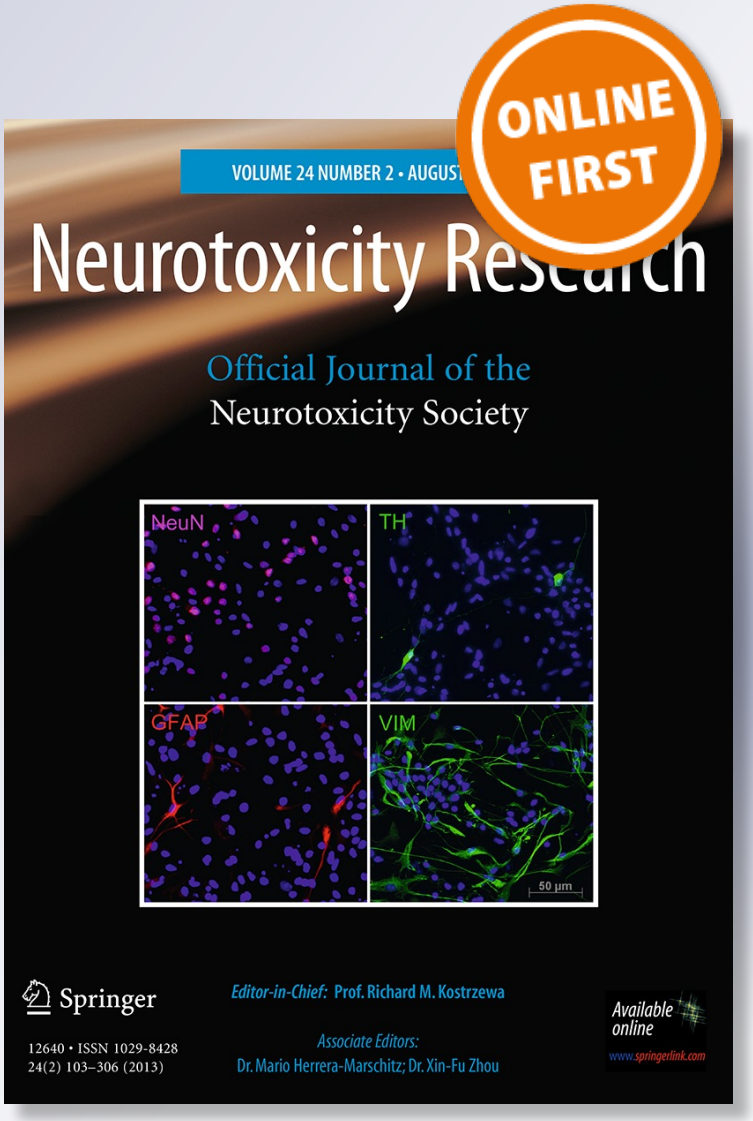
The Yeast Product Milmed Enhances the Effect of Physical Exercise on Motor Performance and Dopamine Neurochemistry Recovery in MPTP-Lesioned Mice

Trevor Archer & Anders Fredriksson

Neurotoxicity Research
Neurodegeneration, Neuroregeneration, Neurotrophic Action, and Neuroprotection

ISSN 1029-8428

Neurotox Res
DOI 10.1007/s12640-013-9405-4



Springer

Neurotox Res
DOI 10.1007/s12640-013-9405-4

The Yeast Product Milmed Enhances the Effect of Physical Exercise on Motor Performance and Dopamine Neurochemistry Recovery in MPTP-Lesioned Mice

Trevor Archer · Anders Fredriksson

Received: 6 June 2013 / Accepted: 13 June 2013

Abstract Both clinical and laboratory studies have demonstrated that different types of physical exercise may alleviate Parkinsonism yet evidence for complete restoration of motor function and biomarker integrity are difficult to identify. MPTP (1 × 30 mg/kg, s.c., 4 groups) or saline (vehicle 1 × 5 ml/kg, s.c., 1 group) were administered in a single dose regime over three consecutive weeks on Fridays. Three MPTP groups were given four 30-min periods/week (Mondays to Thursdays), of these two groups, MPTP + Exer + M(i) and MPTP + Exer + M(ii); the former were introduced to exercise and Milmed (oral injection) on the week following the 1st MPTP injection and the latter on the Monday prior to the 1st injection of MPTP onwards. One MPTP group, MPTP + Exer, was given access to exercise (running wheels) from the week following the 1st MPTP injection onwards. The fourth MPTP group, MPTP–NoEx, and the Vehicle group were only given access to exercise on a single day each week (Wednesdays, exercise test) from the week following the 1st MPTP injection onwards. The exercise/exercise + Milmed regime was maintained for a further 9 weeks. It was observed that exercise by itself ameliorated MPTP-induced deficits regarding motor function and dopamine loss only partially whereas in the groups combining exercise with twice weekly dosages of Milmed the MPTP-induced deficits were abolished by the 10th week of the intervention. The three main conclusions that were drawn from correlational analyses of individual mice were: (i) that DA integrity was observed to be a direct function of ability to express running exercise in a treadmill wheel-running arrangement, and (ii) that 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 mg/kg) induced activity in the motor activity test chambers, and (iii) that the extent to which running exercise in a running wheel was predictive of later motor performance in the activity test chambers was highly convincing.

Keywords MPTP · Running wheels · Motor activity · Amelioration · Dopamine · Mice–yeast

Introduction

Physical exercise has been shown to be useful in the treatment of Parkinsonism both in clinical practice and in the laboratory (Archer et al. 2011; Bilowit 1956; Hurwitz 1989; Palmer et al. 1986). The facilitatory effects of long-term, or moderately long-term, exercise upon motor functioning in Parkinson's disease (PD) are manifested in several ways, including (i) increased blood and oxygen flow to the brain associated with angiogenesis, (ii) the mobilisation of growth factors promoting neuroreparation, neurogenesis and synaptic plasticity (Hunsberge et al. 2007), (iii) facilitation of performance, whether motor, motivational or cognitive, through neurotransmitter release and turnover (Morishima et al. 2006; Waters et al. 2008). Several lines of research indicate that the "intensity" or "endurance" properties of exercise/activity (repetitiveness, velocity, complexity, duration, frequency, etc.) all contribute to the CNS restorative effects upon brains afflicted

T. Archer (✉)
Department of Psychology, University of Gothenburg,
Box 500, 430 50 Göteburg, Sweden
e-mail: trevor.archer@psy.gu.se

A. Fredriksson
Department of Neuroscience Psychiatry, Uppsala University,
751 85 Uppsala, Sweden

Published online: 27 July 2013

with Parkinson-type damage (Adkins et al. 2006; Fisher et al. 2004; O'Dell et al. 2007). For example, Corcos et al. (2013) have shown that 'progressive resistance exercise' induced a statistically and clinically significant reduction in the Unified Parkinson's Disease Rating Scale, motor subscale (UPDRS-III) in PD patients. A 30-min exercise schedule over 20 days was found to alleviate haloperidol-induced akinesia, as well as the neuroleptic-induced shortened stride length and increased stance width, thereby reducing extrapyramidal symptoms also (Baptista et al. 2013). Shulman et al. (2013) observed that physical exercise improved gait speed, muscle strength, and fitness in PD patients with combinations of treadmill and resistance exercises manifesting the greatest benefits. Finally, in a series of studies applying the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD and employing a treadmill running set-up with different treatment regimes, it was shown that a 4- to 5-day/week, 30-min/day exercise regime invariably attenuated the MPTP-induced hypokinesia of spontaneous motor activity, the lack of effect of subthreshold L-Dopa and the loss of striatal dopamine (Archer and Fredriksson 2010, 2012, 2013a; Fredriksson et al. 2011).

