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Research report

Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss

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Abstract

The herbicide paraquat, bearing structural similarity to the known dopaminergic neurotoxicant MPTP, has been suggested as a potential etiologic factor in Parkinson's disease. Consideration of paraquat as a candidate neurotoxicant requires demonstration that systemic delivery produces substantia nigra dopaminergic neuron loss and the attendant neurobehavioral syndrome reflecting depletion of dopamine terminals within the striatum. To address these issues paraquat was administered systemically into adult C57 bl/6 mice, ambulatory behavior monitored, substantia nigra dopamine neuron number and striatal dopamine terminal density quantified. The data indicate that paraquat like MPTP elicits a dose-dependent decrease in substantia nigra dopaminergic neurons assessed by a Fluoro-gold prelabeling method, a decline in striatal dopamine nerve terminal density assessed by measurement of tyrosine hydroxylase immunoreactivity; and neurobehavioral syndrome characterized by reduced ambulatory activity. Taken together, these data suggest that systemically absorbed paraquat crosses the blood–brain barrier to cause destruction of dopamine neurons in the substantia nigra, consequent reduction of dopaminergic innervation of the striatum and a neurobehavioral syndrome similar to the well characterized and bona fide dopaminergic toxin MPTP.

Keywords: Parkinson's; MPTP; Paraquat; Tyrosine hydroxylase

1. Introduction

Exposure to environmental herbicides has been associated with an increased risk for the development of Parkinson's disease [4,23,26,42–45]. These studies provide the rationale to explore the mechanism(s) through which candidate herbicides are absorbed, possibly metabolized, penetrate into the central nervous system (CNS) and elicit damage to the dopaminergic neurons with cell bodies in the substantia nigra and projections to the striatum. Paraquat (PQ), a widely used herbicide, has been extensively studied as a both a pulmonary and neurotoxicant [3,6–8,14,25,27,29,32,34,38,39,46,50]. Unlike accidental and high level PQ exposure which produces acute pulmonary toxicity, it is presumed that perhaps chronic lower levels, non-pulmonary toxic doses, produce a different syndrome defined by damage to the basal ganglia and Parkinsonism. While the linkage between PQ exposure and disease appears significant in some populations, the demonstration that systemic administration to mammals leads to dopaminergic neuronal cell loss is lacking. Prior investigations implicating PQ as a dopaminergic neurotoxicant have involved direct intraparenchymal injection thus removing the important contribution of biotransformation and delivery across the intact blood–brain barrier. The present study addresses this issue. Specifically, it examines whether systemic administration of PQ to inbred C57bl/6 mice produces a neurobehavioral syndrome and dopaminergic neurotoxicity. The data indicate that like the established dopaminergic neurotoxicant MPTP, PQ causes dose-dependent reduction in tyrosine hydroxylase (TH) labeled cell bodies and diminished ambulatory activity, a behavioral change correlated with damage to the basal ganglia circuitry.

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2. Materials and methods

2.1. Drug administration

Thirty C57BL/6J adult male mice were treated with either saline, MPTP, or paraquat. All drugs were delivered i.p. with a 26 GA needle in a volume of 0.3 ml. Upon completion of baseline behavioral assessment, animals were randomly distributed into five groups consisting of the following treatments: saline, low dose paraquat (5 mg/kg), high dose paraquat (10 mg/kg), low dose MPTP (10 mg/kg) or high dose MPTP (30 mg/kg). Paraquat (RBI, Natick, MA) was reconstituted in saline and administered in a total of three doses, with each dose separated by 1 week. The drug administration schedule for both compounds was empirically derived utilizing doses and injection times from previous reports as a guide [13–15,41]. Behavioral assessments were carried out 2, 24 and 48 h after the final injection. MPTP (RBI, Natick, MA) was reconstituted in saline and administered in two doses, with each dose consisting of two injections separated by 16 h. It is important to note that the cumulative dose of the entire treatment schedule for low and high dose MPTP were 40 mg/kg and 120 mg/kg, respectively; the cumulative dose for low and high dose paraquat were 15 mg/kg and 30 mg/kg, respectively. Behavioral assessments were carried out 1 week after the final injection. There was no mortality from drug treatments. Fig. 1 depicts the temporal flow of the protocol used.

2.2. Methods of behavioral testing

Horizontal, vertical, and ambulatory locomotor activity measurements were made in an automated device based on infrared beam breaks (Opto-Varimex Minor, Columbus Instruments International, Columbus, OH) at 5 min intervals over the course of a 60 min experimental session on a single day. Each beam was separated by 25.4 mm, with the width of each beam being 3 mm in diameter. Interruption of any horizontal or vertical beam generated an electrical impulse that was collected using the Opto-M data software. Horizontal beams registered only when the mouse moved in a horizontal plane of the test cage; jumping and rearing were registered by the vertical beam. Ambulatory readings were registered when two different consecutive beams were broken, providing an overall assessment of locomotor activity. These devices were located in a sound-attenuated and dimly-lit room. The order of testing of animals was counterbalanced across treatment conditions and activity devices were thoroughly cleaned between all test sessions.

