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Grid performance test to measure behavioral impairment in the MPTP-treated-mouse model of parkinsonism

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Abstract

Behavioral impairments in mice following administration of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) require large depletions in striatal dopamine content and are often transient. In this paper, we describe a simple and inexpensive test that measures long-term behavioral deficits in mice treated with moderate doses of MPTP. These measures are significantly correlated with the loss of striatal dopamine and immunoreactivity of the dopamine transporter, vesicular monoamine transporter and tyrosine hydroxylase. In addition, behavioral impairments on the measures were reversed following L-DOPA administration. Employment of this test will allow for more efficacious use of mice in PD research, as well as provide more sensitive measures of behavioral improvement following potential therapeutic or neuroprotective interventions.

Keywords: Behavioral assessment; MPTP; Mice; Parkinson's disease; Motor impairment

Parkinson's disease (PD) is behaviorally characterized by akinesia, tremor, rigidity, postural instability and abnormalities of gait. The neuropathological manifestations of the disorder have been well characterized and include a relative selective loss of dopaminergic neurons in the nigrostriatal pathway, complex I inhibition and the presence of inclusion bodies (Olanow and Tatton, 1999). Several rodent models of PD have been developed in both rats and mice. Two of the most common rat models are the reserpinized rat and the unilateral 6-hydroxydopamine rat. Both of these models have been invaluable tools in advances in pharmacotherapy, as well as for testing new intervention, such as growth factors (Cotzias et al., 1967; Zigmond and Stricker, 1989; Olsson et al., 1995; Tillerson et al., 2001). Both of these models provide dopaminergic degeneration, as well as reflect many of the behavioral manifestations of the disorder (Schallert et al., 1978; Olsson et al., 1995; Schallert and Tillerson, 2000; Ballermann et al., 2001).

The primary mouse model of PD is produced by systemically administering the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Administration of MPTP dose-dependently results in decreased content of striatal dopamine and its metabolites, decreased striatal DA terminals and complex I inhibition (Heikkila et al., 1984; Fuller and Hemrick-Luecke, 1985; Sundstrom et al., 1987; Willis and Donnan, 1987). The use of MPTP in animal models of PD began following the inadvertent injection of the drug by several humans that resulted in a Parkinsonian syndrome (Langston et al., 1983). In particular, these patients suffered from a selective depletion of dopamine and a loss of dopaminergic neurons resembling that of idiopathic PD. Some of the major differences between MPTP induced parkinsonism and idiopathic PD include the absence of motor impairments in some species treated with MPTP and the rapid and static nature of the deficit (Heikkila and Sonsalla, 1992). However, the remarkable selectivity of the anatomical lesions, neurological manifestations in many species and the response to L-Dopa therapy have led some to conclude that an 'MPTP-like' molecule

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could underlie many cases of idiopathic PD (Snyder and D'Amato, 1986).

MPTP is a highly lipophilic compound and thus can easily cross the blood–brain barrier following systemic administration. Once in the brain, it is converted to its active metabolite 1-methyl-4-phenylpyridinium (MPP+) via monoamine oxidase B contained within non-dopaminergic cells, such as glial. MPP+ enters the dopaminergic neurons through the dopamine plasma membrane transporter (DAT) (Kopin and Markey, 1988; Zigmond et al., 1990). Once in the cytoplasm, MPP+ is packaged into vesicles by the vesicular monoamine transporter (VMAT2), which is normally responsible for transporting dopamine from the cytosol into the vesicles for re-use (Edwards, 1993). Blocking the DAT using specific antagonists to the transporter or genetic deletion results in prevention of MPTP-induced toxicity (Melamed et al., 1985a,b,c; Gainetdinov et al., 1997; Bezard et al., 1999), conversely increased DAT expression results in an increase in MPTP-induced toxicity (Kitayama et al., 1993). It is interesting to note that there is inherent variation in DAT density in humans and nonhuman primates and this variability in expression may reflect an inherent risk of developing PD (Haber et al., 1995). Once MPP+ accumulates in the neurons of the SNc, it inhibits complex I of the electron transport chain (Nicklas et al., 1985, 1987; Vyas et al., 1986; Ramsay et al., 1991). This inhibition of mitochondrial respiration results in many widespread effects on ATP-dependent cellular processes. One such effect is a perturbation of calcium homeostasis resulting in the loss of mitochondrial capacity to buffer intracellular calcium (Carafoli, 1987; Kass et al., 1988). In addition, inhibition of complex I via MPP+ leads to an increase in free radical formation (Hasegawa et al., 1990; Cleeter et al., 1992). A similar cascade of events including complex I inhibition and free radical formation is believed to contribute to the formation of idiopathic PD.

One interesting feature of the MPTP mouse model of PD is the transient nature of the striatal damage in young mice (Willis and Donnan, 1987; Mitsumoto et al., 1998; Fornai et al., 2000). In contrast, administration of the drug to older mice, such as retired breeder mice, results in a permanent loss of nigrostriatal terminals, as well as cell bodies (Langston et al., 1987). The cause of the transient nature in young mice is speculative. One possibility may be age-dependent changes in growth factor expression within the striatum (Ho and Blum, 1998). This spontaneous recovery in young mice allows for a model of both striatal DA loss and subsequent recovery.

Recent advances, such as the generation of mice that express the putative genetic factors involved in PD, reinforce the importance of an established mouse model of PD. Though the pathophysiological alterations in adult mice are very similar to idiopathic PD, the MPTP mouse model of PD is limited by its inability to demonstrate persistent motor deficits. Indeed, to obtain consistent behavioral deficits on standard mouse motor tests such as the rotorod, hangtest and general activity monitoring, very large doses of MPTP (e.g. 30 mg kg^{-1} twice a day for 5 consecutive days) must be used and behavioral alterations are often still transient (Ogawa et al., 1985; Willis et al., 1988; Heikkila et al., 1989; Colotla et al., 1990; Sundstrom et al., 1990; Heikkila and Sonsalla, 1992; Fredriksson and Archer, 1994; Fredriksson et al., 1997; Rozas et al., 1998; Spooren et al., 1998; Sedelis et al., 2001). Unfortunately, without measurable behavioral correlates to striatal dopamine integrity, this model fails to replicate a vital characteristic of the disorder and limits its utility in PD studies. The present paper describes a new method for measuring behavioral deficits following moderate doses of MPTP in both retired breeder and young C57BL/6J black mice.

