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Restoration of MPTP-induced deficits by Exercise and Milmed Co-treatment

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Abstract

Following the administration of either two or four injections of the dopamine (DA) neurotoxin, MPTP, at a dose of 40 mg/kg, C57 BL6 mice were given access to running-wheels (30-min sessions, four times/week, mon.-thrurs.) and treatment with treated yeast, Milmed (four yimes/week, mon.-thrurs.), or simply running-wheel exercise by itself, over ten weeks. It was observed that the combination of physical exercise and Milmed treatment, the MPTP+Exercise+Yeast(2) group [MPTP+Exercise+Milmed(2)], restored spontaneous motor activity markedly by test day 10, restored completely subthreshold L-Dopa-induced activity, and DA concentration to 76% of control values, in the condition wherein two administrations of MPTP (2 x 40 mg/kg) were given prior to initiation of exercise and/or Milmed treatment. Physical exercise by itself, MPTP+Exercise(2) group, attenuated these deficits only partially. Administration of MPTP four times (i.e. 40 mg/kg, s.c., once weekly over four weeks for a total of 160 mg/kg, MPTP+Exercise+Yeast(2) group [MPTP+Exercise+Milmed(4)] and MPTP+Exercise(4), induced a lesioning effect that far far too severe for either exercise or the exercise+Milmed combination to affect. Nevertheless, these findings indicate a powerful effect of physical exercise reinforced by Milmed treatment in restoring MPTP-induced deficits.

Key-words: MPTP – exercise – Milmed – running-wheels – locomotion – rearing – total activity – dopamine – attenuation – restoration.

Although physical exercise by itself ameliorates functional, motor activity, and neurochemical, dopamine (DA), deficits induced by the selective DA neurotoxin, MPTP (Archer and Fredriksson, 2010, 2012, 2013b, c; Fredriksson et al., 2011), the combination of physical exercise with Milmed provided evidence of the complete restoration of these deficits (Archer and Fredriksson, 2013a). The efficacy of physical exercise (Archer, 2011; Archer et al., 2011; Earhart and Falvo, 2013) and physiotherapy (Cholewa et al., 2013) for Parkinsonism and other neurodegenerative conditions has been well-documented with ever-increasing evidence from both clinical and laboratory studies (Park et al., 2014; Wang et al., 2013; Zigmond and Smeyne, 2014). Three main conclusions were obtained from the Archer et al. (2013a) study: (i) DA integrity was observed to be a direct function of ability to express running exercise in a treadmill running arrangement, (ii) DA integrity was observed to be a direct function of the capacity for motor performance as measured by spontaneous motor activity and subthreshold L-Dopa (5 ml/kg) induced activity in the motor activity test chambers, and (iii) running exercise in the treadmill running wheel predicted later motor performance in the motor activity test chambers to an extremely high degree. In this respect, it has been demonstrated in rats that caffeine, both hyperthermic and ergogenic, in combination with physical exercise increased extracellular DA and noradrenaline (NA) in the preoptic area and anterior hypothalamus (Zheng et al., 2014). Treadmill exercise ameliorated also the nigrostriatal dopaminergic neuronal loss in adolescent rats following neonatal hypoxic brain ischemia which improved spatial learning ability (Park et al., 2013). Finally, using the 10 x 25 mg/kg MPTP model of PD it was indicated that four weeks of treadmill running decreased the levels of the inducible form of nitric oxide and neuronal nitric oxide in the brains of MPTP-treated mice (AL-Jarrah et al., 2013).