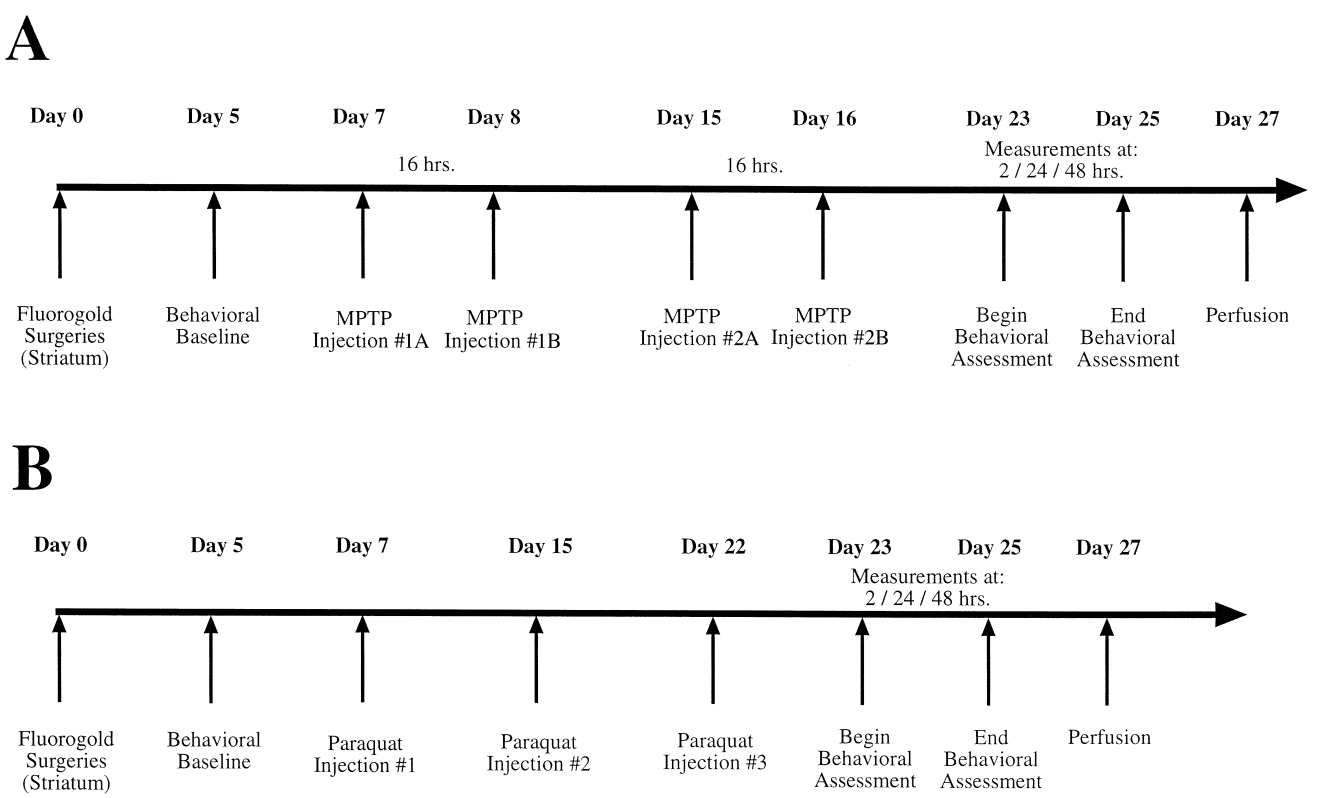

Fig. 1. Drug treatment and behavioral assessment time line. (A) Adult C57bl/6 mice were subjected to FG injections, MPTP treatment and behavioral assessment according to this schedule. (B) Adult C57bl/6 mice were subjected to FG injections, paraquat treatment and behavioral assessment according to this schedule.

2.3. Stereotactic injections of Fluoro-gold

Mice from all groups were anesthetized with 3% halothane in 70% N_2O and 30% O_2 in an induction anesthesia chamber and maintained at 2% halothane during stereotactic intrastriatal injections. After positioning in a mouse stereotactic frame (ASI Instruments, Warren, MI) the skull was exposed via a midline incision, and a unilateral burr hole was drilled over the designated coordinates; Bregma: +0.5 mm; lateral: −2.0 mm; and deep: +3.0 mm. All stereotaxic coordinates were derived using a mouse brain atlas as a guide [12]. A 33 GA needle was gradually advanced to the desired depth over a 5 min period. One hundred nl of a 2% Fluoro-gold solution (in