MPTP induces Parkinsonism in human and nonhuman primates (Jonsson et al. 1985; Langston 1985) that results in the loss *substantia nigra* cells in the *pars compacta* of adult animals. Repeated administration of MPTP to C57/BL6 mice induces selective and long-lasting lesions of dopamine (DA) in nigrostriatal regions of the brain (Jackson-Lewis et al. 1995; Jones-Humble et al. 1994). The neurotoxin destroys nigrostriatal neurons selectively thereby causing acute, sub-acute and long-term effects that resemble certain features of PD, in particular the hypokinesic effects upon motor function and the selective loss of striatal DA (Jackson-Lewis and Przedborski 2007; Ogawa et al. 1987). Systemic administration of MPTP (2×40 mg/kg, s.c.) to C57 BL/6 mice induced L-Dopa reversible hypoactivity (Fredriksson et al. 1990; Sundström et al. 1990). Less rigorous dosage regimes, such as 2×20, or 2×25 or 2×30 mg/kg of MPTP were shown not to affect motility in the C67 Black mice although losses of DA concentrations up to 50–80 % may be observed (Heikkila et al. 1989; Sonsalla and Heikkila 1986). The parameters of MPTP treatment neurotoxicity are virtually permanent (up to and beyond 52 weeks following administration) with marked relationships between the functional motor deficits, hypokinesia of spontaneous motor activity and L-Dopa, the DA precursor, activity, the neurochemical concomitant, severe depletions of DA, and a dose- and time-dependent recovery of several parameters of motor behaviour after treatment with L-Dopa and other agents (Archer and Fredriksson 2003, 2006; Fredriksson and Archer 1994, 2003; Fredriksson et al. 1999). The multiple, chronic MPTP mouse model has been developed to provide a progressive aspect to both the motor and biomarker deficits in order to study the ameliorative influences of physical exercise in experimental Parkinsonism (Gorton et al. 2010; Lau et al. 2011; Patki and Lau 2011; Petzinger et al. 2007, 2010; VanLeeuwen et al. 2010; Vuković et al. 2010).

The product, designated Milmed, is a patent-protected treatment of yeast cultures but for any synergistic anti-parkinson effect a regime of physical exercise must be incorporated: the basis of 'personalised medicine' builds upon this particular combination whereas the pharmacogenetic aspect involves the selective susceptibility of the C57/Bl6 mouse strain for the DA neurotoxin, MPTP. The yeast cultures, *Saccharomyces cerevisiae* or *Saccharomyces carlsbergsis*, have been utilised as industrially important cell factories (Nielsen and Jewett 2008), with many regulatory pathways conserved between these yeasts and humans (Zhang et al. 2010). Cell death studies using yeast apoptosis increasingly provide a model for analysing the cascade of molocular events that contribute to neurodegenerative disorders (Carmona-Gutierrez et al. 2010; Petranovic et al. 2010). Several features of PD have been reproduced in yeast with cell death promotion in a concentration-dependent manner (Outeiro and Lindquist 2003) with possibilities for facilitating the development of both therapeutic targets and compounds (Braun et al. 2009; Tenreiro and Outeiro 2010). It has been observed that specific biological and therapeutic effects of Extremely High Frequency (EHF) electromagnetic fields may be transferred to living organisms by specially treated suspension of living yeast cells (Gedymin et al. 1999; Kolosova et al. 1998). From a neurodegenerative perspective, both cognitive-protective and cognitive-enhancing, as well as reduced brain amyloid-beta deposition effects of microwave exposure were found in transgenic mice with Alzheimer-like disease progression and control mice (Arendash et al. 2010; Arendash 2012). The treatment of yeast cell cultures themselves, as the "transference-agent" provides an agent that may provide an anti-parkinson effect. The treatment and preparation of *S. cerevisiae* or *S. carlsbergsis* with electromagnetic waves in the Extreme High Frequency (EHF) range of 30–300 GHz produces a treated yeast extract, given the name Milmed (i.e. Milmed®). This treatment was developed through the pioneering work of Golant (Golant 1994; Golant et al. 1994; Ragimov et al. 1991) upon the genesis and reparation of cells. The co-administration of Milmed with daily physical exercise has been reported to attenuate MPTP-induced motor deficits (Oscarson et al. 2009). Subsequently, several experiments have shown that weekly administrations of the compound in combination with daily exercise was observed to act synergistically in restoring

completely both motor function and DA integrity as indexed by striatal DA concentration (unpublished observations).

The major purpose of the present study was to ascertain the extent to which DA integrity ensures (i) the ability to express running exercise in a treadmill wheel-running arrangement, (ii) the capacity for motor performance as measured by spontaneous motor activity and subthreshold L-Dopa (5 mg/kg) induced activity in motor activity test chambers and (iii) the extent to which running exercise in a running wheel was predictive of motor performance in the activity test chambers. The ancillary purpose was to examine whether or not the prepared yeast suspension, Milmed, may reinforce the ameliorative effects of physical exercise upon the MPTP-induced deficits through four times weekly administration over 10 weeks.

Materials and Methods

Animals

Male C57 Bl/6 mice were purchased from B&K, Sollentuna, Sweden, and were maintained, five-to-a-cage, in plastic cages in a room at temperature of 22 ± 1 °C and a 12 h/12 h constant light/dark cycle (lights on between 06.00 and 18.00 h). They were placed and maintained in groups of 4–6 animals in a room maintained for male mice only following arrival at the laboratory for about 2 weeks in order to acclimatise. Free access to food and water was maintained throughout, except for the day previous to the initiation to wheel-running exercise which occurred at the end of the second week following arrival. They were housed in groups of 6 animals, wheel-running exercised and activity chamber tested only during the hours of light (08.00–15.00 h). All exercising and testing was performed in a normally lighted room. Half of the mice in each treatment condition (MPTP-Exer, MPTP-Exer-Milmed and Vehicle) were given wheel-running exercise whereas the other half were placed in a clean laboratory cage for the same period in a room in which the running wheels were placed. Motor activity was tested in a specially arranged test room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e. motor activity test cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and a small double-glass window to allow observation; each box had a dimmed lighting.

Three weeks following arrival, four groups ($n = 10$) of DSP4-treated and two groups of vehicle-treated mice were administered either MPTP (3 × 30 mg/kg, s.c., 24 h between injections) or vehicle (0.9 % physiological saline injected s.c. in a volume of 2 ml/kg body weight). Milmed (see below for details of preparation) or vehicle were administered twice weekly.

Experiments were 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 Biomedical Inc., MA, USA, 3 × 30 mg/kg, s.c., with a 24-h interval between injections in each case) was dissolved in saline and administered s.c. in a volume of 2 ml/kg body weight.

Milmed Preparation and Administration

Milmed was obtained through treatment and preparation of the yeast fungi, *S. cerevisiae*, a strain that is obtained from the International Research Center "Beer and Beverage XXI Century", Moscow, Russia. The yeast was cultured in wort which was produced through meaded malt extract. The treatment of the yeast in a electromagnetic field of super-high frequencies with electromagnetic waves in the EHF range of 30–300 GHz to produce the treated yeast extract (cf. Golant 1994), after which the yeast was re-cultured at 25–28 °C for 48 h. Saline was used as vehicle in each case. Following this, the cell concentration in the completed yeast suspension was measured using NucleoCounter YC-100 (ChemoMetec A/S, Denmark) and the extent to which the suspension should be diluted was decided. The yeast suspension was sent to the Department of Neuroscience Laboratory at the University of Uppsala from Production Unit at Milmed AB Company (Färjestad, Sweden). A bottle with sterilised wort for dilution was sent concurrently. Mice were orally treated with 0.5 ml/kg Milmed containing a cell concentration of approximately 2×10^6 yeast cells daily according to the preparation protocol and design developed from previous observations regarding stability and viability of compound (each dose contained 1×10^6 yeast cells). Each mouse was administered Milmed once per day 4 times/week, per orally (p.o.), during the 10 (or 11 for the MPTP + Exer + M(ii) group): The administered suspension was prepared twice weekly and sent to the Uppsala University on Mondays and Tuesdays. The suspension delivered on Mondays was administered to the mice in the respective groups on Mondays and Tuesdays whereas that suspension delivered on Tuesdays was administered on Wednesdays and Thursdays. Throughout,

the suspension delivered to the laboratory was maintained at 5 °C in the refrigerator.

Design and Treatment

At the start of the experiment, mice were administered single weekly doses of MPTP (1 × 30 mg/kg) during three consecutive weeks, directly after a 10-min session in the running wheels followed by testing in motor activity test chambers (ADEA) over 60 min. Wheel-running exercise was initiated during Week 1 (Mondays to Thursdays) as well as treatment with Milmed (see above) for the MPTP + Exer + M(ii) group only (see Table 1); the other four groups received access to the running wheels once only (Wednesday) over 30 min. For the MPTP + Exer and MPTP + Exer + M(i) groups wheel-running exercise was initiated during Week 2 (Mondays to Thursdays) as well as treatment with Milmed (see above) while the Vehicle and MPTP–NoEx received only the single access (Wednesday) till the running wheel. On each Friday (Weeks 2 and 3) all the MPTP-treated mice, Groups MPTP–NoEx, MPTP + Exer, MPTP + Exer + M(i) and MPTP + Exer + M(ii), mice were administered single weekly doses of MPTP (1 × 30 mg/kg) during these two consecutive weeks thereby ensuring that these mice had received MPTP (3 × 30 mg/kg) at a total of 90 mg/kg. During Weeks 4 to 10, the identical exercise—test sessions regime, maintained during Weeks 2 and 3, was carried out: Groups MPTP + Exer, MPTP + Exer + M(i) and MPTP + Exer + M(ii) receiving four sessions/week of wheel running, Groups MPTP + Exer + M(i) and MPTP + Exer + M(ii) receiving in addition the Milmed administration regime and the Vehicle and MPTP–NoEx groups receiving only the Wednesday 30-min test session in the running wheels. Every Friday (Weeks 1 to 10), all the mice in the five groups were placed in the running wheels for 10 min and distance run was registered. After this they were placed in motor activity test chambers for 60 min (except Week 10). For Week 10, following the 60 min they were injected L-Dopa (5 mg/kg) and replaced in the chambers for a further 180 min.