1. Methods

1.1. Subjects

Thirty-seven retired-breeder C57BL/6J (between 9 and 10 months of age; average weight 32 g) and 30 young C57BL/6J mice (3 months; average weight 23 g) (Jackson Laboratory, Bar Harbor, Maine), singly housed, were used for these experiments. Animals were handled daily for 2 weeks prior to injection, in order to make them amenable to behavioral testing. All mice were maintained on a standard 12:12 light/dark cycle and given ad libitum access to lab chow and water. All behavior testing was performed during the animals' dark cycle.

1.2. Injections

Mice were injected (s.c.) twice with either 7.5 mg kg^{-1} (2×7.5), 15 mg kg^{-1} (2×15) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (M0896, Sigma, St. Louis, MO) or saline with an inter-injection interval of 12 h (first injection, 18:00 h; second injection, 06:00 h) as outlined in Section 1.7. A subset of retired breeder mice were also given a 15 mg kg^{-1} dose of L-Dopa (D1507, Sigma) on day 28 post-injection (20 min before L-Dopa administration, animals were given 12.5 mg kg^{-1} benserazide (B7283, Sigma) a peripheral dopa decarboxylase inhibitor) and subjected to behavioral tests 20 min post-L-Dopa injection. The dose of L-Dopa used in these animals has been previously demonstrated to improve hypoactivity in MPTP treated mice without causing hyperactivity (Fredriksson et al., 1990).

1.3. Grid test

The grid apparatus consisted of a horizontal grid mesh (total size 12 cm^2: openings 0.5 cm^2) mounted 20 cm above a hard surface, thus discouraging falling, but not leading to injury in the case of falling. The apparatus was equipped with a 3-inch wall that can be made of any opaque, sturdy material, such as black plexi-glass. The wall was mounted onto the grid at a 90° angle and did not restrict movement on the grid. Finally, the edges of the grid were blunted to prevent any injury to the animal if they placed their paws between the wall and the edge of the grid (Fig. 1A). When constructing this apparatus, special attention was given to the size of the openings on the grid. The described dimensions above allowed for the animal to completely place its paw within one square. Smaller openings made grasping the grid more difficult, while larger openings made it more difficult for the animal to cross the grid. In addition, the wire mesh used to make the grid must be sturdy enough to easily maintain the weight and movement of the animals.

Mice were lifted by their tail and slowly placed in the center of the horizontal grid and supported until they grabbed the grid with both their fore- and hind-paws. The grid was then inverted so that the mice were hanging upside down (Fig. 1A). Animals were videotaped while hanging upside down for 30 s and videos

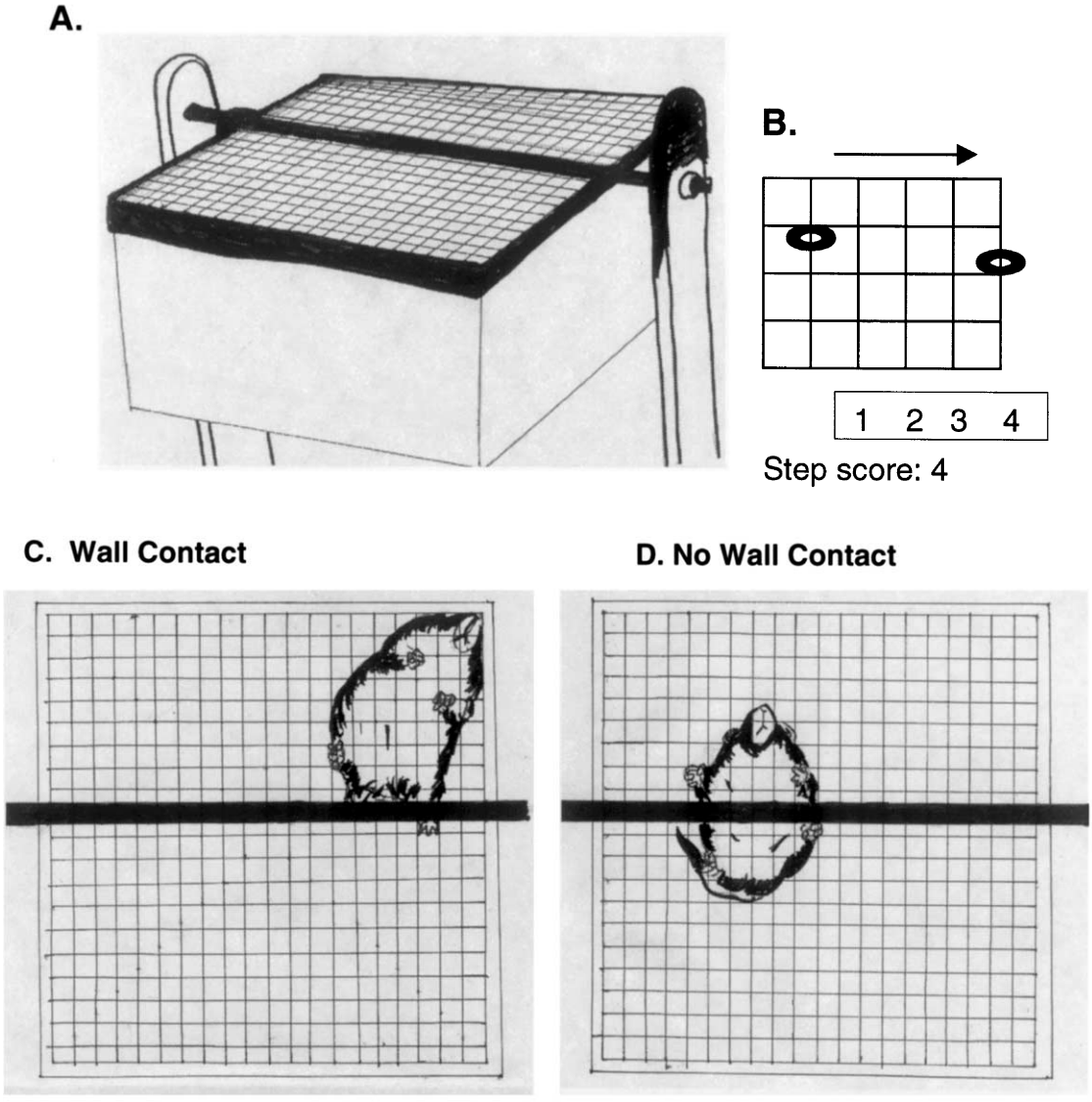

Fig. 1. Grid design and rating. (A) Line drawing of inverted grid, as positioned during testing (grid size: 12 cm^2, openings: 5 cm^2). (B) Example of forepaw step. This diagram displays a section of the grid. Each oval represents the forepaw of one animal. The successful shift and replacement of the forepaw results in a forepaw step score of 4. The size of the grid openings in this diagram are representative of the actual grid openings. (C) Line drawing depiction of an animal in contact with the wall. (D) Line drawing depiction of an animal in the center of the grid, not in contact with the wall.

replayed for analysis of the following measures using a recorder with slow motion and frame-by-frame option.