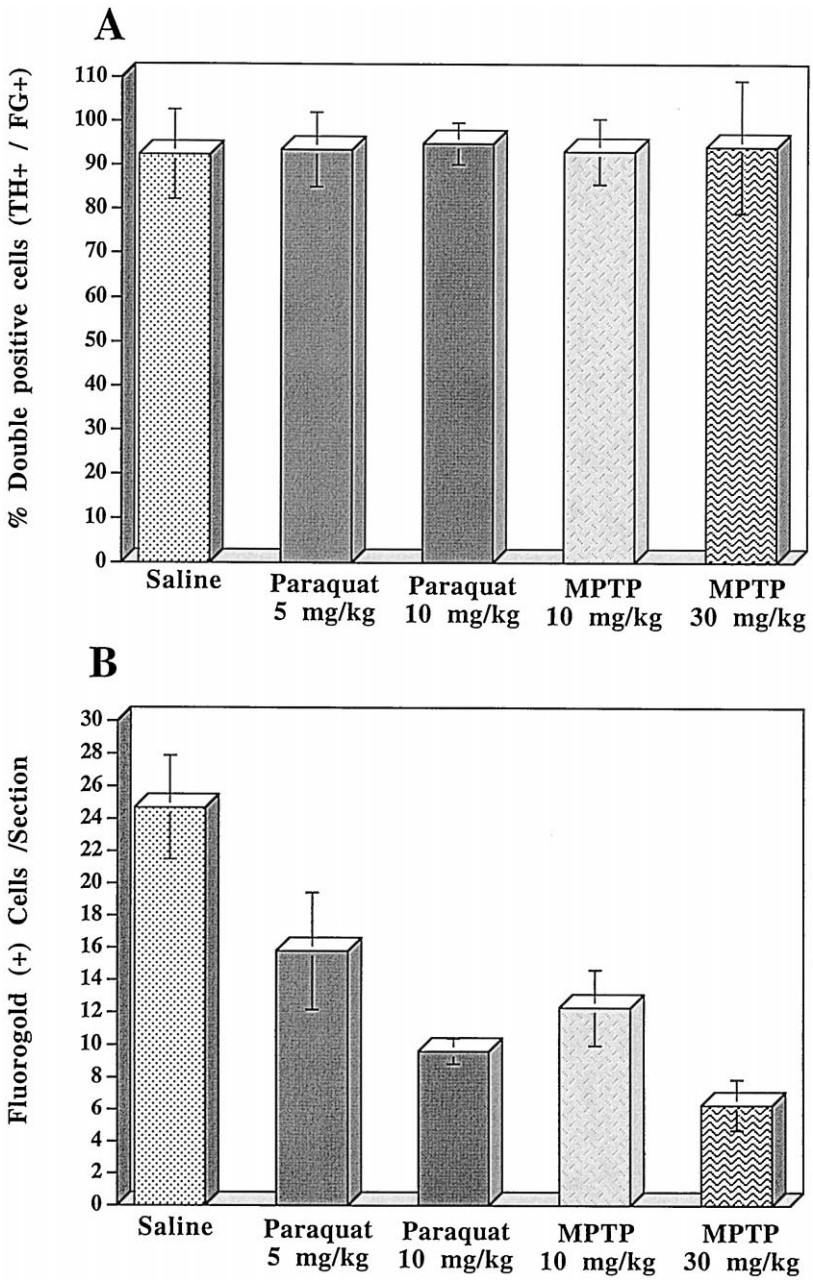

Fig. 2. Fluoro-gold method for assessment of neurotoxicant damage to substantia nigra neurons. FG was injected unilaterally into the striatum of C57bl/6 mice (coordinates relative to Bregma AP +0.5, ML 2.0, DV 3.0). Five days later mice were multiply injected with MPTP or paraquat. Approximately 12 days after the last neurotoxicant dose animals were sacrificed, brains removed, paraffin embedded and sectioned at 6 μm prior to immunocytochemistry. Digitized images of representative sections demonstrating FG and TH labeling were captured. Quantitation of FG + and TH + cell bodies throughout the entire substantia nigra was performed. (A) Double positive (FG + /TH +) cells were scored and expressed as a percentage of FG labeled cells that were also TH labeled ± SEM. (B) FG counts were made on every fourth section and the data expressed as means of FG + cells/section ± S.E.M. The differences in FG + cell number between saline and both MPTP doses ($p = 0.0001$) and saline and both paraquat doses ($p = 0.0077$).

saline; Flurochrome, Englewood, CO) was injected at a constant rate over 7.5 min (10 nl/min). All injections were performed with a microprocessor controlled pump, Ultra-MicroPump (WPI Instruments, Sarasota, FL.; [5]). Upon completion of the injection the needle was removed slowly over 10 min. Animals were returned to the vivarium after recovery from surgery.