Behavioural Measurements and Apparatus

Activity test chambers: An automated device, consisting of macrolon rodent test cages (40 × 25 × 15 cm) each placed within two series of infrared beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous motor activity (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). The distance between the infrared beams was as follows: the low levels beams were 73 mm apart lengthwise and 58 mm apart breadthwise in relation to the test chamber; the high level beams, placed only along each long side of the test chamber were 28 mm apart. According to the procedures described previously (Archer et al. 1986), the following parameters were measured: *Locomotion* was measured by the low grid of infrared beams. Counts were registered only when the mouse in the horizontal plane, ambulating around the test cage. *Rearing* was registered throughout the time when at least one high level beam was interrupted, i.e. the number of counts registered was proportional to the amount of time spent rearing. *Total Activity* was measured by a sensor (a pick-up similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was constantly in contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching and grooming. All three behavioural parameters were measured over three consecutive 20-min periods. The motor activity test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e. activity cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels, and day-lighting. Motor activity parameters were tested on one occasion only, over three consecutive 20-min periods, at the age of 3–4 months. Groups of mice were treated with MPTP and then given access to running wheels (30-min/day, 4 times/week), with or without concomitant treatment with Milmed ([Milm(1)-charged] or [Milm(0)]-uncharged = yeast itself), as displayed in Table 1.

Computer-Linked Running-Wheel Units

Small rodent exercise wheels for treadmill-type running behaviour, purchased from a Pet store and considered suitable for the C57/Bl6 mice to run upon. The wheels were adapted and modified for use by laboratory mice; they were placed altogether in a large sound-proofed room within the animal housing section of the laboratory. All 25 running wheels were placed equidistant from each other with adjacent wheels in two long rows such that the sounds of the 'wheel-revolving noises' emitted by any single wheel could be heard easily by the occupants of all the other 24 wheels. A photograph of the types of running wheels utilised in all our physical exercise studies, presenting a double row of the activity-enhancing running wheels applied in all the experiments as well as the "holding" cages in which the non-exercised groups remained, was depicted previously (Archer and Fredriksson 2010). In previous neuroteratology studies that observed wheel-running exercise following different types of perinatal treatments, it was observed that each wheel needed to be placed in isolation from each of the others because the noise emitted from the wheel running of any one animal served to evoke wheel-running behaviour by the other animals. In the study by Archer and Fredriksson (2012,

Table 1 Chronological and procedural description of exercise schedules and Milmed treatment for MPTP-treated and control mice

Week/day	Vehicle	MPTP–NoEx	MPTP + Exer	MPTP + Exer + M(i)	MPTP + Exer + M(ii)
Monday	Cage	Cage	Cage	Cage	Exer + M
Week 1	Cage	Cage	Cage	Cage	Exer + M
Wed. test	Cage	Cage	Cage	Cage	Exer + M
	Cage	Cage	Cage	Cage	Exer + M
Friday	Test + sal	Test + MPTP	Test + MPTP	Test + MPTP	Test + MPTP
Monday	Exer	Cage	Exer	Exer + M	Exer + M
Week 2-3	Exer	Cage	Exer	Exer + M	Exer + M
Wed. test	Exer	Cage	Exer	Exer + M	Exer + M
	Exer	Cage	Exer	Exer + M	Exer + M
Friday	Test + Sal	Test + MPTP	Test + MPTP	Test + MPTP	Test + MPTP
Monday	Exer	Cage	Exer	Exer + M	Exer + M
Week 4-10	Exer	Cage	Exer	Exer + M	Exer + M
Wed. test	Exer	Cage	Exer	Exer + M	Exer + M
	Exer	Cage	Exer	Exer + M	Exer + M
Friday	Test	Test	Test	Test	Test
	Test	Test	Test	Test	Test

M Milmed was administered 4 times/week, p.o., (see above); *MPTP* 3 × 30 mg/kg; *Exer* exercise condition, 4 sessions/week; *Cage* non-exercise condition, single session/week

Experiment II), one group "MPTP + Wheel-NoEx" was placed in the running wheels for the 30-min exercise periods but in this case the wheels had been fixed and remained immobile despite efforts by each mouse to get the wheel to revolve. Thus, each mouse would initially attempt to run up the slope of the wheel but would soon give up and remain unexercised. It was observed that placement in the 'non-revolving' wheel result in similar MPTP-induced deficits in function and striatal DA concentration. The Rodent running wheels with computer-controlled devices adapted for measurement of running exercise per unit time (Ödman et al. 2013) are depicted in Fig. 1. The arm of the upright (1) registers every revolution which is fed into a laptop computer that monitors all running activity by each mouse during the wheel-running session.

Procedure

In present experiment, mice in the non-exercised groups were placed in the holding cages singly for 3 out of the 4 days (Mondays, Tuesdays and Thursdays) that the exercised mice were given access to the running wheels. On Wednesdays, all the mice, in both the exercised and non-exercised groups were given 30-min access sections in the running wheels: Thus, the exercise condition is defined by four 30-min sessions/week in the running wheels whereas the non-exercise condition is defined by a single 30-min session/week in the running wheels. The single 30-min session (Wednesdays) was taken as an exercise test session in order to monitor the extent of exercise by each group. Each of the running wheels was monitored by a laptop computer that registered every revolution (utilizing arm responding to each wheel revolution) and counted revolutions per unit time during each session (see Fig. 1). On Fridays, prior to the tests of spontaneous motor activity in the motor activity test chambers (60-min test sessions), a single 10-min test session/week was given all the mice in the exercise and non-exercise conditions. Following the 60-min spontaneous motor activity test during Week 10 (Friday), each mouse was injected a subthreshold dose of L-Dopa (5 mg/kg, s.c.) and replaced in its motor activity test chamber and locomotor, rearing and total activity counts were registered over a further 180 min. During Week 11, all the mice were killed and striatal regions taken for analysis of DA concentrations. Each of mice in each of the five different was identified through careful marking procedures so that the DA concentration, running distances from the 30-min (Wednesday) and 10-min (Friday) tests during Weeks 1 to 10, locomotor, rearing and total activity counts from the tests of spontaneous motor activity tests during Weeks 1 to 10, locomotor, rearing and total activity counts from the L-Dopa-induced motor activity test on Week 10 only and DA concentrations of each mouse were registered and identified.

Neurochemical Analysis

Mice were killed by cervical dislocation within 2 weeks of completion of behavioural testing. Determination of DA was performed using an high-performance liquid chromatograph with electrochemical detection (HPLC-EC), according to (Björk et al. 1991), as modified by (Liu et al.

Fig. 1 Rodent running wheels with computer-controlled devices adapted for measurement of running exercise per unit time (Ödman et al. 2013) by individual mice over 30-min daily exercise sessions (Mondays, Tuesdays and Thursdays) or 10-min (Friday) or 30-min (Wednesday) test sessions. The *arm* of the *upright* (*1*) registers every revolution which is fed into a laptop computer that monitors all running activity by each mouse during the wheel-running session

1995). Striatal regions were rapidly dissected out and stored at -80(C until neurochemical analysis. DA concentration was measured as follows: The frozen tissue samples were weighed and homogenised in 1 ml of 0.1 M perchloric acid, and alpha-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12,000 rpm, i.e. 18,600×*g*, 4 °C, 10 min) and filtration, 20 µl of the supernatant was injected into the HPLC-EC to assay DA. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume: 20 µl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 µm), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 µm), and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and a MF-2000 cell) operating at a potential of +0.85 V. The mobile phase, pH 2.69, consisted of K_2HPO_4 and citric acid buffer (pH 2.5), 10 % methanol, sodium octyl sulphate, 40 mg/l, and EDTA. The flow rate was 1 ml/min, and the temperature of the mobile phase was 35 °C. The DA analysis concentration results are expressed as ng/ml wet weight.

