Research paper

Exercise protects synaptic density in a rat model of Parkinson's disease

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ABSTRACT

Background: Parkinson's disease (PD) is characterized by Levy body and neurite pathology associated with dopamine terminal dysfunction. Clinically, it is associated with motor slowing, rigidity, and tremor. Postural instability and pain are also features. Physical exercise benefits PD patients - possibly by promoting neuroplasticity including synaptic regeneration.

Objectives: In a parkinsonian rat model, we test the hypotheses that exercise: (a) increases synaptic density and reduces neuroinflammation and (b) lowers the nociceptive threshold by increasing μ-opioid receptor expression.

Methods: Brain autoradiography was performed on rats unilaterally injected with either 6-hydroxydopamine (6-OHDA) or saline and subjected to treadmill exercise over 5 weeks. [3H]UCB-J was used to measure synaptic vesicle glycoprotein 2A (SV2A) density. Dopamine D2/3 receptor and μ-opioid receptor availability were assessed with [3H]Raclopride and [3H]DAMGO, respectively, while neuroinflammation was detected with the 18kDa translocator protein (TSPO) marker [3H]PK11195. The nociceptive threshold was determined prior to and throughout the exercise protocol.

Results: We confirmed a dopaminergic deficit with increased striatal [3H]Raclopride D2/3 receptor availability and reduced nigral tyrosine hydroxylase immunoreactivity in the ipsilateral hemisphere of all 6-OHDA-injected rats. Sedentary rats lesioned with 6-OHDA showed significant reduction of ipsilateral striatal and substantia nigra [3H]UCB-J binding while [3H]PK11195 showed increased ipsilateral striatal neuroinflammation. Lesioned rats who exercised had higher levels of ipsilateral striatal [3H]UCB-J binding and lower levels of neuroinflammation compared to sedentary lesioned rats. Striatal 6-OHDA injections reduced thalamic μ-opioid receptor availability but subsequent exercise restored binding. Exercise also raised thalamic and hippocampal SV2A synaptic density in 6-OHDA lesioned rats, accompanied by a rise in nociceptive threshold.

Conclusion: These data suggest that treadmill exercise protects nigral and striatal synaptic integrity in a rat lesion model of PD - possibly by promoting compensatory mechanisms. Exercise was also associated with reduced neuroinflammation post lesioning and altered opioid transmission resulting in an increased nociceptive threshold.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease and is associated with symptoms of bradykinesia, rigidity, postural instability and tremor (Tysnes and Storstein, 2017; Morgan and Fox, 2016). It is characterized pathologically by the...
presence of intraneuronal Lewy bodies and neurites which target nigrostriatal dopaminergic neurons but also affect other transmitter systems leading to non-motor symptoms and disruption of terminal vesicle transport (Esposito et al., 2012; Capriotti and Terzakis, 2016; Pfeiffer, 2016). PD is also associated with a reduced threshold to pain (Buhmann et al., 2017) possibly reflecting changes in opioid transmission (Thobois et al., 2018), neuroinflammation in the form of microglial activation (Gerhard et al., 2006), and widespread changes in brain network activity (Tan et al., 2012).

Up to 85% of PD patients experience the distressing non-motor symptom of pain. Loss of dendritic spines from striatal neurons has been observed in PD (McNeill et al., 1988; Stephens et al., 2005) and maladaptive dendritic spine remodeling has been suggested to be a contributing mechanism underlying neuropathic pain (Tan et al., 2012). Additionally, maladaptive structural plasticity in neural pain circuits is associated with the development of chronic pain (Kuner and Flor, 2016).

These observations suggest that synaptic dysfunction plays an important role in reducing the pain threshold in PD. The majority of PD patients are late onset and sporadic but cases associated with susceptibility gene mutations have been identified. Some of these genes are known to play important roles in modulating presynaptic function (Belluzzi et al., 2012; Abellovich and Gitler, 2016), and PD is associated with synaptic loss (Bellucci et al., 2016; Matuskey et al., 2020).