2.4. TH immunocytochemistry and Fluoro-gold visualization

Upon completion of behavioral testing mice were anesthetized, a catheter was placed into the left ventricle, and intracardiac perfusion was initiated with 10 ml of heparanized saline (5000 U/l saline) followed by 60 ml of chilled 4% PFA. Brains were extracted and postfixed for 1–2 h in 4% PFA at 4°C. Brains were then transferred to 70% EtOH at 4°C and subsequently imbedded in Paraffin after ethanol post fixation. Brains were cut in 6 μm sections on a microtome and mounted on subbed slides. Before initiating immunocytochemistry, slides were deparaffinized by heat at 55°C for 30 min followed by three 10 min washes in Propar (Anatech, Battlecreek, MI). Slides were then rehydrated in decreasing concentrations of alcohol and then rinsed briefly in ddH_20 to remove any excess alcohol. At this time slides were mounted with Mowiol for Flouro-gold visualization or processing for Tyrosine Hydroxylase immunohistochemistry was initiated. Slides were washed five times for 5 min in TBS followed by 1–2 h of blocking in 10% goat serum, 0.3% Triton X-100, 1% BSA in 1 × TBS at room temperature The blocking solution was washed from the slides and replaced with primary antibody solution containing a 1:500 dilution of rabbit anti-tyrosine hydroxylase (Chemicon International, Temecula, CA) in 1% goat serum, 0.3% Triton X-100, 1% BSA in TBS. Slides were incubated for 1 h at room temperature and then transferred to 4°C for 48 h. Upon completion of incubation with primary antibody, slides were washed five times in TBS for 10 min each wash. TRITC conjugated goat anti-rabbit (1:500) (Molecular Probes, Eugene, OR) secondary antibody was added to each slide in the same solution as used for the primary antibody. Sections were incubated for 1 h. at 37°C and then washed four times in TBS for 10 min each wash. All slides were mounted with Mowiol and imaged. TH positive cells were visualized with a fluorescent microscope (Axioskop, Zeiss, Thornwood, NY) with a TRITC cube (Chroma Filters, Brattleboro, VT). Fluoro-gold labeled cells were also visualized with fluorescence using wide band ultraviolet excitation and a DAPI cube (Zeiss, Thornwood, NY). All images used for morphological analyses were digitally acquired with a three chip color CCD camera at 200 × magnification (DXC-9000, Sony, Montvale, NJ)

2.5. TH densitometry

Visualization of TH positive terminals in the striatum via TH immunocytochemistry was performed as previously described with two exceptions. First, the primary antibody dilution for TH was used at a 1:50 dilution to detect terminal staining in the striatum. Second, detection was performed colorometrically using DAB as the chromagen. Upon completion of incubation with primary antibody, sections were washed five times for 10 min each wash. Biotinylated goat anti-rabbit secondary antibody (1:500) was added to each slide in the same solution as used for primary antibody. Completion of development was done according to manufacturers protocol (Vector Labs, Burlingame, CA). It is important to note that all sections were developed for 8 min in DAB.

Densitometric measurements were made using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). All images were acquired at a final magnification of 50 × in order to capture the entire striatal area in a single field. Subsequently, all images were converted to grey scale and analyzed for staining intensities. 7–10 sections from each experimental group were analyzed, representing at least three animals per group. All measurements were made from striatum on the side contralateral to FG injection. Nine individual sectors from each section, encompassing 15% of the total area, were analyzed to ensure a representative distribution of the entire structure. Background was established by recording the intensity of a brain region negative for TH and using that intensity as the lower limit for detection. The density for all nine regions were averaged to establish a population density for each section. Statistical analysis of relative densities comparing saline and toxin treated groups was achieved using a one-factor ANOVA. Intra and inter-group analysis were then examined using a Fisher's PLSD.

2.6. Morphological analyses

Cell count analyses were performed on digital images acquired within 24 h of immunohistochemistry processing. Fluoro-gold analyses reflects the number of Fluoro-gold positive cells throughout the substantia nigra. All spatial

Fig. 3. Visualization of neurotoxicant damage to substantia nigra neurons and tyrosine hydroxylase terminals in the striatum. FG was injected unilaterally into the striatum of C57bl/6 mice (coordinates relative to Bregma AP +0.5, ML 2.0, DV 3.0). Five days later mice were sacrificed, brains removed, paraffin embedded and sectioned at 6 μm prior to TH immunocytochemistry. Digital images of representative sections demonstrating FG (A) and TH (B) labeling were captured. A small number of double positive cells are labeled by arrowheads (C). FG(+) cell bodies were imaged in the substantia nigra under UV fluorescence in animals treated with saline (D), high dose MPTP (E) or high dose paraquat (F). Tyrosine hydroxylase terminals in the striatum were visualized in animals treated with saline (G), high dose MPTP (H) or high dose paraquat (I).