Statistical Analysis

Distance run during the 10-min running-wheel tests on Fridays, distance run during the 30-min running-wheel tests on Wednesdays, locomotion, rearing and total activity (from Friday activity tests) over 60-min test periods, locomotion, all over 10 weeks were subjected to split-plot ANOVA (Kirk 1995), while rearing and total activity from L-Dopa-induced activity test periods and striatal DA concentrations were subjected to one-way ANOVA. Pairwise testing was performed using the Tukey's–HSD tests. Pearson's correlation coefficients between (i) distance run during the 30-min running-wheel tests on Wednesdays and striatal DA concentration, (ii) locomotor counts and striatal DA concentration and (iii) distance run during the 30-min running-wheel tests on Wednesdays and locomotor counts, over all 50 mice for Week 10 only, were computed. Pearson's correlation coefficients between (i) distance run during the 30-min running-wheel tests on Wednesdays and striatal DA concentration, (ii) locomotor counts and striatal DA concentration, and (iii) distance run during the 30-min running-wheel tests on Wednesdays and locomotor counts, for each of the five groups ($n = 10$) for Week 10 only, were computed (see Table 2).

Results

Physical exercise (four 30-min sessions/week) ameliorated markedly the MPTP-induced hypokinesic effect in the exercise condition, MPTP + Exer, but not in the non-exercised condition (single 30-min session/week), MPTP–NoEx, over all the motor behaviour parameters measured. By Week 6, the combination of the four 30-min sessions/week exercise regime with weekly Milmed administrations had restored completely motor functioning by the MPTP + Exer + M(i) and MPTP + Exer + M(ii) groups. The MPTP + Exer + M(ii) group continued to increase distance run during the 30- and 10-min tests up to Week 10 to exceed the Vehicle group.

10-min Running Distance Tests (Fridays)

Split-plot ANOVA indicated a significant Treatment Groups × Test days interaction effect for distance run over 10 min: $F(49, 450) = 28.54$, $p < 0.0001$. Figure 2 presents mean (SD) distance run in the running wheels by each of the

Table 2 Correlation coefficients for (i) dopamine (DA) versus distance run in the running wheels during Week 10 (Wednesday test), (ii) dopamine (DA) versus locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10, and (iii) Distance run in the running wheels during Week 10 (Wednesday test) versus locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10 for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI and MPTP + Exercise + MilmedII

Correlations (df = 10)	DA vs Dist	DA vs Locom.	Dist vs Locom.
Groups			
Vehicle	$R = 0.965$	$R = 0.926$	$R = 0.916$
	$p < 0.000005$	$p < 0.0001$	$p < 0.0002$
MPTP	$R = 0.966$	$R = 0.912$	$R = 0.926$
	$p < 0.000005$	$p < 0.0002$	$p < 0.0001$
MPTP + Exercise	$R = 0.942$	$R = 0.898$	$R = 0\ 923$
	$p < 0.0004$	$p < 0.0005$	$p < 0.0002$
MPTP + Exercise + MilmedI	$R = 0.864$	$R = 0.882$	$R = 0.943$
	$p < 0.001$	$p < 0.0008$	$p < 0.000005$
MPTP + Exercise + MilmedII	$R = 0.926$	$R = 0.969$	$R = 0.967$
	$p < 0.0001$	$p < 0.000004$	$p < 0.000004$

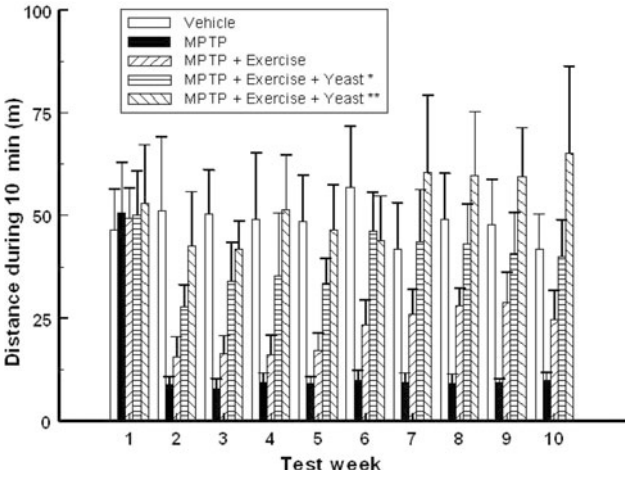


Fig. 2 Mean (SD) distance run in the running wheels by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 10 min on the Friday running test prior to testing in the motor activity test chambers. Vehicle and MPTP groups received access to the running wheels once/week only, whereas the MPTP + Exercise, MPTP + Exercise + MilmedI and MPTP + Exercise + MilmedII received access to the running wheels four times/week (Mondays to Thursdays) over 30-min sessions

five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 10-min on the Friday running test prior to testing in the motor activity test chambers.

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

Week 1: no differences.
Week 2 and 5: Vehicle, MPTP + Exer + M(ii) > MPTP + Exer + M(i) > MPTP + Exer, MPTP–NoEx.
Week 3 and 4: Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer, MPTP–NoEx.
Week 6, 7, 8, 9 and 10: Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

30-min Running Distance Tests/Wednesdays)

Split-plot ANOVA indicated a significant Treatment Groups × Test days interaction effect for distance run over 30 min: $F(49, 450) = 34.35, 28.54$, $p < 0.0001$. Figure 3 presents the mean (SD) distance run in the running wheels by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 30 min on Wednesday running test.

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

Week 1: no differences.
Week 2, 3 and 4: Vehicle, MPTP + Exer + M(ii) > MPTP + Exer + M(i) > MPTP + Exer, MPTP–NoEx.
Week 5: MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer, MPTP–NoEx.
Week 6, 7, 8, 9 and 10: Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

Spontaneous Motor Activity (Friday Tests)

Split-plot ANOVA indicated a significant Treatment Groups × Test days interaction effect for locomotion, rearing and total activity counts: $F(49, 450) = 49.45$, $p < 0.0001$;

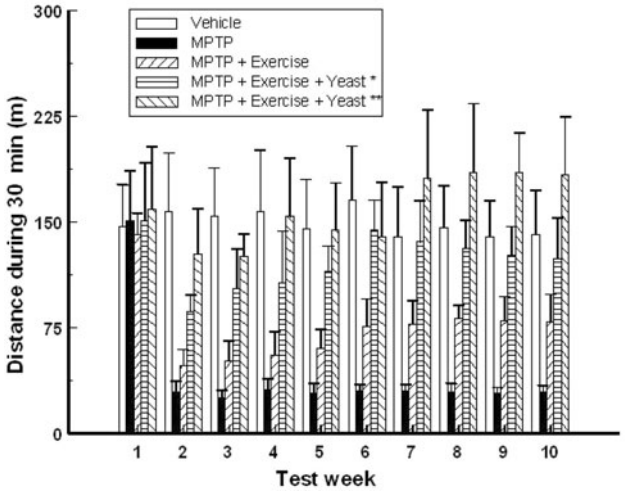


Fig. 3 Mean (SD) distance run in the running wheels by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 30 min on Wednesday running test. Vehicle and MPTP groups received access to the running wheels once/week only, whereas the MPTP + Exercise, MPTP + Exercise + MilmedI and MPTP + Exercise + MilmedII received access to the running wheels four times/week (Mondays to Thursdays) over 30-min sessions

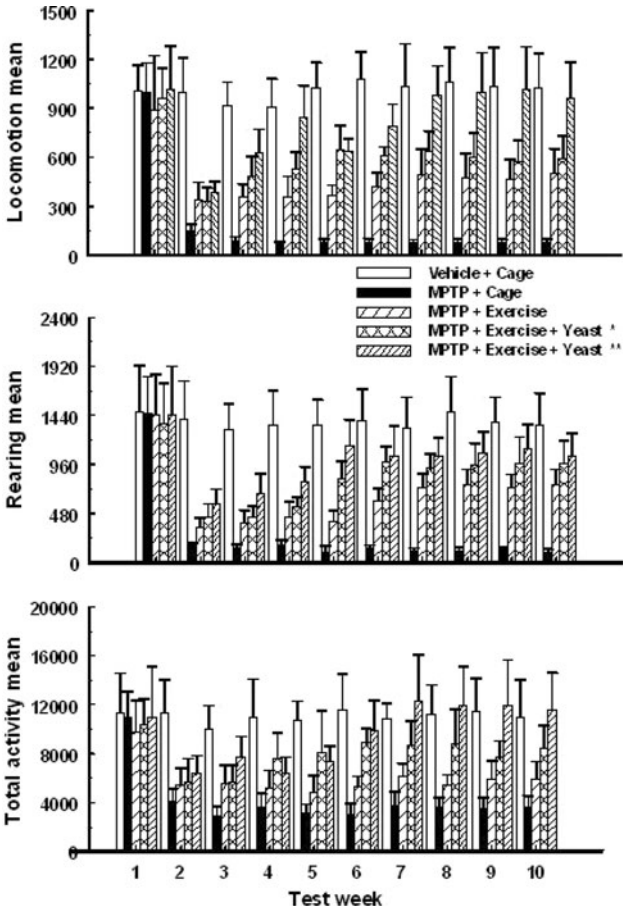


Fig. 4 Mean (SD) locomotion, rearing and total activity counts for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day (Fridays) from Week 1 to 10

$F(49, 450) = 42.08$, $p < 0.0001$; $F(49, 450) = 20.12$, $p < 0.0001$, respectively. Figure 4 presents the mean (SD) locomotion, rearing and total activity counts for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day (Fridays) from Week 1 to 10.