The preparation and application of treated yeast culture, Saccharoyces cerevisiae, to provide the antiparkinson agent, Milmed, in suspension form has been outlined previously (Gedymin et al., 1999; Kolosove et al., 1998). The derivation of Milmed for cellular genesis and reparation has been reported elsewhere (Golant,

1994; Golant et al., 1994; Ragimov et al., 1991). The application of the Milmed suspension (oral administration, twice weekly) in an animal MPTP model of Parkinsonism has been described also (Archer and Fredriksson, 2013a, b). Generally, studies designed to apply exercise intervention using MPTP lesioning to induce Parkinson symptoms, e.g. hypokinesia, in the laboratory have introduced exercise, with (Oscarsson et al., 2009) or without (Archer and Fredriksson, 2010) Milmed co-treatment, prior to administration of the neurotoxin. In the present study, C57 BL7 mice were administered MPTP (40 mg/kg) either twice or four times before access to the exercise (running-wheel, four 30 min/sessions/week, mon.-thurs.) and Milmed co-treatment intervention (once/day, four times/week). The purpose of the present study was to ascertain the extent to which the exercise+Milmed treatment combination would restore MPTP-induced functional and neurochemical deficits following either two (moderate condition) or four (severe condition) injections of the neurotoxin.

Methods and materials

Animals

Male C57 BL6 mice, purchased from B&K, Sollentuna, Sweden, maintained five-to-a-cage in plastic cages in an isolated room at 22 ± 1oC and 12 h/12 H constant light/dark cycle (lights on between 06.00 and 18.00 h), were acclimatized, housed and given access to the running-wheels or holding-cages (30-min sessions) in an identical to that described previously (Archer and Fredriksson, 2013a). The study was carried out in accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) after approval from the local ethical Committee (Uppsala University and Agricultural Research Council), and by the Swedish Committee for Ethical Experiments on Laboratory animals (License S93/92 and S77/94, Stockholm, Sweden).

Drugs

MPTP (Research Biochemical Inc., MA, USA), injected 4 x 40 mg/kg, s.c. (1-week intervals between injections, was dissolved in saline and administered in a volume of 2 ml/kg body weight. Milmed was prepared for administration according to a procedure identical to that described previously (Archer and Fredriksson, 2013a).

Mice were administered oral injections of 0.5 ml/kg Milmed containing a cell concentration of approximately 2 x 10^6 yeast cells daily, according to the preparation protocol and design developed from previous observations regarding stability and viability of the compound (each dose contained 1 x 10^6 yeast cells). Each mouse was administered MIlmed once each day four times/week during the 10 weeks of exercise+Milmed treatment with Design and treatment maintained as described previously (cf. Archer and Fredriksson, 2013a).

Behavioural Measurements and Apparatus

Testing of motor activity in the ADEA test chambers where Locomotion, Rearing and Total activity were measured was performed in an identical manner to that described previously (Archer et al., 1986).

Access to the running-wheels over daily 30-min sessions was maintained as described previously (Archer and Fredriksson, 2010).

Procedure

The identical procedure to that employed previously (Archer and Fredriksson, 2012) was maintained. Access to the running wheel was presented on the 1st four days-of-the-week (mon.-Thurs.) and motor activity testing on the 5th day (Friday), as previously (Archer and Fredriksson, 2010). Testing consisted of spontaneous motor activity test (60 min) and L-Dopa-induced activity test (180-min).

Neurochemical analysis

Analysis of striatal DA concentrations was performed in an identical manner to that described previously (Archer and Fredriksson, 2013a). Mice were killed by cervical dislocation within two weeks of completion of behavioral testing. Determination was carried out using an high-performance liquid chromatograph with electrochemical detection, according to Björk et al. (1991), with modifications (Liu et al., 1995). The DA analysis concentration results are expressed as ng/ml wet weight of tissue.

Statistical analysis

Spontaneous motor activity counts (60-min test sessions) and L-Dopa-induced motor activity counts (180-min test sessions) were subjected Split-plot ANOVA whereas

striatal DA concentrations were subjected to one-way ANOVA (Kirk, 1995). Pairwise testing between groups was performed using Tukey's HSD tests.

Results

The combination of physical exercise with Milmed restored both function, sponateous motor activity and L-Dopa-induced activity, and neurochemical, DA, deficits whereas exercise, by itself, attenuated the deficits.

Spontaneous motor activity

Split-plot ANOVA indicated a significant Groups x Test days interaction for locomotion, rearing and total activity counts: $F(41, 419) = 97.61$, $p < 0.0001$; $F(41, 419) = 69.23$, $p < 0.0001$; and, $F(41, 419) = 50.13$, $p < 0.0001$, respectively. Figure 1 presents the mean (SD) locomotion, rearing and total activity counts for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)], over 60-min test sessions for the spontaneous activity tests.