Physical exercise has beneficial effects in PD patients by maintaining mobility, reducing pain sensitivity, and improving quality of life (Allen et al., 2015; Mak and Wong-Yu, 2019). It remains unclear whether exercise plays a protective role and improves the nociceptive threshold by stimulating synaptic plasticity and regeneration (Mak and Wong-Yu, 2019). Post-mortem studies conducted on a mouse model of PD lesioned with the nigral toxin MPTP showed that exercising on a treadmill facilitated synaptic plasticity of dopaminergic nerve terminals and fibers (Shin et al., 2016) and reversed dendritic spine loss of striatal neurons (Toy et al., 2014). Furthermore, magnetic resonance imaging studies found that exercise promoted neuroplasticity in PD patients, with neuroplastic changes observed following balance exercises (Sehm et al., 2014). This provides preliminary evidence that exercise could contribute to pain management in PD through neuroplastic mechanisms.

Evaluating synaptic density in the PD brain and understanding the synaptic changes induced by interventions such as treadmill exercise and symptomatic or putative protective drug therapies could help rationalise approaches to combat the disease.

A novel marker of synaptic density has recently become available. UCB-J binds to the transmembrane synaptic vesicle glycoprotein 2A (SV2A), expressed ubiquitously by the brain in virtually all of its synapses. The synaptic vesicle pool is thought to be stable across neurons and there is a relatively consistent copy number of SV2A proteins per vesicle which act as transporters (Bajjalieh et al., 1993; Sudhof, 2004). Detection of SV2A reductions in disease states is likely to reflect reduced numbers of synaptic vesicles due to loss of synaptic density. UCB-J can be labeled with the radioisotope 11C allowing its brain uptake to be measured in vivo with positron emission tomography (PET). This potentially provides an in vivo objective marker of synaptic density with which to study disease progression and treatment efficacy in human PD (Matuskey et al., 2020; Wilson et al., 2020) and animal models (Thomsen et al., 2020). The UCB-J molecule can also be labeled with tritium for in vitro autoradiography.

This current work aimed to investigate: (1) The utility of [1H]UCB-J autoradiography for measuring reductions in striatal and nigral synaptic SV2A density after unilateral striatal 6-hydroxydopamine (6-OHDA) injections in rats with a confirmed unilateral dopamine deficit. (2) The maintenance of synaptic density by treadmill exercise post-lesion, serving as a prelude to future in vivo PET imaging studies in PD patients. (3) The potential of treadmill exercise for reducing intrinsic striatal neuroinflammation and modulating thalamic opioid transmission so increasing the low pain threshold associated with the 6-OHDA rat model. We hypothesized that such changes would be associated with changes in synaptic density.

2. Methods

2.1. Animals

Experiments were carried out in accordance with National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and the CONCEA guidelines (CONCEA, Brazil), a constituent body of the Ministry of Science, Technology, and Innovation (MCTI, Brazil). All protocols were approved by the Animal Research Ethics Committee (CEUA) of the Institute of Biomedical Sciences of the University of Sao Paulo (USP) (protocol n° 4,860,310,118). Data was reported according to the ARRIVE guidelines. Twenty male Wistar rats (250-300 g) were supplied by the central animal facility of the Institute of Biomedical Sciences of USP. Since the hormonal cycle of female rats can affect the model induction (Rodriguez-Perez et al., 2015), male animals were used to minimize variation. Animals were housed at room temperature (22 ± 2 °C) with a light/dark cycle of 12/12 h and ad libitum access to food and water. Animals were allowed to acclimatize for at least seven days (Real et al., 2013; Real et al., 2017) prior to study commencement. They were randomly divided into two groups: (1) saline control animals which were injected with vehicle into the right striatum (SAL); and (2) 6-OHDA animals, which were injected with 6-OHDA into the right striatum. Fifteen days after stereotactic surgery, a timepoint selected due to previous evidence of stable neurodegeneration induced by 6-OHDA (Jeon et al., 1995), half of the animals were randomly submitted to treadmill exercise (EX), and half remained sedentary (SED), so the experimental groups were: (1) SAL + SED; (2) SAL + EX; (3) 6-OHDA + SED; (4) 6-OHDA + EX (n = 5/group) (Fig. 1a).

2.2. PD model induction

Rats were anesthetized with isoflurane mixed with oxygen (5% induction, 2% maintenance, 0.8 L/min), and placed in a stereotactic apparatus (Kopf Instruments, Germany). After craniotomy, 1.5 μL of solution containing 9 μg of neurotoxin 6-OHDA hydrochloride (H4381, Sigma) diluted in 0.3% acetic acid in saline or only 0.3% acetic acid in saline was manually injected at a rate of 0.5 μL/min into the right striatum (Cu) at two different brain coordinates: (1) L: 2.7 mm; AP: - 0.5 mm; V: - 4.5 mm; (2) L: 2.7 mm; AP: + 0.5 mm; V: - 5 mm (Paxinos, 2013) with a Hamilton syringe (10 μL, Neuro Syringes - 65,460-02) as previously described (Boraci et al., 2020; Yuan et al., 2005; Real et al., 2019; Garcia et al., 2017). After infusion, the syringe needle was left in the infused region for 5 min to avoid reflux. The incision was then sutured. Analgesia (ketoprofen – 1 mg/kg, s.c.) was administered before and 24 h after surgery. Animals presenting symptoms of pain or discomfort after 48 h received an extra dose of analgesia. All animals survived the surgery.