1.3.1. Average forepaw step distance

The length of each successful forepaw step was measured as the number of grid squares/openings transversed. The average forepaw distance was calculated by summing the distances for each successful step then dividing by the number of successful steps. A successful step was defined by a release of the paw on the grid, movement of the paw to another area on the grid and placement of the paw on the grid with fingers closed around the grid, thus resulting in a shift from the starting grid position to the destined grid position without the paw slipping or the animal not completely grasping the grid (Fig. 1B).

1.3.2. Percent wall time

The time spent in physical contact with the surrounding wall divided by the total time on the grid $\times 100$ was recorded. Contact with the supporting wall was defined as physical contact with the supporting wall with either the head or trunk of the body. Contact with just the tail was not included. When an animal contacted with the wall, there was an apparent leaning or displacement of body weight against the wall. When the animal was exploring the grid and happened to contact the wall, but not stop and displace weight or lean against it, this time was not counted. If an animal moved back and forth on the grid, but only while leaning against the wall, this time was counted. Most measures of wall contact were obvious to the rater (Fig. 1C, D).

1.3.3. Percent forepaw faults

The number of unsuccessful forepaw steps divided by the total number of attempted forepaw steps was recorded. An unsuccessful step was defined as an attempt to step or place the forepaw during a weight shifting (place shifting) movement in which the paw was unsuccessfully placed onto its destined position and had to be replaced on the grid by the animal so as to regain weight-bearing control. The designation of a weight shift movement is very important in this measure. Often times, mice will hang and shuffle their paws across the grid, without an initiation of movement. This measure is not correlated to DA loss and should not be included as a forepaw fault.

1.4. High performance liquid chromatography

Animals were anesthetized with carbon dioxide and decapitated. A 1-mm section of the striatum (+1 mm to bregma) was dissected for both the left and right hemispheres. The left hemisphere was used in HPLC analysis, while the right hemisphere was used for Western blot analysis. Dissected left striata were sonicated in 0.1 M perchloric acid containing 347 μM sodium bisulphite and 134 μM EDTA disodium salt. Homogenates were centrifuged at $16{,}000 \times g$ for 20 min at 4 °C and the supernatant removed. The supernatants were centrifuged at $16{,}000 \times g$ and the supernatants analyzed for levels of DA, 3,4-dihydroxiphenylacetic acid (DOPAC) and homovanillic acid (HVA) by HPLC (Column, HR-80, 4.6 mm × 8 cm, 4-channel coulometric electrode array (Model 5600, ESA Inc., Chelmsford, MA) with sensitivity to femtomole levels). The mobile phase consisted of 16 mM citric acid monohydrate, 32 mM ammonium acetate, 215 μM EDTA disodium salt, 850 μM 1-octanesulfonic acid sodium salt monohydrate (final pH 2.5) and 5% methanol (delivered at a constant flow rate of 1 ml min^{-1}). Quantification was made by reference to calibration curves made with monoamine standards (dopamine hydrochloride (H8502, Sigma), DOPAC (D9128, Sigma) and HVA (H1252, Sigma)).

1.5. Western blot analysis for DAT, VMAT2 and TH

Right striata samples were homogenized in buffer (320 mM sucrose and 5 mM HEPES containing protease inhibitor cocktail). Homogenized samples were centrifuged at $2000 \times g$ for 5 min and supernatant centrifuged at $30{,}000 \times g$ for 30 min. The final pellet was resuspended in homogenization buffer and subjected to polyacrylamide gel electrophoresis (NuPage, 10% Invitrogen). Samples were electrophoretically transferred to a polyvinylidene difluoride (PVDF) membrane and nonspecific sites blocked in 7.5% nonfat dry milk in Tris-buffered saline (135 mM NaCl, 2.5 mM KCl, 50 mM Tris and 0.1% Tween-20, pH 7.4). Membranes were then incubated in a polyclonal antibody to the C-terminus of VMAT2 (pAB VMAT2-Ct; AB1767, Chemicon, Temecula, CA) in Tris-buffered saline with 2% nonfat dry milk. VMAT2 antibody binding was detected using a goat anti-rabbit horseradish peroxidase secondary antibody (170-6515, Bio-Rad, Hercules, CA) and enhanced chemiluminescence (CG50450, Pierce, Rockford, IL). The chemiluminescent signal was captured on an Alpha Innotech ChemiImager (San Leandro, CA) and stored as a digital image. Densitometric analysis was performed and calibrated to co-blotted dilutional standards of control striata and exposures performed within the linear range. Control striata for the standards were pooled from all saline treated animals. Membranes were then stripped for 20 min at 80 °C (8 M urea, 100 mM 2-mercaptoethanol and 62.5 mM Tris, pH 6.8) and re-probed with a monoclonal antibody to the N-terminus of DAT (rat anti-dopamine transporter antibody ((Miller et al., 1997) (MAB369, Chemicon) (goat anti-rat secondary (56400, ICN, Costa Mesa, CA))), a polyclonal TH antibody (rabbit anti-tyrosine hydroxylase (AB152, Chemicon)) and alpha-tubulin ((T9026,

Sigma) goat anti-mouse secondary (170-6516, Bio-Rad)).

1.6. Statistics

Neurochemical measures were analyzed by applying a one-way ANOVA for each group. When significance between treatment groups was achieved, post-hoc comparisons of each treatment group to saline control and when applicable, comparisons to treatment at different time-points were performed. Behavioral observations were first subjected to traditional overall, repeated measures ANOVAs. Interactions for all retired breeder behavioral data were a result of the lesion-induced shift from pre-lesion measures. Therefore, elimination of pre-test from the analysis resulted in a loss of interaction, and only Dunnett's post-hoc comparison of group, using saline controls as reference group, was performed. Young C57BL/6J mouse behavior was analyzed by comparing each post-injection day to the pre-lesion scores for treated animals. This analysis allowed for demonstration of both significant MPTP-induced behaviors and recovery over time. All post-hoc measures were Bonferroni corrected to keep the overall error rate per group at 0.05. Finally, neurochemical measures were correlated to behavioral scores on the final day of behavioral testing using a two-tailed person product moment correlation. Animals included in the correlational data were both retired breeder and young mice in all treatment groups.