A FG (+)
B TH (+)
C Double (+)
D Saline
E MPTP
F Paraquat
G Saline
H MPTP
I Paraquat

measurements were acquired using an image analysis program (Image-Pro Plus, Silver Spring, MD) at a final magnification of 200×. Each field was analyzed by a computer macro to count cells based on the following criteria: object area, image intensity (fluorescent signal) and plane of focus. Only cells in which the cell body was unequivocally labeled and nucleus clearly defined were counted. No correction for double counting was made because the sections in which the neurons were counted were 80 μm apart. The distance is significantly larger than the diameter of a single neuronal cell nucleus. Serial sections from each brain that encompassed the anatomical boundary stated above were analyzed. In addition, a watershed separation technique was applied to every field to delineate overlapping cell bodies. The watershed method is an algorithm that is designed to erode objects until they disappear, then dilates them again so they do not touch. Double labeled cells, TH+ and FG+, were counted in the same manner following image superimposition: two images for every field were acquired (one in TRITC wavelength for TH detection and one in the UV wavelength for FG detection). These images were subsequently superimposed and double labeled cells were analyzed using the parameters described above.

2.7. Statistical analysis

Activity counts were analyzed using two factor repeated measures analyses of variance (ANOVA) with treatment condition (dose of drug) serving as a between groups factor and time interval (5 min block) as a within-group variable. These analyses were conducted separately for each treatment (paraquat, MPTP) and for each activity measure (vertical, horizontal and ambulatory). Based on the outcomes, subsequent analyses were carried out using least squares means tests or one-factor ANOVAs as appropriate. Cell counts were analyzed by one-factor ANOVA with dose as the between-group variable.

3. Results

3.1. Paraquat and MPTP elicit dose-dependent reduction of nigrostriatal projection neurons

To facilitate measurements of the extent of substantia nigra dopaminergic cell loss we utilized a retrograde tracing method. Fluoro-gold (FG) stereotactically delivered to the striatum of mice was allowed to retrogradely label substantia nigra projecting neurons prior to compound dosing. We reasoned that most of the projecting neurons that would acquire the tracer would be dopaminergic and thus our analysis could be confined to counting the number of FG cell bodies. The postulate that most FG cell bodies were dopaminergic was confirmed by performing immunostaining for tyrosine hydroxylase (TH) and demonstrating that in all groups 93–95% of FG-labeled cells were also TH immunopositive (Fig. 2A). The high degree of concordance of FG and TH is appreciated by examination of double labeled cells as shown in Fig. 3A–C. These micrographs taken through the substantia nigra reveal that virtually all cells retrogradely labeled by striatally applied FG are indeed immunolabeled for TH.

Analysis of FG labeled substantia nigra cell bodies disclosed a dose dependent decline in animals treated with PQ as well as in those treated with MPTP (Fig. 3D–G). When subjected to quantitation these data (Fig. 2B) revealed that in the low dose PQ-treated mice FG labeled cells were reduced by 36%. In the high dose PQ group FG positive cell bodies were reduced by 61%. MPTP as expected produced a decline in dopaminergic neuron number: In low dose MPTP mice FG labeled cell bodies were lowered to 50% of control; whereas, in high dose MPTP the labeled cell bodies were reduced by 75%.

3.2. Paraquat and MPTP reduce substantia nigra dopaminergic nerve terminal density in the striatum

Loss of substantia nigra neurons should be accompanied by a decline in the striatal density of dopaminergic fibers. To examine this issue sections of striatum from both PQ and MPTP treated animals were immunostained with TH

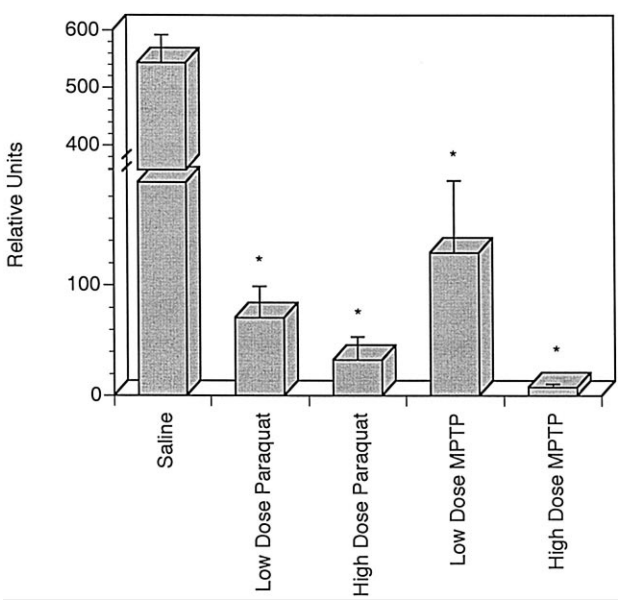

Fig. 4. Neurotoxicant damage to tyrosine hydroxylase containing terminals in the striatum measured by densitometric analysis. Serial sections from the striatum of mice were processed for TH immunocytochemistry. After normalization to background, density measurements were made from digitized images encompassing the entire striatum. Nine independent areas from each striatum were sampled to generate a population density. Densities were compared with a one-factor ANOVA which revealed significant differences between the saline injected animals and low dose paraquat ($p < 0.0001$), high dose paraquat ($p < 0.0001$), low dose MPTP ($p < 0.0002$), and high dose MPTP ($p < 0.0001$) groups.