2.3. Evaluation of mechanical hyperalgesia

The mechanical nociceptive threshold was assessed using a pressure apparatus on the hind paws (Analgesy-Meter Ugo Basile, Italy), as previously described (Randall and Selitto, 1957). In this test, a force in grams (g) of increasing magnitude (16 g/s) was continuously applied to the contralateral and ipsilateral hind paws of the rats. The mass (g) required to induce a paw-withdrawal response represented the mechanical nociceptive threshold. This test was applied prior to the beginning of the treatments (baseline) and at 7, 14, 21 and 28 days after induction of the PD model, selected based on our previous work (Sinda et al., 2020). The last two tests were held one day after the 3rd and 6th treadmill exercise sessions (Fig. 1A).
2.4. Treadmill exercise

The ‘exercised group’ ran on a treadmill three mornings a week, every other day, starting 15 days after surgery, for a total number of 15 running sessions, as previously reported (Garcia et al., 2017; Ferreira et al., 2020). All animals, including the sedentary groups, were familiarized with the treadmill (KT 3000 - IMBRAMED) prior to the surgery day by treadmill exercise for 15 min on two consecutive days (6.66 m/min for 1 min, then 8.3 m/min for 1 min and 10 m/min for 13 min). Fifteen days after surgery both exercised groups (SAL and 6-OHDA) were subjected to the exact same light/moderate exercise protocol at a speed of 10 m/min for 40 min (approximately 400 m/day) (Real et al., 2017; Garcia et al., 2017). Then, the two groups actively ran for a comparable amount of time and distance. It is important to note that all exercise sessions were conducted in the absence of challenge or noxious stimulus, in order to avoid any stressful interference. The animals from SAL + SED and 6-OHDA + SED groups were placed in cages near the treadmill to become familiarized with the new environment and the sound of the machine, but did not perform exercise.

2.5. Brain removal and storage

The brains were removed 49 days after the surgery, and 1 day after the last treadmill session. Brains were frozen in isopentane cooled to −40 °C with dry ice, cryosectioned into 20 μm coronal sections (Thermo Scientific, CryoStar NX70, Axlab) mounted on poly-l-lysine coated slides (Thermo Scientific) and stored at −80 °C.

2.6. Autoradiography

We performed experiments on sequential slides in the striatum, substantia nigra, and hippocampus/thalamus (AP: +0,5 mm, −6.5 mm and -4 mm, respectively (Paxinos, 2013)). For each autoradiography protocol with [3H]Raclopride (dopamine receptor D2/D3), [3H]PK11195 (neuroinflammation/18kDA translocator protein (TSPO)), [3H]UCB-J (SV2A) or [3H]DAMGO (μ-opioid receptor (μOR)), we selected two slides with 4–6 sequential brain slices per slide for total binding and one additional slide for nonspecific binding, where the experiment was performed in the presence of unlabeled drug at a dose at least 1000-fold higher than the radioactive tracer dose.

For [3H]Raclopride binding studies, slides at the level of the striatum were pre-incubated for 10 min in a 50 mM Tris-HCl buffer (pH 7.4) with 0.1% ascorbic acid and 150 mM NaCl, and then incubated for 45 min with the same buffer containing 2 nM [3H]Raclopride (Specific Activity 78.1 Ci/mmol; PerkinElmer). Adjacent sections were used for control, in which non-specific binding was defined as the [3H]Raclopride binding in the presence of 10 μM Butaclamol (Sigma-Aldrich).