1.7. Experimental design

Retired breeder males received two injections (separated by 12 h) of either 7.5, 15 mg kg^{-1} MPTP or saline, and behavioral analysis was performed prior to injection, and on days 7, 14, 21 and 28 post-second injection. A subset of animals received L-Dopa, following normal testing on day 28 and were tested for behavioral impairments on the grid. They were then sacrificed on day 30 post-injection. Young C57BL/6J mice were exposed to two injections of either 15 mg kg^{-1} MPTP or saline. Twelve animals (saline = 6; MPTP = 6) had behavioral analysis performed prior to injection, 48 h, 7 and 28 days post-injection, similar to retired breeder mice. These animals were also sacrificed on day 30-post injection. Young C57BL/6J mice exposed to moderate doses of MPTP display 'spontaneous' recovery of striatal DA content and no SN cell loss (Mitsumoto et al., 1998; Fornai et al., 2000). Because of this, nine young mice were sacrificed 48 h post-second injection (saline = 3; MPTP = 6) and nine mice were sacrificed 7 days post-second injection to verify transient DA loss. No behavioral analysis was performed on young mice sacrificed either 48 h post-injection or on day 7.

2. Results

2.1. MPTP injection in retired breeder mice resulted in long-term behavioral deficits

Upon removal of pre-injection measures, an overall group effect for step distance on the grid remained (F (2,34) = 115.786, $P < 0.001$). Post-hoc comparisons revealed a significant overall decline in step distance across testing days in animals given 2 × 7.5 MPTP ($P < 0.001$) as well as animals given 2 × 15 MPTP ($P < 0.001$) when compared to saline controls. Thus, administration of either dose of MPTP resulted in long-term decreased step distance on the grid compared to saline control (Fig. 2A).

When pre-injection scores were eliminated from the analysis, an overall Group effect for percent wall time (F (2,34) = 52.887, $P < 0.001$) remained. Post-hoc analysis demonstrated a significant increase in reliance on the wall in both 2 × 7.5 ($P < 0.01$) and 2 × 15 ($P < 0.001$) groups compared to saline controls. In addition, a dose effect in wall contact time was also measured, with animals given 2 × 15 mg kg^{-1} MPTP spending significantly more time in contact with the wall than animals given 2 × 7.5 MPTP ($P < 0.001$) (Fig. 2B).

As seen in step distance and percent wall time measures, the initial shift from pre-injection scores in both treatment groups was responsible for the Day effect and Group × Day interaction and removal of this time point from the analysis resulted in a sustained Group effect (F (2,34) = 28.101, $P < 0.001$) for percent forepaw faults on the grid. Post-hoc comparisons again revealed a significant long-term impairment as measured by increased forepaw faults on the grid in both 2 × 7.5 ($P < 0.001$) and 2 × 15 ($P < 0.001$) MPTP treatment groups compared to saline controls (Fig. 2C).

2.2. Behavioral performance on the grid is significantly improved following L-DOPA administration in retired breeder mice

L-DOPA administration on day 28 post-injection resulted in significant improvement on step distance scores in both 2 × 7.5 (F (1,10) = 34.916, $P < 0.001$) and 2 × 15 (F (1,18) = 6.359, $P < 0.05$) animals compared to pre-L-DOPA scores (Fig. 3A). In addition, 2 × 15 animals also displayed a significant decrease in the percent wall time following L-DOPA administration (F (1,18) = 10.535, $P < 0.01$) (Fig. 3B). Animals administered 2 × 7.5 MPTP did not show a decline in time against the wall following L-DOPA administration; this could be due to several factors. We suspect this was due to the already apparent decline in contact with the wall by 28 days post-lesion in this group, thus allowing for only a limited amount of recovery potential. Finally, L-DOPA administration led to an apparent decrease in the

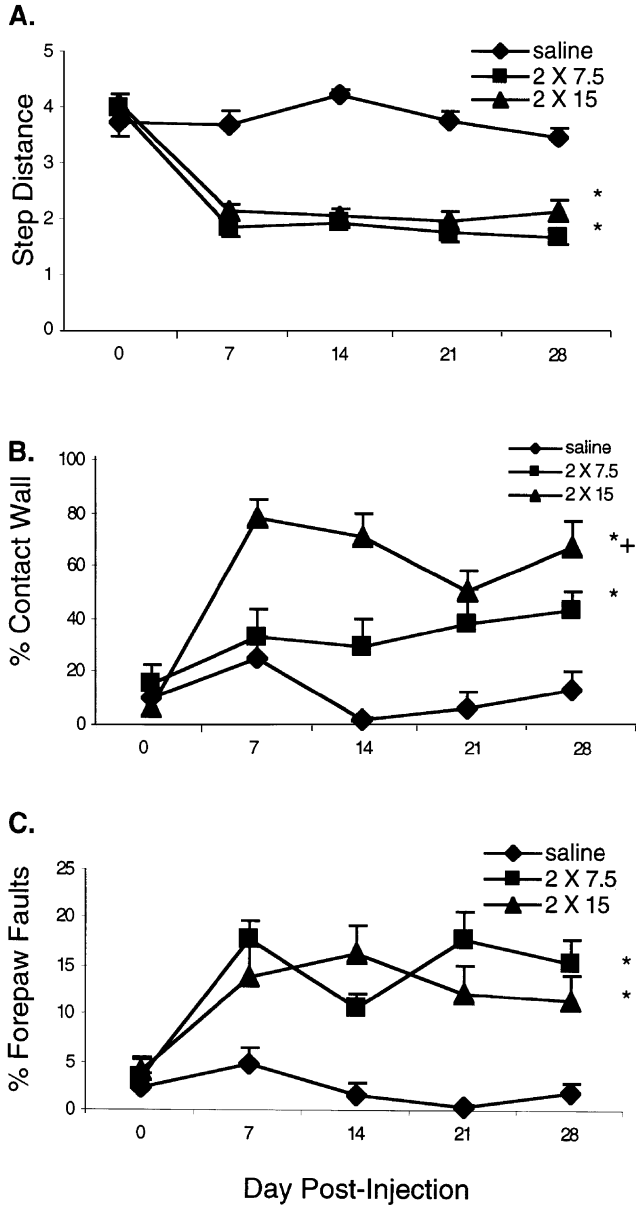

Fig. 2. Moderate doses of MPTP resulted in sustained behavioral impairments in retired breeder mice. (A) Both treatment groups displayed sustained and significant decreases in step distance on the grid following MPTP injection. (B) Both treatment groups showed sustained increases in forepaw faults following MPTP injection. (C) Both MPTP treatment groups spent more time in contact with the supporting wall following injection. This reliance lasted across testing days. (* <0.05 compared to saline controls; + <0.05 compared to 2×7.5 group).