(Fig. 3G, D, I) and the density of labeling quantitated (Fig. 4). Analyses revealed that low dose PQ reduced the density of striatal dopaminergic terminals by 87%, whereas, high dose PQ produced greater than a 94% decrease. Similar dose-dependent decrements were observed in the MPTP treated groups: Low dose resulted in a 76% decline and high dose led to a 98% reduction. The decreases in TH immunostaining measured for all drug treated groups were significantly different than the saline injected control group.

3.3. Paraquat and MPTP produce similar neurobehavioral effects

In this study C57bl/6 were given repeated injections of two different doses of PQ and MPTP. Motor activity was measured after the last dose of each compound. The most pronounced changes in motor activity occurred during the final sessions in which these assessments were made and were most systematically observed in ambulatory activity

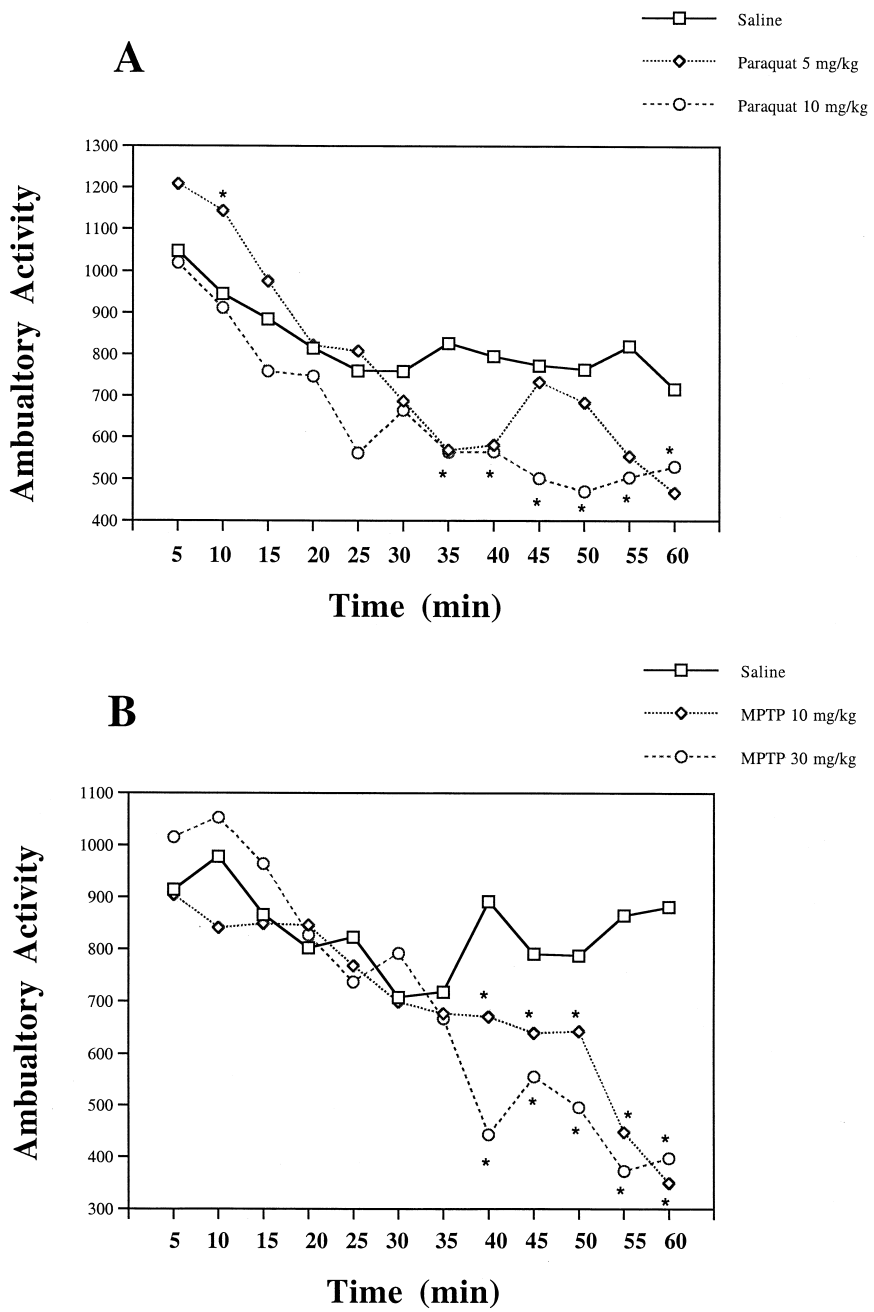

Fig. 5. Decreased locomotor activity in animals treated with paraquat and MPTP. Adult C57bl/6 mice were injected i.p. with paraquat (5 mg/kg or 10 mg/kg thrice) (A), MPTP (10 mg/kg or 30 mg/kg twice) (B), or saline. Ambulatory activity was determined 48 h after the final injection during the course of a 60 min experimental session. Activity counts were recorded in successive 5 min blocks over the session in an automated device. Both doses of paraquat and MPTP decreased ambulatory activity. These effects were confirmed in the corresponding repeated measures analyses of variance for each compound as an interaction of time block by treatment for MPTP ($p = 0.0001$) and as a main effect of treatment in the case of paraquat ($p = 0.003$).

counts which are presented here. The direction of effects presented below was paralleled by similar changes in both horizontal and vertical activity counts.

Changes in ambulatory activity 48 h after the final treatment for both the PQ and the MPTP-treated groups are shown in Fig. 5A and B. While activity levels declined over the course of the 1 h test session following MPTP, the magnitude of the decline was significantly greater in treated mice relative to controls. This was particularly evident during the last 30 min of the session, with activity counts of both treated groups declining to less than 400 counts in each 5 min interval during the final 10 min while control values remained twice that value (interaction of MPTP and time, $F(22,165) = 4.03$, $p = 0.0001$). Subsequent least squares means analyses demonstrated that activity was decreased by both the 10 and 30 mg/kg doses of MPTP (both p's < 0.05) relative to control.

Despite what appeared to be a similar decline in ambulatory activity counts in response to PQ administration, as well as an initial increase in activity in the 5 mg/kg group, the RMANOVA revealed a p value of only 0.10 for the main effect of PQ. This was no doubt due to the collapsing of the PQ effect across time intervals, since analyses of the 0 vs. 5 mg/kg treatment group revealed a significant interaction of PQ over time ($F(11,110) = 1.97$, $p = 0.038$). Subsequent least squares means tests revealed marginally higher activity counts for the 5 mg/kg group during the first 5 min ($p = 0.07$) and significantly higher counts during