For [3H]PK11195 autoradiography, slides at the level of the striatum were pre-incubated for 5 min in 50 mM Tris-HCl buffer (pH 7.4), and then incubated for 1 h with the same buffer containing 1 nM [3H]PK11195 (Specific Activity 82.7 Ci/mmol; PerkinElmer). Adjacent sections were used as control, in which non-specific binding was defined as...
2.8. Statistical analysis

Data were statistically interrogated using GraphPad Prism® 7.0 software. Comparisons between groups for autoradiography data were carried out with a two-way analysis of variance (ANOVA). The variables considered for analysis were 6-OHDA injection and treadmill exercise. For the analysis of mechanical nociception, the baseline data for each animal were compared to the postoperative (PO) data with repeated measures, and data between groups were compared (Kazuba et al., 2017). ANOVA was followed by a Tukey’s test where appropriate. The data are represented in graphs as mean and standard error of the mean (GraphPad Prism 7.0 software). A significance level of 5% (p < 0.05) was adopted for all statistical analyses. Data will be made available by the corresponding author upon reasonable request.

3. Results

3.1. Behavioral testing

The injection of 6-OHDA significantly reduced the nociceptive threshold at 7 and 14 days after surgery in both hind paws compared to baseline measures and saline-injected animals (Fig. 1B). However, after only three exercise sessions, there was an improvement in pain threshold leading to partial restoring of non-lesioned levels in contralateral 25% (p < 0.0001) [F (12, 64) = 11.45] and ipsilateral 25% (p = 0.01) [F (12, 64) = 5.52] hind paw compared to sedentary PD rats. In a separate cohort of rats that we unilaterally injected using identical parameters, there was a clear asymmetry in the use of the forelimbs detected by the cylinder test in the 6-OHDA injected rats (Binda et al., 2020), demonstrating the efficacy of unilateral 6-OHDA lesioning in inducing behavioral symptoms relevant to PD.

3.2. PD model induction validation: dopamine D2/3 receptor binding and tyrosine-hydroxylase staining

To confirm model induction by 6-OHDA, we performed [3H]Raclopride autoradiography of striatal dopamine D2/D3 receptors (Fig. 2A and B) and TH staining of the substantia nigra (Fig. 2C and D). There was a significant 20% increase in striatal binding [3H]Raclopride in the ipsilateral vs contralateral striatal ratio in the sedentary 6-OHDA injected rats compared to the sedentary saline-injected rats (p < 0.01). We also detected 54% reduced TH immunopositive area in the ratio of ipsilateral to contralateral substantia nigra in the sedentary 6-OHDA injected rats compared to the sedentary saline-injected rats (p < 0.001). The same was found in the animals that had performed exercise with an 18% increase in the ratio of ipsilateral to contralateral [3H]Raclopride binding in 6-OHDA vs saline-injected rats and 49% decrease in substantia nigra TH immunopositive area (p < 0.05 and p < 0.01, respectively), indicating that the PD model was established [F (1, 16) = 24.41; p = 0.0001; F (1, 16) = 43.05; p < 0.0001]. There was no effect of the exercise protocol per se on availability of striatal dopamine D2/3 receptors or the TH immunopositive area in the substantia nigra in either the saline or 6-OHDA-injected rats.

3.3. Exercise decreases striatal [3H]PK11195 binding in lesioned rats

We then performed autoradiography using [3H]PK11195 as a marker of activated microglia, known to be upregulated in animal models of PD (Lillethorup et al., 2018a; Lillethorup et al., 2018b; Pain et al., 2019) and PD patients (Gerhard et al., 2006). We observed a significantly higher ratio of ipsilateral to contralateral striatal binding in sedentary 6-OHDA rats compared to sedentary saline controls (25%, p < 0.01) (Fig. 3A,B). The 20% rise in microglial activation induced by 6-OHDA was not present in the 6-OHDA rats who performed treadmill exercise (p < 0.05) [F (1, 16) = 5.758]. No changes in ipsi- vs contralateral striatal [3H]PK11195 binding were observed in the saline-injected rats after exercise.
3.4. Exercise increases $[^3]$HUCB-J binding in striatum and Substantia Nigra of lesioned rats

There was significantly lower $[^3]$HUCB-J binding in 6-OHDA-lesioned sedentary rats compared to saline-injected sedentary rats, reflected by the ratio of specific ipsilateral to contralateral binding in the striatum (20%, $p < 0.0001$) (Fig. 4A,B) and substantia nigra (13%, $p < 0.05$) (Fig. 4C,D). In lesioned rats that were exposed to the treadmill exercise protocol, binding was comparable to that present in saline-injected control rats [$F (1, 16) = 5.758$], however, there was no effect...
of exercise on $[^3]$HUCB-J binding in saline-injected control animals.