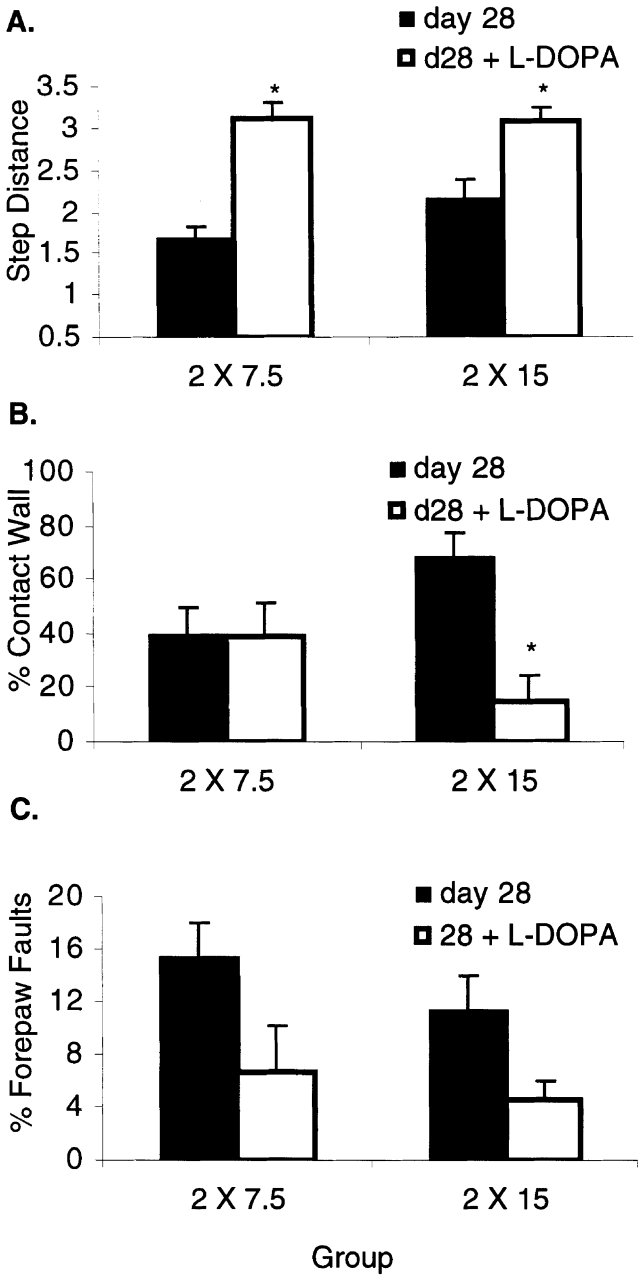

Fig. 3. Behavioral performance in retired breeder mice given MPTP was improved following L-Dopa administration. (A) In both treatment groups, a significant increase in step distance was observed following administration of L-Dopa. (B) Animals given 2×15 MPTP showed a significant decline in contact time with the supporting wall following administration of L-Dopa (* <0.05 compared to day 28 score). (C) An apparent decrease in the number of forepaw faults was observed following L-Dopa treatment, though these analyses did not reach statistical significance.

percent of faults in both treatment groups, but this difference did not reach statistical significance (Fig. 3C).

2.3. MPTP injection resulted in significant loss of striatal DA and DA markers in retired breeder mice

Injections of either 2×7.5 or 2×15 mg kg^{-1} MPTP resulted in a significant decline in striatal DA content (Fig. 4A) (overall: $F(2,34) = 57.384$, $P < 0.001$; saline versus 2×7.5, $P < 0.001$; saline versus 2×15, $P < 0.001$; 2×7.5 vs. 2×15, $P < 0.05$). In addition to overall DA content, immunoreactivity of striatal DA

