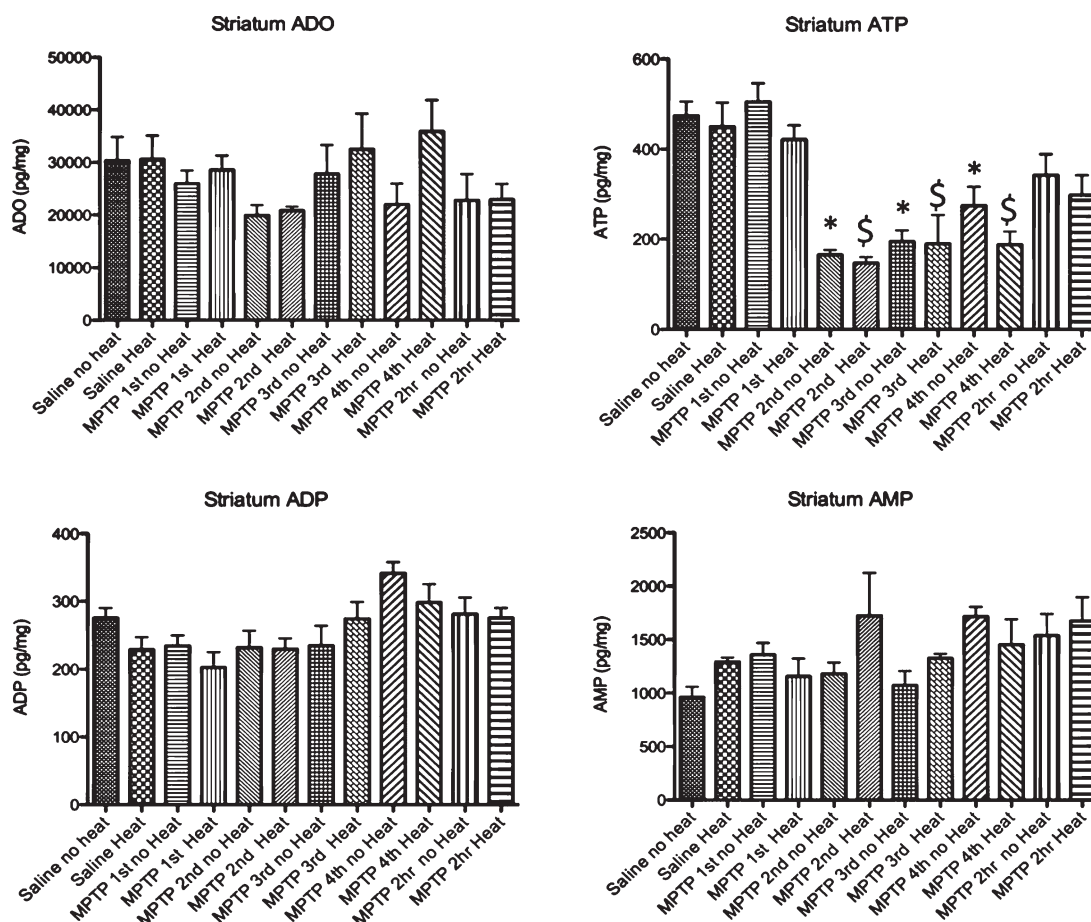


Fig. 3. Effect of MPTP on purine levels in the striatum. Intraperitoneal administration of 4 injections of 20 mg/kg MPTP spaced 2 hours apart does not alter adenosine, ADP or AMP levels. Starting 15 minutes after the 2nd injection of MPTP (135 minutes after the first injection), there is a significant loss of ATP that is maintained until 2 hrs after the last injection, when this purine begins to recover to baseline levels. The MPTP-induced changes in ATP are not affected by the addition of an external heat source. * $p \leq 0.05$ compared to no heat controls; $\$p \leq 0.05$ compared to heat controls.

core body temperature. This hypothermia is slightly more profound than other studies that have examined this variable. Satoh et al. [39] used several concentrations of MPTP in ddY mice and showed no more than a 5% loss of core temperature. Freyaldenhoven et al. [28] using single injections of 50 mg/kg MPTP or 12.5 mg/kg MPP+ reported a maximum loss of 28% in body temperature in 6 month C57BL/6N and a 17% loss in CD-1 mice given MPTP. What is interesting is that it appears that MPP+ is the critical mediator of temperature change since mice pretreated with Deprenyl, a MAO-B inhibitor that inhibits conversion of MPTP to MPP+ significantly reduces the hypothermia [28].