3.5. Exercise increases $[^3]$HUCB-J binding in thalamus and Hippocampus of 6-OHDA-lesioned rats

Sedentary rats injected with 6-OHDA had significantly reduced $[^3]$HUCB-J binding in the ipsilateral hippocampus (13%, $p < 0.01$) (Fig. 5A, B,C) compared to saline-injected rats, but no effects in the thalamus were observed (Fig. 5A,D,E). Lesioned rats performing the treadmill exercise protocol had higher $[^3]$HUCB-J binding bilaterally in hippocampus and thalamus (hippocampus ipsilateral 22% ($p < 0.0001$) [F (1, 16) = 43.17], hippocampus contralateral 13%, ($p < 0.001$) [F (1, 16) = 12.01], thalamus ipsilateral 22% ($p < 0.001$) [F (1, 16) = 33.58], and thalamus contralateral 24% ($p < 0.0001$) [F (1, 16) = 24.28]) compared to sedentary 6-OHDA-injected rats. However, no changes in hippocampus and thalamus $[^3]$HUCB-J binding were observed in the saline-injected rats in response to exercise.

3.6. Exercise increases $[^3]$HDAMGO binding in thalamus of lesioned rats

In sedentary 6-OHDA-injected rats, the neurotoxin led to lower $[^3]$HDAMGO binding in ipsilateral (20%, $p < 0.01$) (Fig. 6A,B) and contralateral (13%, $p < 0.05$) (Fig. 6A,C) thalamus compared to saline-injected rats. The exercise protocol blocked or reversed this 6-OHDA effect in lesioned animals in both ipsilateral (14%, $p < 0.05$) [F (1, 16) = 26.44] and contralateral (13%, $p < 0.05$) [F (1, 16) = 8.692] thalamus. In saline rats that were exposed to the treadmill exercise, binding was similar to sedentary saline-injected rats in both ipsilateral and contralateral thalamus.

Supplementary table 1 shows regional raw data from both hemispheres for all autoradiography analyses.

4. Discussion

In the current study, we present evidence that regular exercise leads to increased synaptic density in a rat lesion model of PD, using the $[^3]$HUCB-J radioligand. Not only did we show maintenance of the synaptic density in striatum and substantia nigra in exercised relative to sedentary rats with a 6-OHDA lesion, but we also found bilateral increases in $[^3]$HUCB-J binding in their hippocampus and thalamus.

The nigrostriatal toxin 6-OHDA is widely used to produce lesion models of PD. When injected unilaterally into the striatum, 6-OHDA acts more selectively and less aggressively on the dopaminergic nigrostriatal pathway compared with injections into the substantia nigra or medial forebrain bundle (Yuan et al., 2005; Alvarez-Fischer et al., 2008). Striatal injections also promote prolonged retrograde degeneration of the nigrostriatal neurons (Sauer and Oertel, 1994) resulting in motor deficits (Blum et al., 2001) and provide a model with which to evaluate the symptomatic efficacy of both drugs and non-pharmacological treatments designed to treat PD (Yuan et al., 2005; Buhidma et al., 2020). In a previous study using an identical injection protocol, we showed the validity of this 6-OHDA model using the cylinder test to assess motor function (Binda et al., 2020). Here, we demonstrate that the 6-OHDA lesion reduced the TH immunopositive area in the substantia nigra, which was not restored with treadmill exercise. We further confirmed the dopamine deficit induced by 6-OHDA by performing autoradiography with the $[^3]$Hraclopride ligand, and found increased striatal D2/3 receptor availability due to reduced synaptic dopamine. This increase was present in both sedentary 6-OHDA rats and in the 6-OHDA rats that had been subjected to treadmill running, suggesting that there was no modulation of dopamine D2/3 receptor availability by the treadmill exercise protocol used in this study.
Striatal dopamine and its metabolites have previously been found to increase in response to acute exercise, but return to baseline within 2 h of the cessation of activity (Basso and Suzuki, 2017). Dopamine release has been implicated in the acute rewarding effects of volitional exercise (Greenwood et al., 2011), however, in the current study, the effects of forced exercise were investigated and the exercise persisted for 5 weeks so acute effects could not be analyzed. Our data however are in line with a human [11C]Raclopride PET study that found no effect on dopamine release in response to 30 min of treadmill running (Wang et al., 2000) and suggested that the lack of effect was due to the subjects exercising regularly. Our previous study using the same rat model corroborates TH data in the substantia nigra pars compacta, revealing dopaminergic loss in response to 6-OHDA which was not rescued by exercise (Binda et al., 2020). The lack of a difference in TH expression between sedentary and exercised animals was also described by other groups where the exercise protocol started 2 weeks after PD induction (Wang et al., 2013; Shi et al., 2019; Churchill et al., 2017). These data suggest that a start of the exercise protocol after dopamine has already been depleted does not exert neuroprotective effects on the dopamine system in PD rats.