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

Locomotion:
Week 1: no differences.
Week 2: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 3: Vehicle > MPTP + Exer + M(ii) > MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 4: Vehicle, MPTP + Exer + M(ii) > MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 5: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
Week 6: Vehicle > MPTP + Exer + M(ii) > MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
Week 7, 8, 9 and 10: Vehicle, MPTP + Exer + M(ii) > MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.

Rearing:
Week 1: no differences.
Week 2: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 3 and 4: Vehicle > MPTP + Exer + M(ii) > MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 5: Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
Week 6 and 7: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
Week 8, 9 and 10: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.

Total activity:
Week 1: no differences.
Week 2: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.

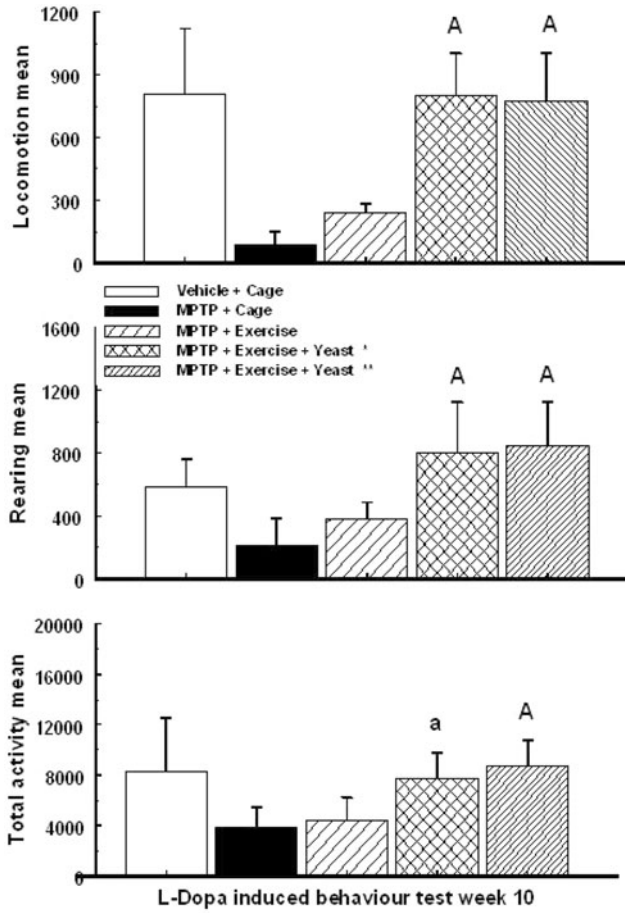


Fig. 5 Mean (SD) locomotion, rearing and total activity counts for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 180-min test sessions for L-Dopa-induced activity at testing day (Fridays) during Week 10 only. For locomotion, MPTP + cage < MPTP + Exercise; *A* versus MPTP + Exercise ($p < 0.01$), *a* $p < 0.05$

Week 3: Vehicle > MPTP + Exer + M(ii) > MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 4: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i), MPTP + Exer > MPTP–NoEx.
Week 5: Vehicle > MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
Week 6, 7, 8, 9 and 10: Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

L-Dopa-Induced Motor Activity (Friday Tests)

One-way ANOVA indicated a significant between-groups effect for L-Dopa-induced motor activity: $F(4, 45) = 24.70$, $p > 0.0001$; $F(4, 35) = 11.33$, $p > 0.0001$; $F(4, 35) = 6.65$, $p > 0.0004$, for locomotion, rearing and total activity, respectively. Figure 5 presents the mean (SD) locomotion, rearing and total activity counts for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) over 180-min test sessions for L-Dopa-induced activity at testing day (Fridays) during Week 10 only.

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

Locomotion:
Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

Rearing:
Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

Total activity:
Vehicle, MPTP + Exer + M(ii), MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.

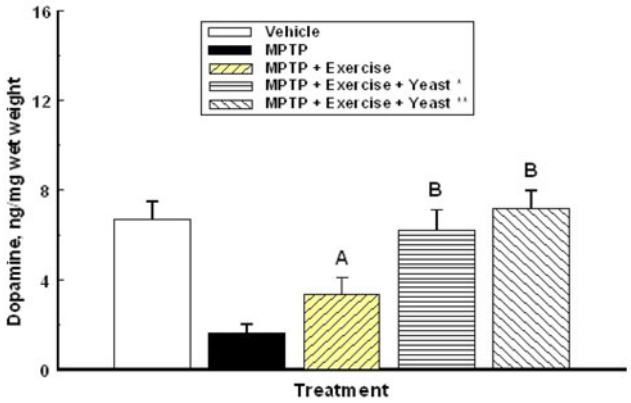


Fig. 6 Mean (SD) concentrations of striatal dopamine for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**). *A* versus MPTP; *B* versus MPTP + Exercise

Striatal Dopamine Concentration

One-way ANOVA indicated a significant between-groups effect for striatal dopamine concentration: $F(4, 45) = 109.08$, $p < 0.0001$. Figure 6 presents the mean (SD) concentrations of striatal dopamine for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**).

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

MPTP + Exer + M(ii), Vehicle, MPTP + Exer + M(i) > MPTP + Exer > MPTP–NoEx.
MPTP + Exer + M(ii) > MPTP + Exer + M(i).

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

MPTP–NoEx = 24 %; MPTP + Exer = 51 %; MPTP + Exer + M(i) = 93 %.
MPTP + Exer + M(ii) = 108 %.

Correlational Analyses

Pearson's correlation coefficients between (i) distance run during the 30-min running-wheel tests on Wednesdays and striatal DA concentration, (ii) locomotor counts and striatal DA concentration and (iii) distance run during the 30-min running-wheel tests on Wednesdays and locomotor counts, over all 50 mice for Week 10 only, were 0.945 ($N = 50$, $p < 0.0001$), 0.914 ($N = 50$, $p < 0.0001$), and 0.930 ($N = 50$, $p < 0.0001$). Figures 7, 8 and 9 present the correlational relationship between (i) distance run in the running wheels during Week 10 (Wednesday test) and striatal dopamine concentration (ng/mg), (ii) Spontaneous Motor activity test during Week 10 (Friday test) and striatal dopamine concentration (ng/mg) and (iii) distance run in the running wheels during Week 10 (Wednesday test) and locomotion counts in the Spontaneous Motor activity test during Week 10 (Friday test), respectively, by each mouse in each of the five groups: Vehicle, MPTP–NoEx, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) groups, and by all 50 mice studied. Table 2 presents Correlation coefficients for (i) dopamine (DA) versus distance run in the running wheels during Week 10 (30-min Wednesday test), (ii) dopamine (DA) versus locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10 and (iii) Distance run in the running wheels during Week 10 (Wednesday test) versus locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10 for each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI and MPTP + Exercise + MilmedII.

It will be noted that all the within group correlations were highly significant (highest: 0.969, lowest: 0.864, $N = 10$, $p < 0.001$–0.000004), implying that, within each treatment group, (i) mice showing lower levels of striatal DA ran shorter distances in the running wheels during the 30-min Wednesday during Week 10 test sessions, (ii) mice showing lower levels of striatal DA showed less locomotor activity during the testing during Week 10 and (iii) mice running shorter distances in the running wheels during the 30-min Wednesday during Week 10 test sessions showed less locomotor activity during the testing during Week 10.