Insert Figure 1 here

Pairwise testing using Tukey's HSD test indicated that over all three motor activity parameters that: (i) the MPTP+Exercise(2) and MPTP+Exercise+Yeast(2) groups made more counts than the MPTP, MPTP+Exercise(4) and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)] groups but fewer counts than the Vehicle group, (ii) the MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)] group made more counts than the MPTP+Exercise(2) group over test days 6, 8, 10, and (iii) the MPTP+Exercise(2) and MPTP+Exercise+Yeast(2) groups increased the numbers of counts made from test days 3 to days, an indication of gradual recovery.

L-Dopa-induced activity

Split-plot ANOVA indicated a significant Groups x Test days interaction for locomotion, rearing and total activity counts: $F(17, 143) = 72.81$, $p < 0.0001$; $F(17,$

143) = 15.81, $p < 0.0001$; and $F(17, 143) = 15.97$, respectively. Figure 2 presents the mean (SD) locomotion, rearing and total activity counts for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)], over 180-min test sessions for the L-Dopa-induced activity test.

Insert Figure 2 here

Pairwise testing using Tukey's HSD test indicated that over all three motor activity parameters that: (i) the MPTP+Exercise+Yeast(2) group made more counts than all the other MPTP-injected groups and as many counts as the vehicle group, (ii) the MPTP+Exercise(2) [MPTP+Exercise+Milmed(2)] group made more counts than the the MPTP, MPTP+Exercise(4) and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)] groups, and (iii) the motor activity of the MPTP+Exercise+Yeast(2) group was restored completely.

Neurochemical analysis

One-way ANOVA indicated a significant between-groups effect for striatal DA concentrations: $F(5, 30) = 55.53$, $p < 0.0001$. Figure 3 presents the mean (SD) DA concentrations for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4).

Insert Figure 3 here

Pairwise testing using Tukey's HSD test indicated the following differences:

The MPTP group that received the exercise – Milmed combination, i.e. MPTP+Exercise+Yeast(2), showed higher DA concentrations than the MPTP+Exercise(2) [MPTP+Exercise+Milmed(2)] group which in turn showed higher DA concentrations than the MPTP, MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)] groups.

Expressed as percent of control (Vehicle) values, the following were obtained:-

MPTP = 20%; MPTP+Exercise(2) = 40%; MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)] = 76%; MPTP+Exercise(4) = 19%; and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)] = 23%.

Discussion

The purpose of this study was to ascertain whether or not the combination of exercise with Milmed treatment would restore MPTP-induced functional and neurochemical deficits. The results showed that wheel-running exercise (30-min sessions, 4 days/week) combined with the treated yeast Milmed suspension (administered 4 times/week), the MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(2)] group, restored spontaneous motor activity markedly by test day 10, restored completely subthreshold L-Dopa-induced activity, and DA concentration to 76% of control values, in the condition wherein two administrations of MPTP (2 x 40 mg/kg) were given prior to initiation of exercise and/or Milmed treatment. Physical exercise by itself, MPTP+Exercise(2) group, attenuated these deficits only partially, as has been observed several times previously (Archer and Frefriksson, 2010, 2012, 2013a, b, c; Fredriksson et al., 2011). Administration of 4 injections of MPTP each week (4 x 40 mg/kg) induced deficits that were far too severe for amelioration by exercise and Milmed: .i.e. groups MPTP+Exercise(4) and MPTP+Exercise+Yeast(4) whereas the MPTP group received no exercise access.