the second 5 min ($p = 0.026$). The significant interaction of PQ by time for this group is also indicative of differential slopes for the activity values of the two groups across time. Furthermore, direct statistical comparison of the saline and 10 mg/kg paraquat groups revealed a highly significant decrease in activity counts ($F(1,9) = 16.61$, $p = 0.0028$), with values of the 10 mg/kg group declining to levels generally between 400–600 counts while controls remained between 700–800 counts.

4. Discussion

An epidemiological linkage between herbicide use and Parkinson's disease has been established in some populations [4,26,45]. That the risk for developing Parkinson's disease is increased in individuals exposed to herbicides like PQ suggests these compounds must gain access to the CNS where they compromise dopaminergic neurons. Exposure to PQ could occur via inhalation, per oral ingestion or perhaps transdermally. Prior studies in which dopaminergic neuronal toxicity has been suggested have injected PQ intracerebrally to elicit CNS toxicity [25]. Moreover, in these studies the direct neurotoxic effect on the viability of projecting nigrostriatal neurons was not examined. Since Parkinson's disease is histopathologically defined by loss of substantia nigra dopaminergic neurons we sought to establish that PQ is a dopaminergic neurotoxin following systemic administration. A fluorescent retrograde tracing method was employed which involved deposition of FG in the striatum to prelabel a subfraction of dopaminergic nigrostriatal cell bodies that serve as a reporter population for toxicant damage [35,36]. Our studies employed the C57 bl/6 mouse since it has been previously used for MPTP studies [9,10,15,16,28,30,37] and is amenable for behavioral analyses [2,15,31,47,49]. The results of these experiments indicate that several repeated doses of PQ and MPTP are sufficient to produce a neurobehavioral syndrome characterized by reduced locomotor activity. Most importantly, PQ, like MPTP systemic administration, elicits a dose-dependent decline in dopaminergic neurons in the substantia nigra and reduction of dopaminergic nerve terminals in the striatum.

For PQ to have produced dopaminergic neuronal demise the compound must have crossed the blood–brain barrier (BBB). Studies using ^{14}C PQ indicate that the herbicide when given systemically does partition in the CNS presumably by penetration of the endothelium comprising the BBB (Lindquist et al. [24]). In our studies we breached the barrier by the stereotactic delivery of FG and it is possible that it had not resealed at the time (5 days) when the first dose of PQ was given. This is unlikely given preliminary data from our laboratory which indicates that 24 h after similar stereotactic surgery to deliver saline the BBB can exclude serum-derived immunoglobulins (Olschowka, Brooks and Federoff, unpublished data) and as well other data using horseradish peroxidase [48]. These data would then indicate that after the 5 days, the period between FG stereotactic delivery and systemic PQ injection, the BBB had reformed. Thus, the dopaminergic neuronal degeneration we measured after PQ administration reflects penetration of the BBB and access to dopaminergic neurons in the substantia nigra.