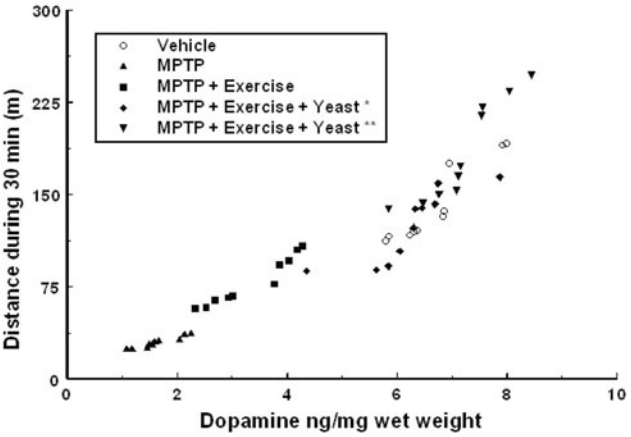


Fig. 7 Mean distance run in the running wheels during Week 10 (Wednesday test) in relation to mean striatal dopamine concentration (ng/mg) by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) groups, and by all 50 mice studied. Yeast* = MilmedI; Yeast** = MilmedII. Correlation coefficients for each of the five treatment groups are presented in Table 2

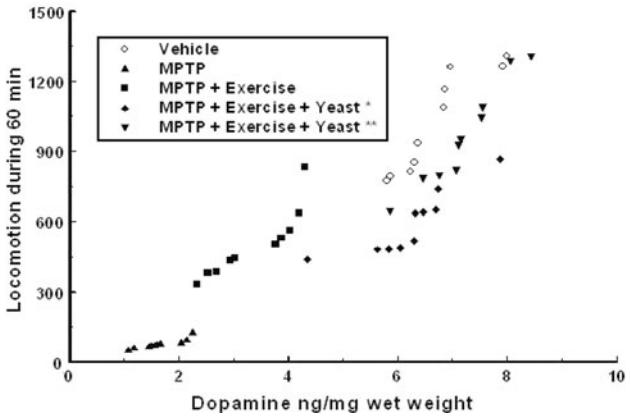


Fig. 8 Mean locomotion counts in the Spontaneous Motor activity test during Week 10 (Friday test) in relation to mean striatal dopamine concentration (ng/mg) by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) groups, and by all 50 mice studied. Yeast* = MilmedI; Yeast** = MilmedII. Correlation coefficients for each of the five treatment groups are presented in Table 2

Correlational analyses performed to compute correlation coefficients over all 50 mice in the study between: (i) dopamine (DA) versus distance run in the running wheels during Week 10 (10-min Friday test): correlation coefficient = 0.725 ($N = 50$, $p < 0.0001$), (ii) dopamine (DA) versus locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10: correlation

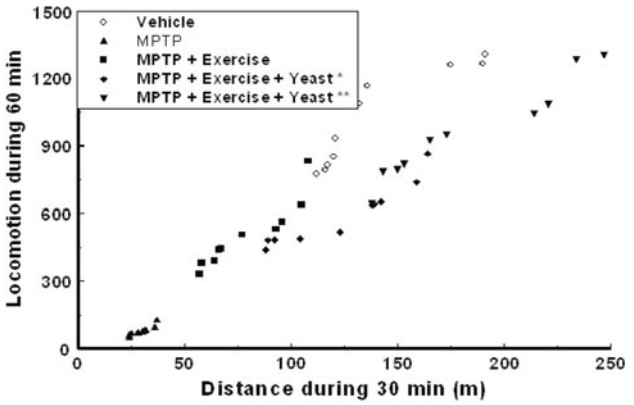


Fig. 9 Mean distance run in the running wheels during Week 10 (Wednesday test) in relation to mean locomotion counts in the Spontaneous Motor activity test during Week 10 (Friday test) by each of the five groups: Vehicle, MPTP, MPTP + Exercise, MPTP + Exercise + MilmedI (MPTP + Exercise + Yeast*) and MPTP + Exercise + MilmedII (MPTP + Exercise + Yeast**) groups, and by all 50 mice studied. Yeast* = MilmedI; Yeast** = MilmedII Correlation coefficients for each of the five treatment groups are presented in Table 2

coefficient = 0.914 ($N = 50$, $p < 0.0001$) and (iii) Distance run in the running wheels during Week 10 (10-min Friday test) and locomotion counts over 60-min test sessions for spontaneous motor activity in the Motor Activity test chambers at testing day during Week 10: correlation coefficient = 0.622 ($N = 50$, $p < 0.0001$). These strongly significant correlation coefficients indicate that the 10-min wheel-running sessions prior to the testing of spontaneous and L-Dopa-induced motor activity also provide an important parameter to support the notion that DA integrity is essential expressions of both motor performance and physical exercise capacity.

Discussion

The results of this experiment to ascertain the extent of physical exercise, wheel-running and 4 times weekly administrations (p.o.) of the treated yeast suspension, Milmed, combination (Exer + Milmed) in protecting and restoring motor function and DA integrity may be summarised as follows:

(i) Introduced prior to the 1st injection of MPTP, Exer + MilmedII attenuated noticeably the extent of functional deficits induced by MPTP and restored completely motor function as assessed through the 30-min running session (Wednesday), the 10-min running session (Friday), spontaneous motor activity and L-Dopa-induced activity. Furthermore, striatal DA concentrations were slightly above vehicle values after 10 weeks of the Exer + MilmedII treatment combination; it appears that this intervention provided both protection and restoration.

(ii) Introduction of the Exer + MilmedI treatment combination, after the 1st injection of MPTP, affected a small, but significant, attenuation of deficits in motor function immediately during Week 2, and an almost full restoration of motor function by Week 10. DA concentration, although slightly less than that of the Vehicle mice did not differ from them; thus, the protective effects of Exer + Milmed introduction 70 h after the 1st injection of MPTP were marginal and the restorative effects did not quite reach full completion but very nearly so.

(iii) The physical exercise intervention was introduced also 70 h following the 1st injection of MPTP. It was observed that physical exercise, by itself, did not significantly attenuate the motor deficit in running behaviour until Weeks 3 or 5 in the tests of running behaviour whereas motor activity in the test cages, though less than the Exer-Milmed groups, was significantly greater than the MPTP–NoEx group. The DA deficit was attenuated also.

(iv) The correlational analyses indicated that within each treatment group, (a) mice showing lower levels of striatal DA ran shorter distances in the running wheels during the 30-min Wednesday during Week 10 test sessions, (b) mice showing lower levels of striatal DA showed less locomotor activity during the testing during Week 10 and (c) mice running shorter distances in the running wheels during the 30-min Wednesday during Week 10 test sessions showed less locomotor activity during the testing during Week 10. (d) The 10-min (Friday) running sessions also showed a very high level of positive correlation between distance run and DA integrity in the striatum.

(v) All the correlations between the main parameters: 10- and 30-min running sessions, motor activity in the test chambers and DA concentrations, both over all 50 mice and within each particular group of 10 mice, underline the consistency and reliability of the observations.

(vi) With regard to the major purpose of the present experiment: DA integrity was observed to be a direct function of (a) ability to express running exercise in a treadmill wheel-running arrangement (see Fig. 7), and (b) the capacity for motor performance as measured by spontaneous motor activity and sub-threshold L-Dopa (5 mg/kg) induced activity in motor activity test chambers (see Fig. 8). Furthermore, the extent to which running exercise in a

running wheel was predictive of motor performance in the activity test chambers was shown also to be overwhelming (see Fig. 9).

(vii) Regarding the ancillary purpose, the combination of twice weekly administrations of Milmed with physical exercise (4 days/week, 30-min/day) restored completely MPTP-induced deficits regards all measures of motor function and striatal DA concentrations.

The loss of striatal DA following the different regimes of MPTP administration has been found to vary markedly, but quite systematically, as a function of neurotoxin dosages and the number of administrations. For example, after the administration of MPTP (2 × 40 mg/kg, with a 24-h interval between injections) the striatal concentration of DA was 17 % of control values, whereas applying the more severe administration of MPTP (4 × 40 mg/kg, with 1 week intervals between injections DA level was 11 % of controls (cf. Archer and Fredriksson 2010, 2012; Fredriksson et al. 2011); applying an MPTP dose regime of 3 × 30 mg/kg, with 1 week between injections, it has been shown that DA levels were 24–25 % of control values, whereas with a 4 × 30 mg/kg MPTP dose regime DA levels were 20–21 % of control values (unpublished data). All these conditions involving "no exercise" schedules for the MPTP-treated mice where in each case an "exercise" condition had been presented that consisted of four 30-min sessions/week of wheel-running activity. In each case, the introduction of weekly exercise schedules in the running wheels attenuated, but did not restore completely the deficits in striatal DA concentrations, with levels of recovery that were dependent upon the duration of exercise schedules. Gerecke et al. (2010), using a MPTP dose regime of 4 × 20 mg/kg, injected a 2-h intervals, showed that the DA deficit was attenuated in the exercise condition by about a 20–24 % increase in concentration, expressed as % of control values. Throughout the series of experiments both published (Archer and Fredriksson 2010, 2012; Fredriksson et al. 2011), in press (Archer and Fredriksson 2013a, b) and unpublished, the percentage increase, significant in each case, in striatal DA level has varied, over the different MPTP regimes, 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) and in the present experiment 27 % (10 weeks of exercise). Although there seems clear evidence that physical exercise in the running wheels induced reliable in DA concentrations, it is clear that exercise, itself, is insufficient. Nevertheless, for DA neurons exercise plays an essential and central role: "use it or lose". Tuon et al. (2012) have indicated that the effects of exercise upon PD deficits may be due to a modulation of the neurochemical status in the striatum of the rats used, possibly through improvement of oxidative stress parameters. They found that their Exercise-PD group showed increased the levels of brain-derived neurotrophic factor (BNDF), anti-α-synuclein, anti-sarcoplasmic reticulum Ca^{2+}-ATPase SERCA II, anti-superoxide dismutase (SOD), and anti-catalase (CAT) as well as decreased oxidative damage in lipids and protein. Fredriksson et al. (2011) observed that 14 weeks of wheel-running exercise increased significantly the concentration of BDNF in the parietal cortex of MPTP-mice. Silva et al. (2013) showed that trained eccentric running improved mitochondrial function but did not reduce oxidative stress, muscle damage, or inflammation induced by eccentric contractions.