Several studies have reported restoration of reduced TH levels and improvements in motor behavior when initiating the treadmill exercise protocol immediately after injection of a neurotoxin (Shin et al., 2016; K.H. Binda et al.)

Fig. 5. A) Representative autoradiograms of [3H]UCB-J binding in hippocampus and thalamus. Quantification of specific [3H]UCB-J binding in ipsilateral (B,D) and contralateral (C,E) regions. Quantification is not presented as a ratio due to bilateral effect of exercise. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (n = 5).
They selected timepoints before peak dopaminergic degeneration and demonstrated a potential neuroprotective effect (Jeon et al., 1995). However, work by other groups has reported that treadmill exercise promotes improvement in motor function in MPTP-injected mice in the absence of recovery of striatal dopamine levels (Petzinger et al., 2007). The authors did however note a downregulation of the dopamine transporter (Petzinger et al., 2007; Fisher et al., 2004) and suggested a compensatory increased synaptic dopaminergic turnover may occur in response to exercise in MPTP-injected mice. Interestingly, they also found increased dopamine levels in saline-injected mice in response to exercise which suggests that exercise acts differently on dopamine transmission in healthy compared to disease states (Petzinger et al., 2007). Other studies have found no recovery of dopamine neuron loss in response to treadmill exercise, despite improved motor function (Fisher et al., 2004; O’Dell et al., 2007). The lack of an effect of exercise on striatal dopamine levels is consistent with our findings of no treadmill exercise-induced preservation or restoration of the level of TH immunopositive nigral loss, nor reversal of the increased [3H]raclopride binding which occurred in response to 6-OHDA lesioning, whereas the binding after treadmill running remained at non-lesioned levels. The mechanism of this protective effect is unclear but may be associated with release of neurotrophic factors that have anti-inflammatory properties (Real et al., 2013; da Silva et al., 2016; Cotman and Berchtold, 2002; Di Benedetto et al., 2017). Exercise reduces pro-inflammatory monocytes and increases the number of regulatory T cells. Furthermore, levels of anti-inflammatory and neuroprotective molecules are enhanced in peripheral blood after physical activity (Di Benedetto et al., 2017) Alternatively, endogenous ligands acting at the isooquinoline site of the TSPO (Graeber and Korkhov, 2019) may have been responsible.

Evaluation of the loss of synaptic density in the parkinsonian brain and an understanding of the synaptic changes induced by effective long-term treatments are crucial. The PET tracer [11C]UCB-J, which binds to the transmembrane SV2A protein universally expressed in synapses, has been used as a quantitative marker of synaptic density in healthy humans (Finnema et al., 2016), temporal lobe epilepsy subjects (Finnema et al., 2016), Alzheimer’s disease (Chen et al., 2020), depression (Holmes et al., 2019), schizophrenia (Onwordi et al., 2020), and more recently, in PD patients (Matuskey et al., 2020; Wilson et al., 2020).

At post-mortem, PD subjects show microglial activation in the substantia nigra, striatum, and frontal association cortex (Marinova-Mutafchieva et al., 2009; Venneti et al., 2006; McGee et al., 1988). Activated microglia express TSPO which can be detected in vivo using [11C]PK11195 or [11C]PBR28 PET. Previously, [11C]PBR28 PET imaging has shown that treadmill exercise reduces microglial activation in the 6-OHDA rodent model, and this imaging marker correlates well with post mortem Iba-1 staining (Real et al., 2019). These data are consistent with our current data showing increased striatal [3H]PK11195 binding in response to 6-OHDA lesioning, whereas the binding after treadmill running remained at non-lesioned levels. The mechanism of this protective effect is unclear but may be associated with release of neurotrophic factors that have anti-inflammatory properties (Real et al., 2013; da Silva et al., 2016; Cotman and Berchtold, 2002; Di Benedetto et al., 2017). Exercise reduces pro-inflammatory monocytes and increases the number of regulatory T cells. Furthermore, levels of anti-inflammatory and neuroprotective molecules are enhanced in peripheral blood after physical activity (Di Benedetto et al., 2017) Alternatively, endogenous ligands acting at the isooquinoline site of the TSPO (Graeber and Korkhov, 2019) may have been responsible.