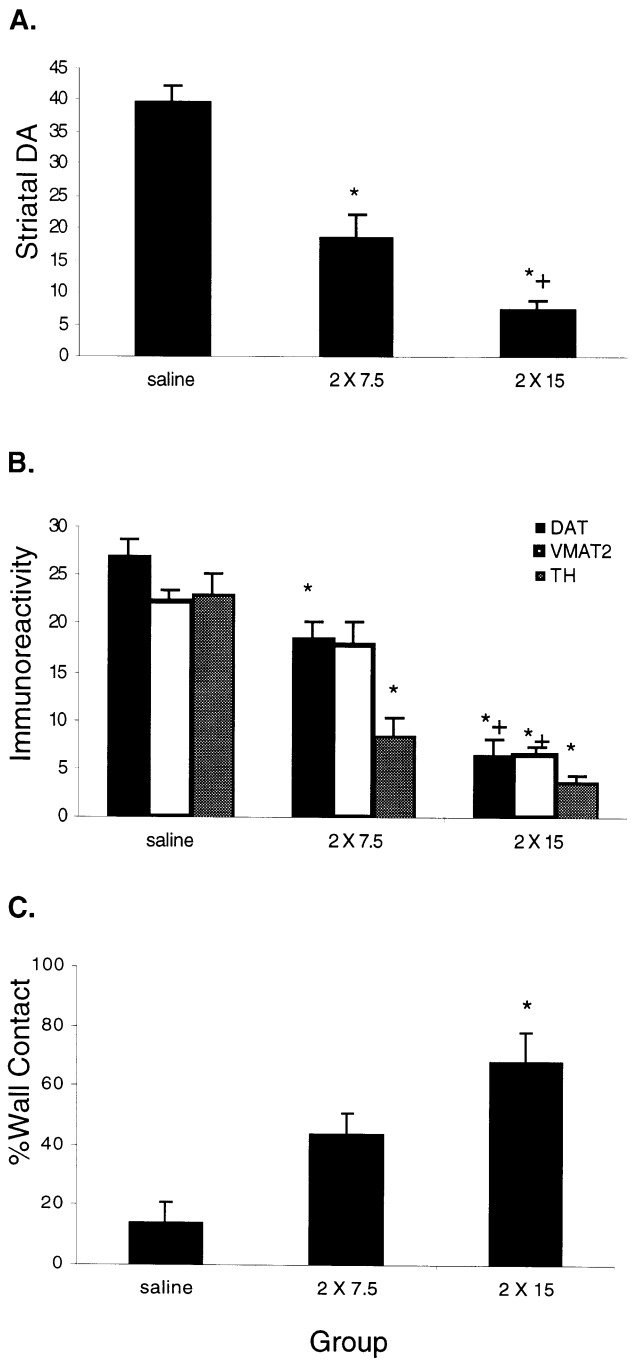

Fig. 4. Moderate doses of MPTP result in significant and sustained loss of striatal DA in retired breeder mice (p.mol mg^{-1} wet tissue). (A) Both 2×7.5 and 2×15 mg kg^{-1} MPTP resulted in significant declines in striatal DA in retired breeder mice at 30 days post-injection. In addition, animals injected with 2×15 mg kg^{-1} had significantly less striatal DA compared to 2×7.5 animals. (B) Immunoreactivity of the DA terminal markers DAT and TH was significantly declined in 2×7.5 animals. Some 2×15 animals showed significant declines in DAT, VMAT2 and TH immunoreactivity on day 30 post-injection (* <0.05 compared to saline controls; + <0.05 compared to 2×7.5 group). (C) Percent wall contact on day 28 post-lesion. As striatal DA and DA terminal markers decline (A, B) the amount of contact with the wall increases.

terminal markers was also significantly decreased in both MPTP treatment groups (Fig. 4B) (DAT: $F(2,33) = 44.116$, $P < 0.001$; saline versus 2×7.5, $P < 0.02$; saline versus 2×15, $P < 0.001$; 2×7.5 vs. 2×15, $P < 0.001$: VMAT2: $F(2,33) = 41.377$, $P < 0.001$; saline versus 2×15, $P < 0.001$; 2×7.5 vs. 2×15, $P < 0.001$: TH: $F(2,33) = 30.068$, $P < 0.001$; saline versus 2×7.5, $P < 0.001$; saline versus 2×15, $P < 0.001$) (Fig. 4C). Thus, the two doses of MPTP used in these experiments resulted in long-term and dose-dependent loss of striatal DA and DA terminal markers in retired breeder mice.

2.4. Behavioral performance on the grid test showed similar recovery to striatal DA in young mice exposed to MPTP

MPTP administration led to a sustained decline in step length on the grid in young mice ($F(3,20) = 30.679$, $P < 0.001$) (Fig. 5A). Post-hoc tests comparing each MPTP time point to saline controls showed significant declines at each time point (48 h, $P < 0.001$; day 7, $P < 0.001$; day 30, $P < 0.001$). The percent wall time showed an overall increase following MPTP injection ($F(3,20) = 5.166$, $P < 0.01$), with significant impairments at 48 h post-injection ($P < 0.01$) that returned to near baseline by 7 days post-injection (Fig. 5B). MPTP administration also led to increased forepaw faults on the grid ($F(3,20) = 5.550$, $P < 0.01$) (Fig. 5C). Performance on this measure was impaired 48 h post-injection ($P < 0.01$) and 7 days post-injection ($P < 0.05$), but recovered by day 30 post-injection. Thus, in young mice exposed to MPTP, behavioral performance on the grid showed a similar 'spontaneous' recovery, as was measured in striatal DA integrity (Fig. 5A–E).

2.5. MPTP (2×15 mg kg^{-1}) in young C57BL/6J mice resulted in transient declines in striatal DA and DA terminal markers

A significant group effect for striatal DA ($F(3,26) = 30.722$, $P < 0.001$) was found using a one-way ANOVA comparing saline treated animals to MPTP treated mice at different days (Fig. 5D). Post-hoc comparisons demonstrated significant declines 48 h post-injection ($P < 0.001$), 7 days post-injection ($P < 0.001$) and 30 days post-injection ($P < 0.01$) compared to saline controls. In addition, a recovery of initial striatal DA loss was demonstrated between the 48 h group versus the day 30 group ($P < 0.01$) and between the day 7 group and the day 30 group ($P < 0.02$). Thus, 2×15 mg kg^{-1} MPTP resulted in significant declines in striatal DA, with a time-dependent increase in striatal DA concentrations (Fig. 5D).

Immunoreactivity of DA terminal markers showed similar time-dependent declines following MPTP administration (Fig. 5E). Overall analysis revealed group