Throughout the published series of experiments (Archer and Fredriksson, 2010, 2012, 2013a, b, c; Fredriksson et al., 2011) and unpublished data, applying different MPTP dose regimes and number of administrations, the percentage increase in striatal DA levels, following the exercise invention, has varied as follows: 15% (5 weeks of exercise), 47% (14 weeks of exercise), 44% (7 weeks of exercise), 21% (14 weeks of exercise), 20% (10 weeks of exercise), 42% (14 weeks of exercise, 27% (10 weeks of exercise) and in the present experiment 20% (10 weeks of exercise. Despite this consistent evidence that running-wheel exercise induced reliable elevations in striatal DA concentration, it is obvious that exercise by itself was not sufficient to ensure complete restoration. Nevertheless, for the integrity of DA neurons, physical exercise throughout exerted an essential and central role: “use it or lose it”. Combining running-wheel exercise with Milmed administration

induced complete restoration of striatal DA concentrations (Archer and Fredriksson, 2013a, b, c). In the present study, the treatment with exercise+Milmed induced a striatal DA level that was 76% of the control value, or a percentage increase of 56% over the 10 weeks of the treatment combination. However, it must be considered that prior to the treatment intervention a total of 80 (40 + 40) mg/kg of MPTP neurotoxin had been administered, after introduction of the treatment intervention, a further 80 (40 + 40) mg/kg MPTP was administered. In the Archer and Fredriksson (2013b, c) studies, the 1st two weeks of exercise+Milmed treatment combination were instituted prior to the 1st administration of MPTP whereas in the Archer and Fredriksson (2013a) study, a 3 x 30 mg/kg dose regime of MPTP was applied and the exercise+Milmed treatment combination was introduced after the 1st administration of MPTP. Thus, the MPTP dose regimes administered in all those studies was substantially milder that that employed in the present experiment; indeed, 2 x 40 mg/kg of MPTP induces a substantial lesion (Archer and Fredriksson, 2003, 2006), whether followed by a further 2 x 40 mg/kg of the neurotoxin or not.

The clinical implications of physical exercise for improving the patients' condition in Parkinsonism are multiple: e.g. progressive high-intensity locomotor training with body weight support improved their clinical status, quality-of-life and gait capacity as well as being practicable and well-tolerated (Rose et al., 2013). A program of 12-week walking both for PD patients and community-dwelling older adults was shown to be effective: it was found that there were velocity and step-length in the PD group (Cheng et al., 2013). In a review of implications for rehabilitation, Ericsson et al. (2013) have forwarded the notion that physical exercise consitutes an essential ingredient in the process of retaining the healthy self in older individuals with PD. Since L-Dopa remains the drug-of-choice, it is important to observe that the exercise+Milmed combination restored completely motor activity after administration of the subthreshold dose (5 mg/kg) of L-Dopa (see Figure 2). Nevertheless, the emergence of side-effects with L-Dopa remains a continual hazard (Cerasa et al., 2014; Pietracupa et al., 2014; Shin et al., 2014). However, it has been shown also in 6-hydroxydopamine-injected rats, an animal model of PD, that L-DOPA-induced dyskinesias were attenuated through the intervention with an exercise schedule (Aguiar et al., 2013). Grazina and Massano have presented three conclusions in conjunction with the putative influences of physical exercise upon the

symptoms expressions and prognosis in PD: (i) exercise is associated with a lower propensity for developing PD symptoms, II) it has been demonstrated that exercise ameliorates, but does not eliminate, disease symptoms, mobility loss, balance problems, gait instability and lesser quality of life (it appears that walking training, tai-chi and tango dancing have demonstrated the highest level of evidence of efficacy); and 3) that neuroprotective effects accumulating from elevated neuroplasticity may be expected in Parkinsonism conditions, despite the occurrence of these observation from animal studies exclusively. The present findings, taken together with previous observations (Archer and Fredriksson, 2013a, b, c), both underline these benefits and implicate the role of Milmed combined with physical exercise to produce more dramatic manifestations of reclaimed DA-integrity following disorder onset.

In summary, the lesioning effects of MPTP upon DA neurons were introduced either twice or four times before access to running-wheel exercise and/or administration of the treated yeast, Milmed. In the former condition, the co-treatment of exercise+Milmed restored both functional, motor activity, and neurochemical, DA levels, integrity to a notable extent. Exercise, by itself, attenuated the motor activity deficit and loss of DA. In the latter condition, the administration of 4 doses of MPTP (40 mg/kg), a total of 160 mg/kg induced an extent of tissue destruction that proved to be far too severe for later exercise+Milmed intervention to affect.