Studies examining the hypothermic effects of peripherally administered MPP+ suggest the hypothermia is not directly mediated through a central CNS mech-

anism, despite the finding that labeled MPTP can bind in the hypothalamus [44], a primary site of central temperature regulation [45]. In the periphery, MPTP is converted to MPP+ by several mechanisms including MAO-B [46], mitochondrion-targeted human cytochrome P450 2D6 (CYP2D6) [47] and rat liver cytochromes P-450IIB1 and P-450IA1 [48]. Once converted to MPP+, this radical can be taken into cells preferentially through the dopamine transporter (DAT), although others have suggested alternative methods for cellular uptake including transport through the organic cation transporter, known in mouse as *Orct3* (*Slc22a1*) [49]. Once in cells, MPP+ interferes with NADH-ubiquinone oxidoreductase (complex I) of the mitochondrial electron transport chain [50, 51] leading to a reduction in cellular ATP [52], a depletion of catecholaminergic stores [53–55] and increase in cellular

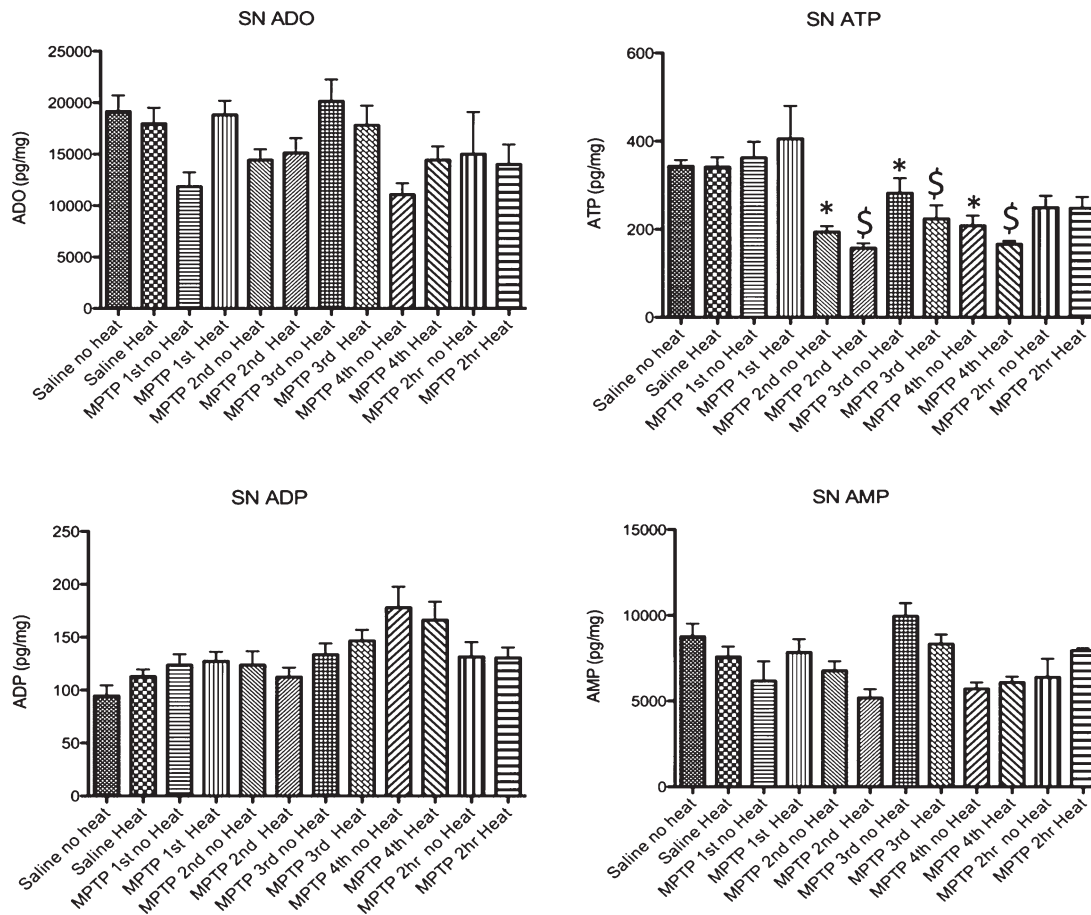


Fig. 4. Effect of MPTP on purine levels in the substantia nigra. Intraperitoneal administration of 4 injections of 20 mg/kg MPTP spaced 2 hours apart does not alter adenosine, ADP or AMP levels. Starting 15 minutes after the 2nd injection of MPTP (135 minutes after the first injection), there is a significant loss of ATP that is maintained until 2 hrs after the last injection, when this purine begins to recover to baseline levels. The MPTP-induced changes in ATP are not affected by the addition of an external heat source. * $p \leq 0.05$ compared to no heat controls; \$ $p \leq 0.05$ compared to heat controls.

adenosine [56]; all of which may contribute to induction of cell death.