To produce dopaminergic neuron loss the PQ would be expected to localize within the substantia nigra or perhaps in the striatum, the field of distribution of the projecting neurons. Prior autoradiographic studies with systemically applied ^{14}C PQ show that the compound is most highly confined to neuromelanin producing cells [24] such as those in the pars compacta of the substantia nigra. Such a pattern of distribution suggests that PQ can preferentially enter and/or be maintained in cells which elaborate neuromelanin. Dopamine biosynthesis and metabolism, notably oxidation is the basis for accumulation of neuromelanin [18]. Whether PQ localization in these cells relates to efficient uptake through the dopamine transporter (DAT) as is the case for MPP + is presently unclear [21]. Regardless of the specific mechanism for its accumulation, PQ is in the appropriate anatomic site to compromise dopaminergic neurons.

PQ has been demonstrated in a number of studies to stimulate free radical generation that subsequently causes lipid peroxidation and promotion of cell death [11,39,50]. It is presumed that PQ through electron transfer reactions with NADH-dependent oxidoreductases results in the gen-

eration of superoxide, a highly reactive oxygen species (ROS; [39,50]). Indeed, mice deficient in Cu/Zn SOD have marked sensitivity to PQ; 100% of SOD knockout mice die with 5 days following the same 10 mg/kg dose used in our study [19]. This is in marked contrast to heterozygous or wildtype mice which do not succumb to this dose of PQ. The critical question in terms of substantia nigra dopaminergic neuronal vulnerability is whether the failure to dismute or otherwise detoxify the superoxide radical is a mechanism underlying PQ-induced neuronal cell loss. One critical feature of the mammalian substantia nigra that may contribute to its susceptibility to ROS injury is its relatively low level of GSH [40]. This appears to be correlated with an increased level of γ-glutamyltranspeptidase, the GSH degradative enzyme [40]. Whether the lower level of GSH is a cause or consequence of the pathogenic sequence leading to PD remains to be determined. However, although speculative, consideration should be given the hypothesis that chronic and low level PQ-induced redox cycling within substantia nigra dopaminergic systems may exceed the oxidative defenses of these cells and thus produce crippling intracellular events including the activation of an apoptotic cascade.

Damage to striatal dopaminergic nerve terminals is observed in PD and is postulated to precede somal loss [20]. In our study, dopaminergic nerve terminal density was examined by TH immunocytochemistry and found to be reduced at the both the low and high doses of PQ and MPTP. The mechanism(s) underlying terminal disruption could include diminished synthesis and/or transport of macromolecular components, reduced responsiveness to a striatally-supplied trophic influence, and localized activation of membrane oxidative events. Given the propensity of PQ to stimulate lipid peroxidation we favor the latter mechanism. This speculation would be supported if PQ, like MPP+, could be internalized by dopamine transporters which are highly expressed within the terminals of substantia nigra dopaminergic neurons [17,33].

An involvement of nigrostriatal dopamine systems in motor function and response initiation has been well-established [1]. Although the specific contribution of nigrostriatal system damage to altered locomotor activity per se is less clearly understood [22], the current decrements in measures of motor activity produced both by MPTP and PQ are consistent with previous reports. Fredriksson et al. [14] reported decreases in spontaneous motor activity at both 60 and 120 days of age following neonatal oral exposures to paraquat or MPTP, demonstrating the permanent nature of the deficits. Exposures of adult mice to MPTP have also been associated with hypoactivity [13,15]. It cannot be determined from the current study whether the decline in spontaneous motor activity could also reflect damage to mesolimbic DA systems. Other investigators have found substantially less impact of high dose MPTP in C57/BL/6 mice on nucleus accumbens as compared to dorsal striatum [41]. When measured 4 weeks after treatment, MPTP depleted striatal dopamine by 80% while the corresponding value for nucleus accumbens was 36%. These data argue for a significant effect of MPTP on nigrostriatal but not mesolimbic dopamine systems. Although our study was not designed to measure mesolimbic effects the histologic picture of this region in PQ treated mice did not disclose discernible damage. Thus our PQ results are most consistent with a predominant nigrostriatal action.

This study adds additional credence to the idea that the widely used herbicide, PQ, can penetrate the CNS and produce lethal injury to substantia nigra dopaminergic neurons and production of a neurobehavioral syndrome. While the clinical epidemiologic data support an etiologic role for PQ in the pathogenesis of PD the observation has been made in only some populations. Altered susceptibility could represent an additional environmental factor or perhaps a genetic contribution to vulnerability. Focus on the oxidative mechanisms that render dopaminergic neurons susceptible to PQ injury may fuel the discovery of new gene candidates that determine vulnerability.

Acknowledgements

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