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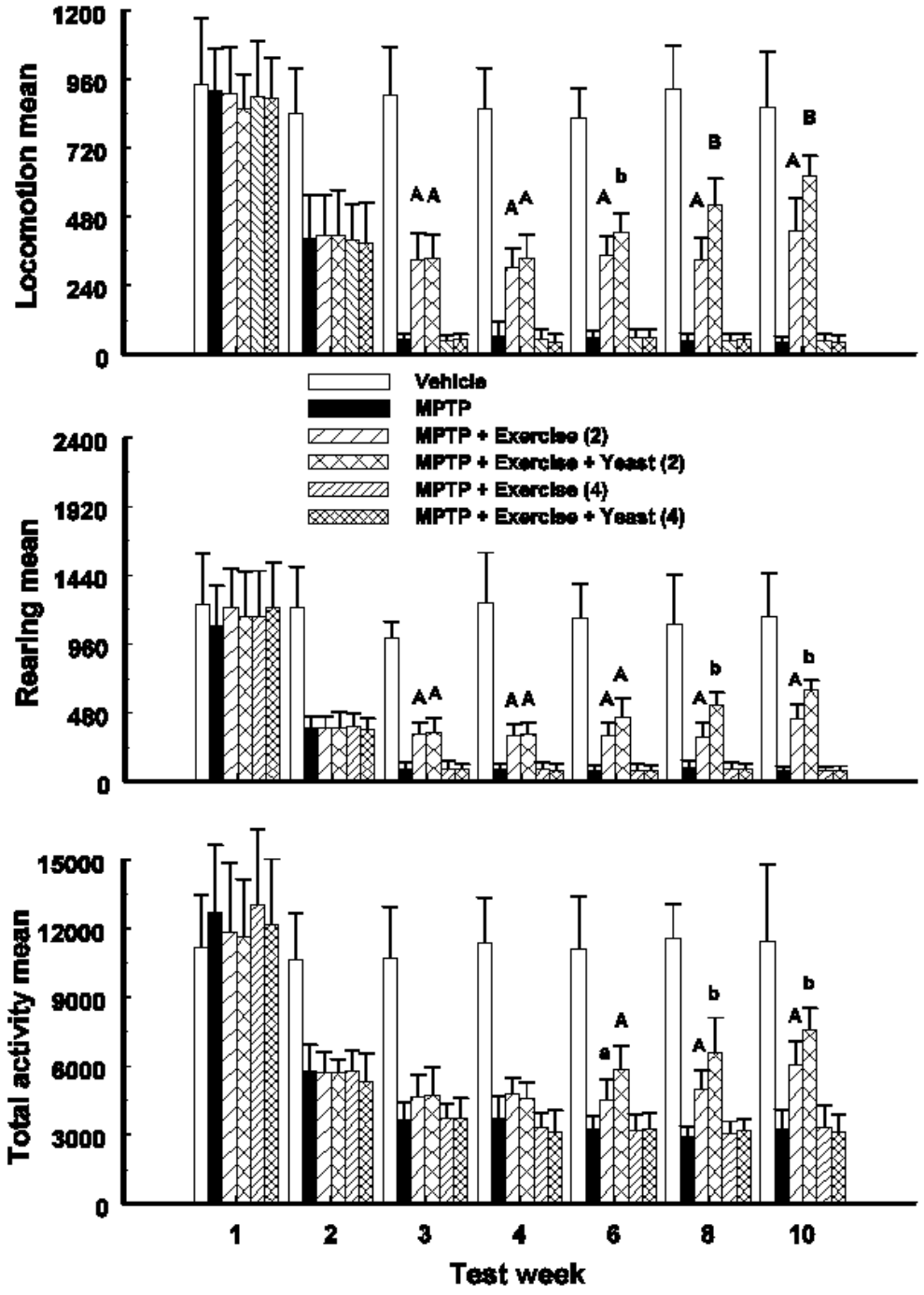
Figure captions

Figure 1. Mean (SD) locomotion, rearing and total activity counts for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(4)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)], over 60-min test sessions for the spontaneous activity tests. MPTP was injected (40 mg/kg, s.c., single weekly injections) either twice or four times prior to initiation of wheel-running exercise (30-min sessions/week, mon.-thurs.) + Milmed treatment (four injections, p.o., each week).