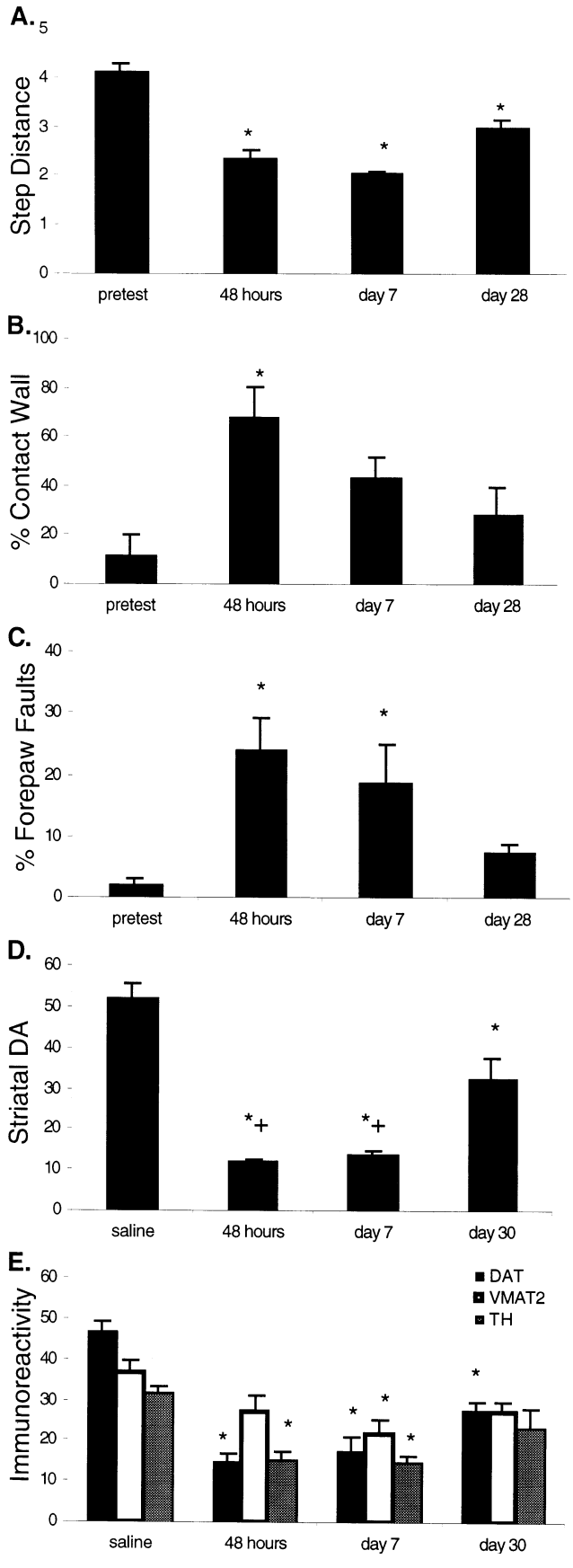

Fig. 5

differences in DAT immunoreactivity (F (3,25) = 29.784, $P < 0.001$). Compared to saline treated animals, all three MPTP time points displayed significant loss of DAT expression (48 h, $P < 0.001$; day 7, $P < 0.001$; day 30, $P < 0.001$). VMAT2 immunoreactivity was significantly decreased 7 days post-injection, but recovered to saline levels by day 30 post injection (overall: F (3,25) = 3.198, $P < 0.05$: saline versus day 7, $P < 0.05$). Similarly, TH immunoreactivity was significantly different between saline treated animals and MPTP time points (F (3,25) = 14.107, $P < 0.001$), with post-hoc analysis revealing declines at 48 h post-injection ($P < 0.001$) and 7 days post-injection ($P < 0.001$), but returning to saline levels by day 30 post-injection (Fig. 5E).

2.6. Grid measures were significantly correlated to MPTP induced injury

The three measures of step distance, percent wall time and percent forepaw fault were significantly correlated to striatal dopamine and dopamine terminal markers (see Fig. 6 for correlations and significance). As the levels of striatal DA and DA terminal markers increased, the size of forepaw steps on the grid also increased. In both the measures of percent wall time and percent forepaw faults, as the levels of striatal DA levels and terminal markers increased, the percent wall time and faults decreased. All three measures on the grid at day 28 of testing were correlated to striatal DA, DAT, VMAT2 and TH as measured on day 30 post-injection using Pearson product moment correlation. Of these analyses only the correlation between percent forepaw fault and DAT immunoreactivity was not significantly correlated (Fig. 6).

Fig. 5. Administration of 2×15 mg kg^{-1} MPTP in young C57BL/6J mice resulted in differential DA loss and behavioral impairments. (A) Moderate doses of MPTP resulted in declines in stride distance on the grid in young mice. (B) Percent contact with the wall was increased following moderate dosing of MPTP in young mice. (C) Time-dependent increases in forepaw faults were measured in young mice given a moderate dose of MPTP. (D) Young C57BL/6J mice given 2×15 mg kg^{-1} MPTP showed significant declines in striatal DA content 48 h, 7 and 30 days post-lesion (p.mol mg^{-1} wet tissue). (D) DA concentrations were significantly lower 48 h and 7 days post-lesion compared to day 30 post-lesion, demonstrating the previously described recovery in nigrostriatal DA content in young mice. (E) Moderate dosing of MPTP in young mice resulted in time-dependent loss of DA terminal markers. Post-injection (48 h), young mice given 2×15 mg kg^{-1} MPTP displayed significant declines in immunoreactivity of both DAT and TH. By day 7 post-injection, significant declines in all three DA terminal markers were observed, but by day 30 post-injection only significant declines in DAT were still present in young mice (* < 0.05 compared to saline controls).

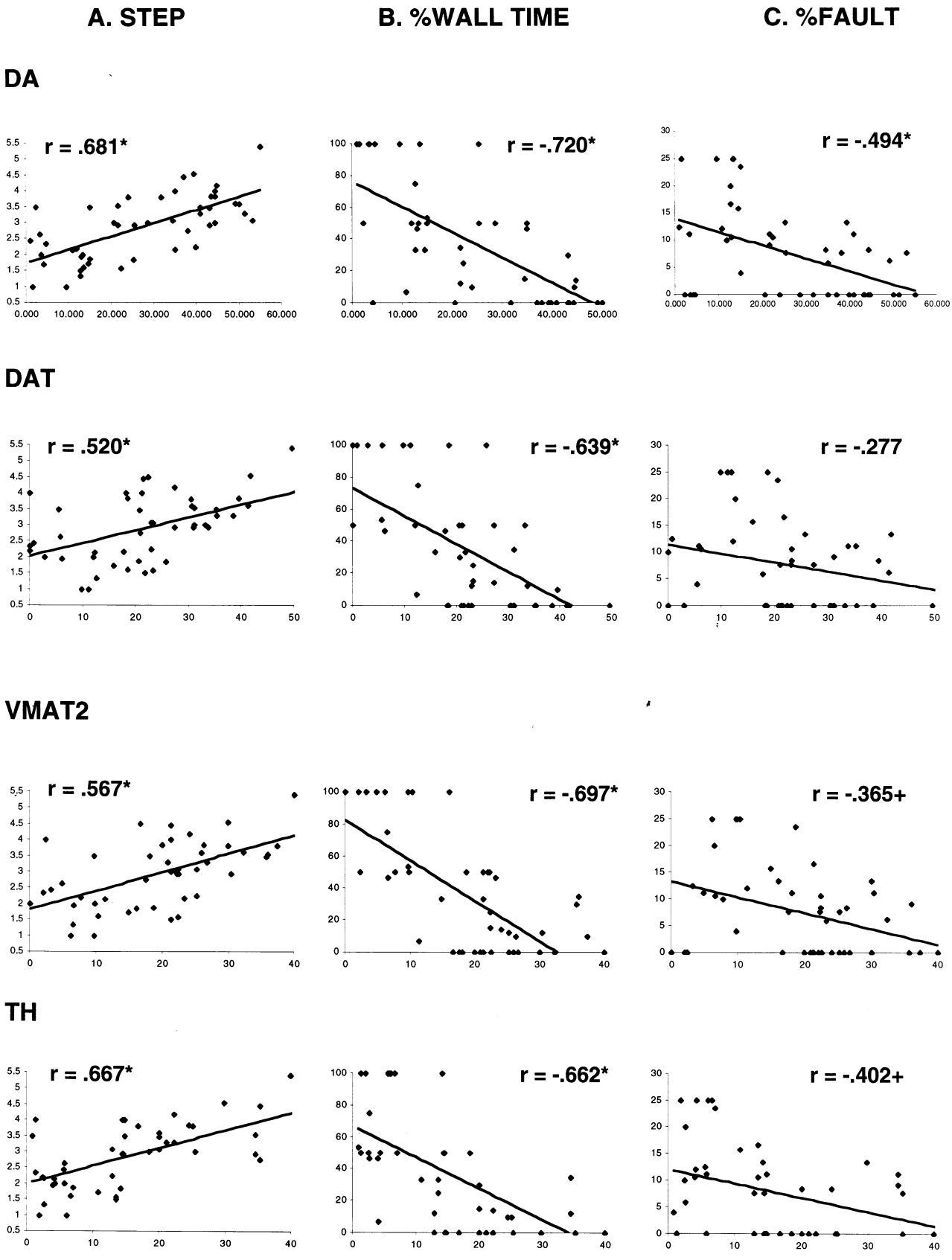
A. STEP
B. %WALL TIME
C. %FAULT
DA
r = .681*
r = -.720*
r = -.494*
DAT
r = .520*
r = -.639*
r = -.277
VMAT2
r = .567*
r = -.697*
r = -.365+
TH
r = .667*
r = -.662*
r = -.402+