Fig. 6. Representative autoradiograms of [3H]DAMGO in thalamus (A) Quantification of specific [3H]DAMGO binding in ipsilateral (B) and contralateral (C) thalamus. Note that quantification is not presented as a ratio due to a clear bilateral effect of exercise. *p < 0.05, **p < 0.01 (n = 5).
Using microPET imaging we recently found reduced synaptic density in both the 6-OHDA and preformed alpha-synuclein fibril rat models of PD (Thomsen et al., 2020; Thomsen et al., 2021). It is a promising PET tracer for monitoring PD with the ability to objectively detect focally altered synaptic density. This finding has prompted future work to study interventions that may restore synaptic loss, including the current study of a rodent model of PD in the absence and presence of a non-pharmacological therapy, treadmill running.

Reduced synaptic density in the ipsilateral striatum and substantia nigra of 6-OHDA-injected animals is not surprising, since the dopaminergic terminals and connected fibers are eliminated (Shin et al., 2016). Physical exercise maintained the normal level of striatal [3H]UCB-J binding in the striatum and substantia nigra of rats despite the injection of 6-OHDA. This suggests that treadmill exercise may have the capacity either to promote compensatory mechanisms associated with restoration of synaptic integrity in the remaining neurons in substantia nigra, for example by increasing connections (Deumens et al., 2002), or to protect against mitochondrial toxins providing potential mechanisms for the efficacy of exercise in PD patients. The increase of connections could be associated with the striatal glutamatergic system as was also observed in hemiparkinsonian rats that ran for 4 weeks and expressed increased striatal mGluR2/3 receptors compared to sedentary rats (Shi et al., 2019). This increase in mGluR2/3 may attenuate glutamate-mediated excitotoxicity and restore striatal spines in striatal medium spiny neurons (Shi et al., 2019).

It should be noted that increased vesicle clustering effects have been reported in animal lesion models of PD. It is unclear whether such effects may have influenced our UCB-J findings since they do not necessarily cause alterations in SV2A protein availability. We detected reduced synaptic density in the ipsilateral hippocampi of the sedentary rats injected with 6-OHDA but raised [3H]UCB-J binding in 6-OHDA-injected exercised rats to levels higher than in saline-injected animals. Interestingly, the higher binding in response to exercise was observed bilaterally in the hippocampus. Indeed, exercise has been reported to promote synaptic plasticity in ipsilateral and contralateral hippocampus and restore spatial memory performance in rats after transient cerebral ischemia (Shih et al., 2013).

Our results suggest that increased synaptic [3H]UCB-J binding promoted by treadmill exercise in the 6-OHDA lesioned animals could be part of a compensatory mechanism to reverse dysfunction in networks involving limbic brain regions due to dopamine deficiency. Our finding of higher [3H]UCB-J binding in the thalamus and hippocampus of 6-OHDA-lesioned rats after exercise suggests a positive additive effect. Such an additive effect has previously been shown where the combination of MPTP and treadmill exercise promoted increased brain and glial-derived neurotrophic factor levels, higher than saline-injected sedentary control animals, in midbrain and striatum (Palasz et al., 2019). In addition, there is an important limbic- basal ganglia interaction in different navigational contexts (Ferbinteanu, 2016; Brown et al., 2012). The dorsal striatal and hippocampal systems may dynamically interact to permit us to fluidly transition between more rigid and flexible navigational behaviors, and to translate declarative memory into guidance of ongoing actions (Wilson et al., 2020). Thus, the increased synaptic density in the striatum and hippocampus promoted by treadmill exercise could suggest improvement of navigational route flexibility.

PD patients have been shown to abnormally activate hippocampus during performance of the Tower of London task (Dagher et al., 1999). Exercise can act to decrease hippocampal neural activity contributing to the prevention of the deleterious effects of 6-OHDA on memory (Real et al., 2019). Previous studies have found a reduction of GABA levels in thalamic subregions in PD, and even lower GABA levels in tremulous compared with akinetic rigid PD (Gong et al., 2018). A postmortem study found a 36% reduction in thalamic GABA concentrations in patients with PD compared with controls (Gerlach et al., 1996). Previous work from our group has shown that injection of 6-OHDA affects both short-term and long-term memory in sedentary animals and that exercise was able to suppress microglial activation in hippocampus which could also have helped prevent the deleterious effects of 6-OHDA on memory (Real et al., 2019).