Figure 2. Mean (SD) locomotion, rearing and total activity counts for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(4)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)], over 180-min test sessions for the L-Dopa-induced activity tests. MPTP was injected (40 mg/kg, s.c., single weekly injections) either twice or four times prior to initiation of wheel-running exercise (30-min sessions/week, mon.-thurs.) + Milmed treatment (four injections, p.o., each week).

Figure 3. Mean (SD) DA concentrations for each of the six groups: Vehicle, MPTP, MPTP+Exercise(2), MPTP+Exercise+Yeast(2) [MPTP+Exercise+Milmed(4)], MPTP+Exercise(4), and MPTP+Exercise+Yeast(4) [MPTP+Exercise+Milmed(4)]. MPTP was injected (40 mg/kg, s.c., single weekly injections) either twice or four times prior to initiation of wheel-running exercise (30-min sessions/week, mon.-thurs.) + Milmed treatment (four injections, p.o., each week).


Locomotion mean
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960
720
480
240
0
Vehicle
MPTP
MPTP + Exercise (2)
MPTP + Exercise + Yeast (2)
MPTP + Exercise (4)
MPTP + Exercise + Yeast (4)
Rearing mean
2400
1920
1440
960
480
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Total activity mean
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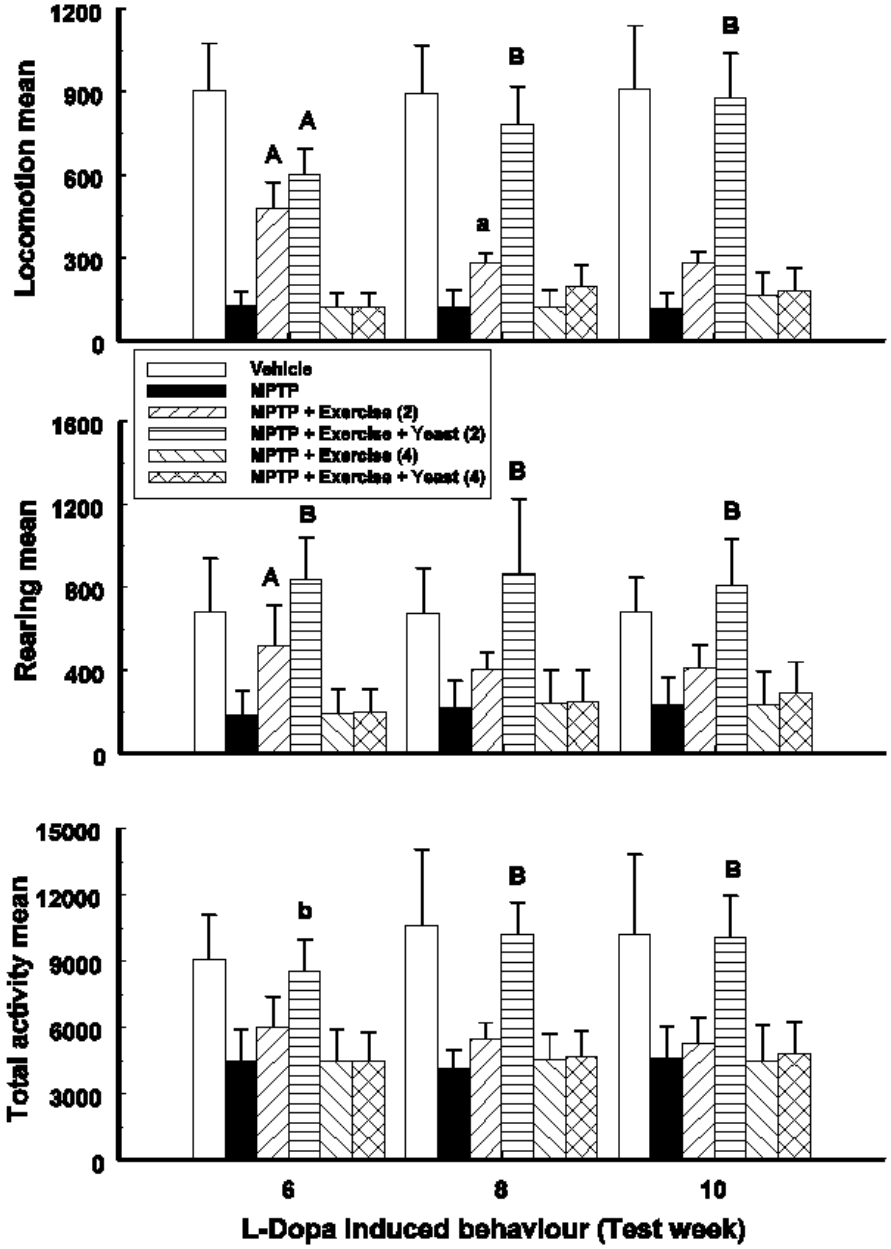
12000
9000
6000
3000
0
1
2
3
4
6
8
10
Test week