To examine if maintenance of body temperature affected purine levels in the brain, both at resting and after MPTP, we used a novel HPLC-ECD detection method that allows simultaneous detection of adenosine, ATP, ADP and AMP from brain tissue [37]. As expected, based on the physiological effects of MPTP on Complex I, we observed a significant drop in ATP starting 135 minutes after the initial administration of MPTP. The timing of this slightly slower than that reported by Chan et al. [57] who reported a significant 20% drop in ATP in the striatum and ventral mesencephalon within 6 minutes, although they did give a dose 2x that of this study. We also find a greater loss of ATP than previously reported (approximately 48% compared to 20%), which could be due to the effects of the multiple administrations of MPTP versus that of a

single injection; further supported by our observation that ATP levels start to recover within 2 hours of the last injection of MPTP.

Like cellular ATP, we also noted a drop in striatal DA occurring 2–4 hours after the start of MPTP administration. This significant loss occurs at the same time as we observed a significant loss of ATP.

Loss of cellular energy (ATP) can reduce the animal's ability to maintain its cellular metabolism and maintain temperature homeostasis as well as maintain critical function of organ systems [58]. The maintenance of core body temperature occurs through a balance between heat generation and heat loss. When heat loss is greater than generation, hypothermia occurs. Since generation of body heat requires energy consumption, including induction of shivering and active vasoconstriction, loss of ATP would be expected to allow for a hypothermic response. Given that there