In addition to motor and cognitive symptoms, pain symptoms affect 40–85% of PD patients and may be associated with abnormal gating by central pain pathways modulated by the opioid system, more specifically μORs (Binda et al., 2020; Cury et al., 2016; Tamaddonfard and Erfanparast, 2017). Our PD model showed bilateral mechanical hyperalgesia, which reinforces the idea that there is a central plasticity and sensitization, and a crossing of the nociceptive pathways (Wooll, 2011). Treadmill exercise has been found to increase the nociceptive threshold and the expression of μORs in the hippocampus of healthy rats (de Oliveira, 2010). Interestingly, we found increased synaptic density and restoration of μOR binding in the thalamus of the exercised animal model of PD, and this was associated with bilateral improvement of hyperalgesia. This is consistent with our previous study which demonstrated that a μOR antagonist can hinder the beneficial effects of exercise on improving pain symptoms (Binda et al., 2020). The thalamus is one of the most highly connected centers of the vertebrate brain, with roles in perception and memory (Jesuthasan, 2018). Unsynchonized thalamic cortical oscillations are directly related to occurrence of central and chronic pain (Walton and Llinas, 2010), reinforcing its importance in the modulation and nociception of pain. Regarding the bilateral effect in thalamus and hippocampus, the hemispheres of the brain have connections through corpus callosum (van der Knaap and van der Ham, 2011). Due to these connections, it is difficult to isolate the effects of the lesion to only one side of the brain, despite the induction of unilateral PD model. The same occurs for an intervention such as exercise which interferes with the whole organism, as was previously seen in the hippocampus in a stroke model (Shih et al., 2013). This may explain the bilateral effect in thalamus and hippocampus.

This autoradiography study supports the utility of the UCB-J molecule as a marker of synaptic function in neurodegenerative disorders (Thomsen et al., 2020) and, for the first time, supports exercise having protective or restorative properties in PD by increasing synaptic density. In the future, the efficacy of neuroprotective strategies designed to restore synaptic integrity in animal models of PD and in the human condition could be examined with [11C]UCB-J PET and correlated with the responses of both motor and non-motor symptoms.

A limitation of this work is the small number of animals (n = 5) in each of the 4 groups, it is unknown if a larger group may have resulted in an effect of exercise in saline-injected rats. Also, a motor behavior test was not performed in these rats, however, we demonstrated a dopamine deficit in each 6-OHDA-injected rat shown by increases in [3H]Raclopride binding and reduced TH positive area in the substantia nigra. In future investigations, the use of additional molecular biology methods to evaluate synaptic proteins, mRNA and counting of dendritic spines could corroborate our findings, and would help to validate the use of SV2A as a marker of synaptic density. Finally, it must be mentioned that these studies were performed in a toxin-induced rat model of PD. The progression of human PD occurs over decades, and therefore any leads on neuroprotective mechanisms obtained in a toxin-induced rat model should be followed up in a cohort of PD patients using longitudinal PET imaging. Although the main advantage of PET imaging is the possibility to perform in vivo longitudinal studies, our autoradiography method also has some strengths, including 1) micron resolution compared to the mm resolution of PET, 2) the ability to study several radioligands in the same population in attempts to use the same interventions between the pathways involved in PD and exercise intervention, it would have been difficult to image subjects with 4 radiotracers in vivo, 3) larger groups of animals can be compared at the same point in time, and 4) changes in blood flow and metabolism are not confounding factors.

In conclusion, our findings in the 6-OHDA rat model of PD suggest that treadmill exercise may promote compensatory mechanisms associated with protecting or restoring synaptic integrity in striatum, substantia nigra, thalamus and hippocampus, which can reduce intrinsic...
 striatal neuroinflammation and normalize thalamus opioid pathways, thus improving nociceptive threshold. This study extends knowledge in the field of synaptic restoration in response to a non-pharmacological intervention and supports the literature suggesting that exercise can enhance the quality of life for PD patients.

**Authors’ roles**

1) Research project: A. Conception, B. Organization, C. Execution; 2) Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3) Manuscript: A. Writing of the first draft, B. Review and Critique.

**Declaration of Competing Interest**

All authors declare that they have no conflict of interest concerning the research related to the manuscript.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.expneurol.2021.113741.

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