Fig. 6

3. Discussion

Adult C57BL/6J mice exposed to moderate doses of MPTP do not show behavioral impairments on measures of general activity, rotorod and hangtest (Ogawa et al., 1985; Willis et al., 1988; Heikkila et al., 1989; Colotla et al., 1990; Sundstrom et al., 1990; Heikkila and Sonsalla, 1992; Fredriksson and Archer, 1994; Fredriksson et al., 1997; Rozas et al., 1998; Spooren et al., 1998; Sedelis et al., 2001). Although the animals experience permanent decreases in dopamine content, they are able to reach the endpoint measures of these tasks efficiently. In addition, we have found that behavioral performance on these standard tests is not correlated to striatal DA content at the same doses of MPTP used in the present studies (data not shown). In the present study, we scored three behavioral measures in MPTP-mice while hanging on a grid. The underlying assumption of the measures outlined in this report is animals with more compromised systems will develop more compensatory strategies, such as decreases in step length and reliance on external means, such as a supporting wall, in order to complete the task.

The measures of forepaw step length, percent wall time and forepaw faults while on the grid, accurately reflected the persistent dopaminergic loss in adult C57BL/6J mice exposed to moderate levels of MPTP and these behavioral deficits were reversed following L-DOPA treatment (Figs. 2–5). Unlike the standard motor tests currently employed for analysis in mice, the tasks described in this paper are significantly correlated to striatal dopamine levels at the doses used in these experiments (Fig. 6). In addition to retired breeder mice, we investigated the behavioral differences found in young C57BL/6J mice exposed to MPTP. As has been thoroughly described, young C57BL/6J mice display only transient losses of striatal dopamine when exposed to moderate doses of MPTP (Willis and Donnan, 1987; Mitsumoto et al., 1998; Fornai et al., 2000). Thus, these animals provide a system of striatal DA recovery. Young C57BL/6J mice exposed to MPTP displayed transient behavioral deficits in the measures outlined in this paper that recovered in a similar fashion as the neurochemical recovery (Fig. 5).

All three performance measures on the grid were significantly correlated to striatal DA levels (Fig. 6). Of the three measures, forepaw faults was the most difficult to rate and the least correlated to striatal DA and terminal markers. It is very important that while videotaping, the camera is close enough to the animal and grid to measure slips of the paw and whether the fingers of the paw close around the wire of the grid. Raters experienced in behavioral analysis of rodents should be able to measure forepaw faults within a few trials. On the other hand, un-experienced behaviorists may find this to be a difficult measure. Both measures of forepaw steps and wall time provided accurate evaluation of nigrostriatal DA integrity; therefore, it is not necessary to evaluate forepaw faults if they are ambiguous to the rater. Indeed, depending on the overall aim of the study, it would be possible to measure wall contact time during the actual hanging trial with use of two stopwatches.

The measures described in this report are the first to allow for critical behavioral evaluation of MPTP mice. As motor symptoms are the hallmark characteristics of PD, an animal model that does not allow one to test partial to full recovery of the characteristic symptoms of a disorder is limited in its experimental usefulness. The behavioral measures outlined in this paper will now allow for more comprehensive behavioral analysis in the mouse MPTP model. We have demonstrated that these measures not only detect behavioral impairment in animals with permanent DA loss, but are also sensitive enough to striatal DA perturbations to measure therapeutic increases in DA levels following L-Dopa treatment in old mice (Fig. 3) and spontaneous recovery in young mice (Fig. 5). The behavioral impairments and recovery in animals with both persistent and transient striatal DA loss using the grid test suggest that these simple measures will: (1) allow researchers to measure motor impairments in MPTP treated mice that have been previously considered behaviorally asymptomatic; and (2) evaluate the ability of different interventions such as novel drugs and/or behavioral manipulations, to result in partial to full recovery of nigrostriatal function. In addition, these measures may be useful in other neurodegenerative models of disease, such as stroke, cerebellar dysfunction and/or amyotrophic lateral sclerosis. Finally, this test may be able to more easily detect motor impairments in intact animals exposed to motor impairing drugs, such as alcohol. Taken together, the demonstrated and proposed potential utility of this test may allow for an overall more efficacious evaluation of many mouse models in research.

Fig. 6. Neurochemical and behavioral correlations: Pearson product moment correlations between striatal DA, DAT, VMAT2 and TH and the three grid measures on day 28 post-injection (* <0.01; + <0.05). Included in the analysis are young and retired breeder mice in all treatment groups ($n = 48$). Column (A): as DA and DA terminal markers increased, the step size also increased. Column (B): as the level of DA and DA markers decreased, the percent wall time increased. Column (C): As striatal DA levels and terminal markers increased, the percent faults decreased.

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