Locomotion mean
1200
900
600
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A
A
a
B
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Vehicle
MPTP
MPTP + Exercise (2)
MPTP + Exercise + Yeast (2)
MPTP + Exercise (4)
MPTP + Exercise + Yeast (4)
Rearing mean
1600
1200
800
400
0
A
B
B
B
Total activity mean
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12000
9000
6000
3000
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6
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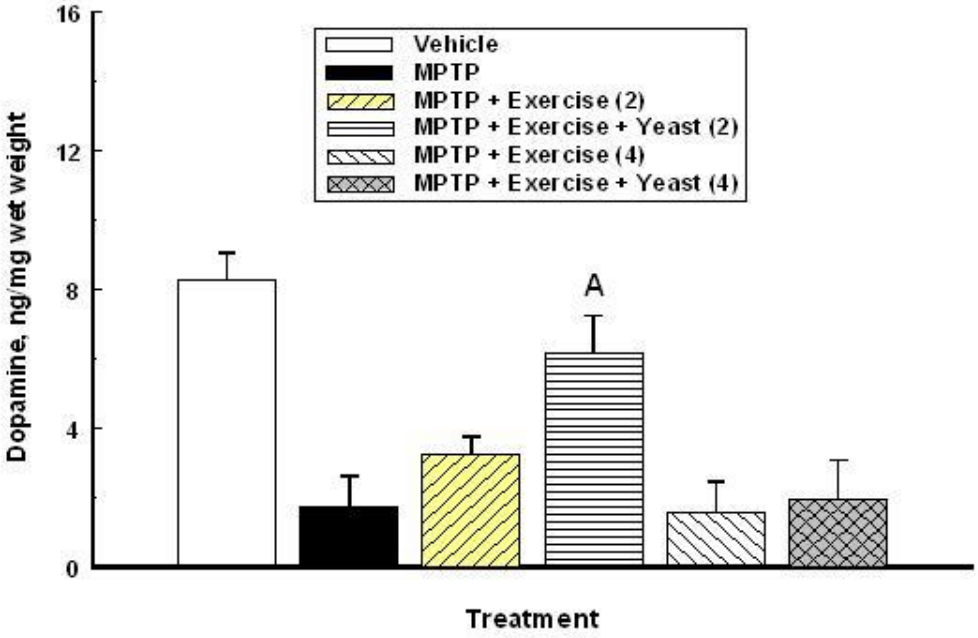
10
L-Dopa Induced behaviour (Test week)


16
12
8
4
0
Dopamine, ng/mg wet weight
Vehicle
MPTP
MPTP + Exercise (2)
MPTP + Exercise + Yeast (2)
MPTP + Exercise (4)
MPTP + Exercise + Yeast (4)
A
Treatment