The combination of exercise intervention with the treated yeast, Milmed, administration two to four times each week of the wheel-running schedule has been found to provide a complete restoration of the functional and DA deficits following MPTP (Archer and Fredriksson 2013a, b). In the present study, the initiation of exercise + Milmed 70 h after the 1st injection of MPTP, affected an almost complete restoration of motor function by Week 10 and a 93 % restoration of striatal DA. The initiation of exercise + Milmed 5 days prior to the 1st MPTP injection in the MPTP + Exercise + MilmedII condition abolished completely the MPTP-induced deficits in motor and restored fully (108 %) striatal DA. It appears that the introduction of the exercise + Milmed combination several days prior to the neurotoxin treatment provided both a neuroprotective effect limiting the extent of damage and disruption of motor function and a neurorestorative effects over the several weeks of continued exercise + Milmed combination treatment. *S. cerevisiae*, "budding yeast", has proven increasingly to offer a valuable tool for the study the molecular basis of several neurodegenerative disorders from model systems that present both causative and interventional perspectives (Tenreiro et al. 2013). Sirtuins, identified from yeast, present a new class of III NAD-dependent histone deacetylases that regulate a number of physiological processes including cellular and metabolic processes involved in neurodegeneration processes linked to Type 2 diabetes, obesity and Alzheimer's disease (Suvarna 2013; see also Fusco et al. 2012). Finally, several studies have indicated that the activation/inhibition Sir2-family of enzymes presents therapeutic possibilities over a broad spectrum of ageing-associated neurodegenerative conditions (Mahajan et al. 2011).

The present study describes the putative transporter role of the yeast, *S. cerevisiae*, for transferring specific biological and therapeutic effects of EHF electromagnetic fields living organisms in the form of Milmed yeast. Electromagnetic treatment of these yeast cells has been

studied (Sebastián et al. 2011) but apparently not in the manner introduced by Golant et al. (Golant et al. 1994). Nevertheless, the above restorative effects of exercise + milmed yeast administration upon function and DA integrity are highly robust, having been replicated several times (unpublished data).

References

Adkins DL, Boychuk J, Remple MS, Kleim JA (2006) Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol 101:1776–1782

Archer T, Fredriksson A (2003) An antihypokinesic action of α2-adrenoceptors upon MPTP-induced behavior deficits in mice. J Neural Transm 110:183–200

Archer T, Fredriksson A (2006) Influence of noradrenaline denervation upon MPTP-induced deficits in mice. J Neural Transm 113:1119–1129

Archer T, Fredriksson A (2010) Physical exercise attenuates MPTP-induced deficits in mice. Neurotox Res 18:313–327

Archer T, Fredriksson A (2012) Delayed exercise-induced functional and neurochemical partial restoration following MPTP. Neurotox Res. doi:10.1007/s12640-011-9261-z

Archer T, Fredriksson A (2013a) Pharmacogenomics and personalized medicine in Parkinsonism. In: Barh D (ed) Pharmacogenomics. Springer, Berlin

Archer T, Fredriksson A (2013b) Physical exercise as intervention in Parkinsonism. In: Kostrzewa RM (ed) Handbook of neurotoxicity. Springer, New York

Archer T, Fredriksson A, Jonsson G, Lewander T, Mohammed AK, Ross SB, Söderberg U (1986) Central noradrenaline depletion antagonizes aspects of d-amphetamine-induced hyperactivity in the rat. Psychopharmacology 88:141–146

Archer T, Johansson B, Fredriksson A (2011) Exercise alleviates Parkinsonism: clinical and laboratory evidence. Acta Neurolog Scand. doi:10.1111/j.1600-0404.2010.01360.x

Arendash GW (2012) Transcranial electromagnetic treatment against Alzheimer's disease: why it has the potential to trump Alzheimer's disease drug development. J Alzheimers Dis 32:243–266. doi:10.3233/JAD-2012-120943

Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C (2010) Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis 19:191–210. doi:10.3233/JAD-2010-1228

Baptista PP, de Senna PN, Paim MF, Saur L, Blank M, do Nascimento P, Ilha J, Vianna MR, Mestriner RG, Achaval M, Xavier LL (2013) Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats. Pharmacol Biochem Behav 104:113–118. doi:10.1016/j.pbb.2012.12.020

Bilowit DS (1956) Establishing physical objectives in rehabilitation of patients with Parkinson's disease. Phys Ther Rev 36:176–178

Björk L, Lindgren S, Hacksell U, Lewander T (1991) (S)-UH-301 antagonises ®-8-OH-DPAT-induced cardiovascular effects in the rat. Eur J Pharmacol 199:367–370

Braun RJ, Büttner S, Ring J, Kroemer G, Madeo F (2009) Nervous yeast: modeling neurotoxic cell death. Trends Biochem Sci 35:135–144

Carmona-Gutierrez D, Jungwirth H, Eisenberg T, Madeo F (2010) Cell cycle control of cell death in yeast. Cell Cycle 9:4046

Corcos DM, Robichaud JA, David FJ, Leurgans SE, Vaillancourt DE, Poon C, Rafferty MR, Kohrt WM, Comella CL (2013) A two-year randomized controlled trial of progressive resistance exercise for Parkinson's disease. Mov Disord. doi:10.1002/mds.25380

Fisher BE, Petzinger GM, Nixon K, Hogg E, Bremmer S, Meshul CK, Jakowec MW (2004) Exercise-induced behavioral recovery and neuroplasticity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse basal ganglia. J Neurosci Res 77:378–390

Fredriksson A, Archer T (1994) MPTP-induced behavioural and biochemical deficits: a parametric analysis. J Neural Transm Park Dis Dement Sect 7:123–132

Fredriksson A, Archer T (2003) Effect of postnatal iron administration on MPTP-induced behavioural deficits and neurotoxicity: behavioural enhancement by L-Dopa-MK-801 co-administration. Behav Brain Res 139:31–46

Fredriksson A, Plaznik A, Sundström E, Jonsson G, Archer T (1990) MPTP-induced hypoactvity in mice: reversal by L-Dopa. Pharmacol Toxicol 67:295–301

Fredriksson A, Palomo T, Chase TN, Archer T (1999) Tolerance to a suprathreshold dose of L-Dopa in MPTP mice: effects of glutamate antagonists. J Neural Transm 106:283–300

Fredriksson A, Stigsdotter IM, Hurtig A, Ewalds-Kvist B, Archer T (2011) Running wheel activity restores MPTP-induced functional deficits. J Neural Transm 18:407–420

Fusco S, Maulucci G, Pani G (2012) Sirt1: def-eating senescence? Cell Cycle 11:4135–4146. doi:10.4161/cc.22074

Gedymin LE, Golant MB, Kuznetsov AP, Mudrik DG, Kolpikova TV, Balakireva LZ (1999) The use of yeast living cells as a biological retranslation media for the therapeutic EHF effects. Millim Waves Biol Med 16:10–15 (in Russian)

Golant MB (1994) Physical laws of medicine and their use in the realization of living organisms with EHF radiation. Radiophys Quantum Electron 37:45–47

Golant MB, Mudrik DG, Kruglyakova OP, Izvol'skaya VE (1994) Effect of EHF-radiation polarization on yeast cells. Radiophys Quantum Electron 37:82–84. doi:10.1007/BF01039307

Gorton LM, Vuckovic MG, Vertelkina N, Petzinger GM, Jakowec MW, Wood RI (2010) Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse. Behav Brain Res 213:253–262. doi:10.1016/j.bbr.2010.05.009