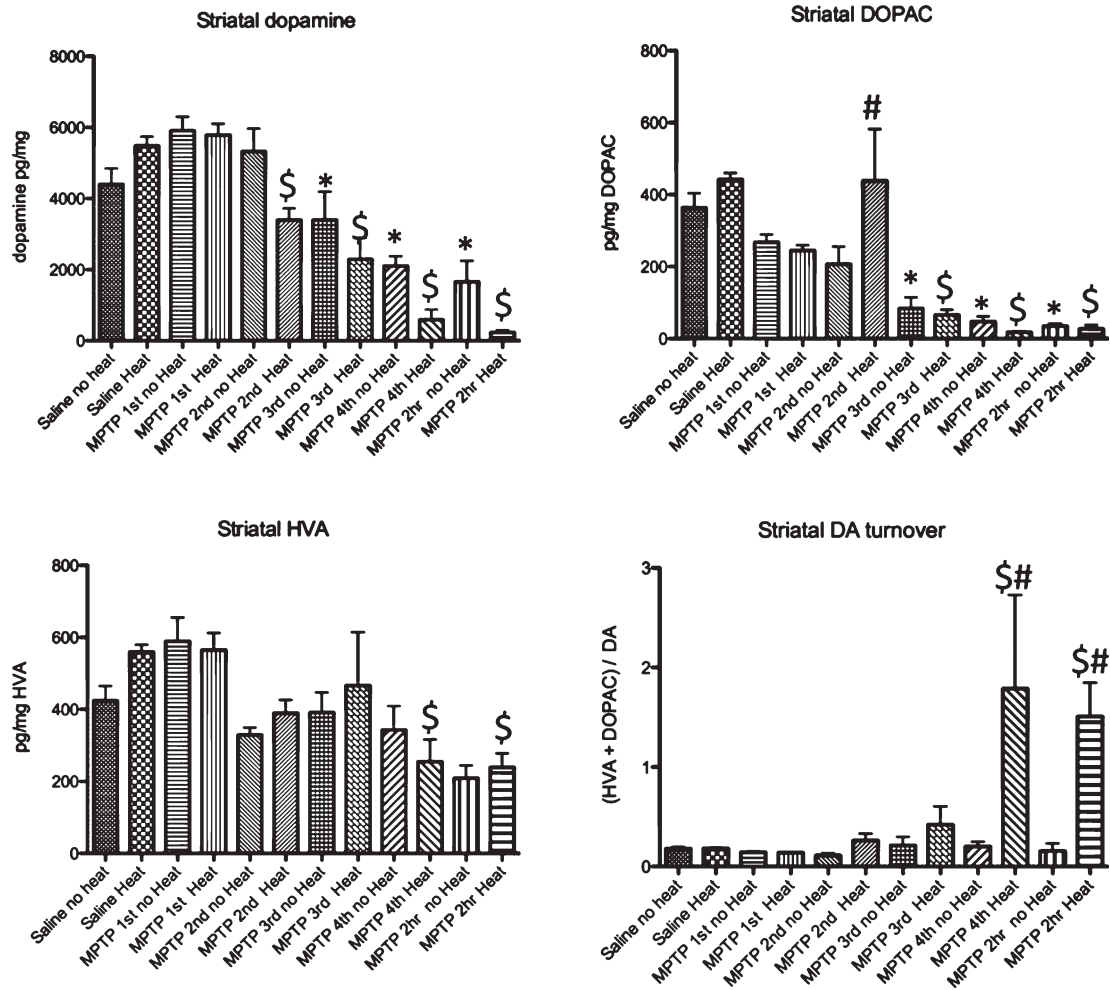


Fig. 5. Effect of MPTP on dopamine DOPAC and HVA levels in striatum.. Intraperitoneal administration of 4 injections of 20 mg/kg MPTP spaced 2 hours apart induces a significant loss of dopamine 15 minutes after the 2nd injection of 20 mg/kg MPTP (135 minutes after the first injection) that is progressive after each subsequent injection. There is a reduction of DOPAC that is seen 15 minutes after the third injection (195 minutes after the first injection). No change in HVA is seen in animals not provided an external heat source, although a small but significant change is seen in animals in heated cages starting 15 minutes after the 4th injection of MPTP (495 minutes after the first injection). 15 minutes after the 4th injection of MPTP, animals in heated cages show an increase in dopamine turnover. * $p \leq 0.05$ compared to no heat controls; $^{\$}p \leq 0.05$ compared to heat controls; $^{\#}p \leq 0.05$ non heated cages compared to heated cages.

appears to be little-to-no expression in skeletal muscle of the dopamine transporter and organic cation transporter 3 [59] or extraneuronal monoamine transporter [60], the effects of peripheral MPTP administration on muscle may be an indirect effects of changes occurring in the CNS. This is supported by studies that have shown that CNS injection of the dopamine antagonist, haloperidol [61], can induce a marked hypothermia.

One major observation we made in this study, and have noted in other experiments in our lab (Smeyne, unpublished observations) was that mice that received MPTP but were not provided with an external heat source did not survive more than 24 hours after the

completion of the MPTP injection regimen. As noted earlier, C57BL/6J mice in this study had a ~31% loss of body temperature that ultimately matched the animal's cage temperature, and this temperature was slightly greater than the reported Fatal Hypothermic Temperature (FHT) of mice [31]. When access was given to an external heat source, these animals still became hypothermic, but the total body temperature loss was only ~17% of its initial core temperature and significantly above the FHT. Since only 20% of these mice died, it appears that this 17% loss in body temperature was sufficiently tolerated by the C57BL/6J mice.

In conclusion, we examined the effect of MPTP and paraquat on core body temperature in C57BL/6J mice. Although both of these chemicals have been reported to induce experimental parkinsonism, only MPTP induced a hypothermic response that without external heat support proved fatal. Since MPTP and PQ work via different cellular mechanisms, it is likely that the peripheral hypothermia that is only seen in mice given MPTP is due its blockade of Complex I and subsequent downstream effects, while PQ, which affects cells via a direct lipid redox mechanism [62], does not affect cellular metabolism and thus has little effect core temperature.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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