Heikkila RE, Sieber B-A, Manzino L, Sonsalla PK (1989) Some features of the nigrostriatal dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in the mouse. Mol Chem Neuropathol 10:171–183

Hunsberger JG, Newton SS, Bennett AH, Duman CH, Russell DS, Salton SR, Duman RS (2007) Antidepressant actions of the exercise-regulated gene VGF. Nat Med 13:1476–1482

Hurwitz A (1989) The benefit of a home exercise regime for ambulatory Parkinson's disease patients. J Neurosci Nurs 21:180–184

Jackson-Lewis V, Przedborski S (2007) Protocol for the MPTP mouse model of Parkinson's disease. Nat Protoc 2:141–151

Jackson-Lewis V, Jakowec M, Burke RE, Przedborski S (1995) Time course and morphology of dopaminergic neuronal death caused by the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neurodegeneration 4:257–269

Jones-Humble SA, Morgan PF, Cooper BR (1994) The novel anticonvulsant lamotrigine prevents dopamine depletion in C57 black mice in the MPTP animal model of Parkinson's disease. Life Sci 54:245–252

Jonsson G, Sundström E, Mefford I, Olson L, Johnson S, Freedman R, Hoffer B (1985) Electrophysiological and neurochemical correlates of the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP) on central catecholamine neurons in the mouse. Naunyn Schmiedebergs Arch Pharmacol 331:1–6
Kirk R (1995) Experimental design: procedures for the behavioral sciences. Brooks/Cole, Belmont
Kolosova LI, Akoev GN, Ryabchikova OV, Avelev VD (1998) Effect of low-intensity millimeter-range electromagnetic irradiation on the recovery of function in lesioned sciatic nerves in rats. Neurosci Behav Physiol 28:26–30
Langston JW (1985) MPTP neurotoxicity: an overview and characterization of phases of toxicity. Life Sci 36:201–206
Lau YS, Patki G, Das-Panja K, Le WD, Ahmad SO (2011) Neuroprotective effects and mechanisms of exercise in a chronic mouse model of Parkinson's disease with moderate neurodegeneration. Eur J Neurosci 33:1264–1274. doi:10.1111/j.1460-9568.2011.07626.x
Liu Y, Yu H, Mohell N, Nordvall G, Lewander T, Hacksell U (1995) Derivatives of cis-2-amino-8-hydroxy-1-methyltetralin: mixed 5-HT1A-receptor agonists and dopamine D2-receptor antagonists. J Med Chem 38:150–160
Mahajan SS, Leko V, Simon JA, Bedalov A (2011) Sirtuin modulators. Handb Exp Pharmacol 206:241–255. doi:10.1007/978-3-642-21631-2_11
Morishima M, Harada N, Hara S, Sano A, Seno H, Takahashi A, Marita Y, Nakaya Y (2006) Monoamine oxidase A activity and norepinephrine level in hippocampus determines hyperwheel running in SPORTS rats. Neuropsychopharmacology 31:2627–2638
Nielsen J, Jewett MC (2008) Impact systems biology on metabolic engineering of *Saccharomyces cerevisiae*. FEMS Yeast Res 8:122–131
O'Dell SJ, Gross NB, Fricks AN, Casiano BD, Nguyen TB, Marshall JF (2007) Running wheel exercise enhances recovery from nigrostriatal dopamine injury without inducing neuroprotection. Neuroscience 144:1141–1151
Ödman T, Ödman N, Rabotchi E, Archer T (2013) Design experiments of a wireless performance measuring system for different sizes of mammals. Int J Commun Antenn Propag (IRECAP) (submitted)
Ogawa N, Mizukawa K, Hirose Y, Kajita S, Ohara S, Watanabe Y (1987) MPTP-induced parkinsonian model in mice: biochemistry, pharmacology and behavior. Eur Neurol 26(Suppl 1):16–23
Oscarson S, Archer T, Fredriksson A (2009) Physical exercise and Milmed: synergism and neuroreparation in antiparkinsonian effects. In: Abstracts of staging neuropsychiatric disorders: implications for etiopathogenesis and treatment. Fundacion Cerebro y Mente, Mojacar, Spain, p 88
Outeiro TF, Lindquist S (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302:1772–1775
Palmer SS, Mortimer JA, Webster DD, Bistevins R, Dickinson GL (1986) Exercise therapy for Parkinson's disease. Arch Phys Med Rehab 67:741–745
Patki G, Lau YS (2011) Melatonin protects against neurobehavioral and mitochondrial deficits in a chronic mouse model of Parkinson's disease. Pharmacol Biochem Behav 99:704–711. doi:10.1016/j.pbb.2011.06.026
Petranovic D, Tyo K, Vemuri GN, Nielsen J (2010) Prospects of yeast systems biology for human health: integrating lipid, protein and energy metabolism. FEMS Yeast Res 10:1046–1059
Petzinger GM, Walsh JP, Akopian G, Hogg E, Abernathy A, Arevalo P, Turnquist P, Vucković M, Fisher BE, Togasaki DM, Jakowec MW (2007) Effects of treadmill exercise on dopaminergic transmission in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. J Neurosci 27:5291–5300
Petzinger GM, Fisher BE, Van Leeuwen JE, Vukovic M, Akopian G, Meshul CK, Holschneider DP, Nacca A, Walsh JP, Jakowec MW (2010) Enhancing neuroplasticity in the basal ganglia: the role of exercise in Parkinson's disease. Mov Disord 25(Suppl 1):S141–S145. doi:10.1002/mds.22782
Ragimov CR, Ter-Asaturov GP, Golant MB, Rogov KA, Balakireva LZ (1991) Stimulation of reparative osteogenesis by millimeter band electromagnetic radiation in experimental traumatic defects of the mandible. Bull Exp Biol Med 111:562–565
Sebastián JL, Muñoz S, Sancho M, Martínez G, Alvarez G (2011) Electromechanical effects on multilayered cells in nonuniform rotating fields. Phys Rev E: Stat Nonlin Soft Matter Phys 84(1 Pt 1):011926
Shulman LM, Katzel LI, Ivey FM, Sorkin JD, Favors K, Anderson KE, Smith BA, Reich SG, Weiner WJ, Macko RF (2013) Randomized clinical trial of 3 types of physical exercise for patients with Parkinson disease. JAMA Neurol 70:183–190. doi:10.1001/jamaneurol.2013.646
Silva LA, Bom KF, Tromm CB, Rosa GL, Mariano I, Pozzi BG, Tuon T, Stresck EL, Souza CT, Pinho RA (2013) Effect of eccentric training on mitochondrial function and oxidative stress in the skeletal muscle of rats. Braz J Med Biol Res 11:1–7
Sonsalla PK, Heikkila RE (1986) Neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and methamphetamine in several strains of mice. Prog Neuropsychopharmacol Biol Psychiatry 12:345–354
Sundström E, Fredriksson A, Archer T (1990) Chronic neurochemical and behavioural changes in MPTP-lesioned C57 BL/6 mice: a model for Parkinson's disease. Brain Res 528:181–188
Suvarna BS (2013) Sirtuins: the future insight. Kathmandu Univ Med J (KUMJ) 10:77–82
Tenreiro S, Outeiro TF (2010) Simple is good: yeast models of neurodegeneration. FEMS Yeast Res 10:970–979
Tuon T, Valvassori SS, Lopes-Borges J, Luciano T, Trom CB, Silva LA, Quevedo J, Souza CT, Lira FS, Pinho RA (2012) Physical training exerts neuroprotective effects in the regulation of neurochemical factors in an animal model of Parkinson's disease. Neuroscience 227:305–312. doi:10.1016/j.neuroscience.2012.09.063
Tenreiro S, Munder MC, Alberti S, Outeiro TF (2013) Harnessing the power of yeast to unravel the molecular basis of neurodegeneration. J Neurochem. doi:10.1111/jnc.12271
VanLeeuwen JE, Petzinger GM, Walsh JP, Akopian GK, Vuckovic M, Jakowec MW (2010) Altered AMPA receptor expression with treadmill exercise in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. J Neurosci Res 88:650–668. doi:10.1002/jnr.22216
Vucković MG, Wood RI, Holschneider DP, Abernathy A, Togasaki DM, Smith A, Petzinger GM, Jakowec MW (2010) Memory, mood, dopamine, and serotonin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned mouse model of basal ganglia injury. Neurobiol Dis 32(2):319–327. doi:10.1016/j.nbd.2008.07.015
Waters RP, Renner KJ, Pringle RB, Summers CH, Britton SL, Koch LG, Swallow JG (2008) Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. Physiol Behav 18:1044–1054
Zhang J, Vemuri G, Nielsen J (2010) Systems biology of energy homeostasis in yeast. Curr Opin Microbiol 13:382–388