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Combinação terapêutica de células-tronco e exercício aeróbico para os déficits mnemônicos de um modelo animal da doença de Parkinson induzido com 6-OHDA

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Combining Stem Cell Therapy and Aerobic Exercise for the mnemonic deficits of a 6-OHDA-induced animal model of Parkinson´s disease

Dissertation submitted to the Graduate Program in Rehabilitation Sciences at the Universidade Federal de Ciências da Saúde de Porto Alegre as a requirement to obtain the Master Degree.

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I dedicate this work to my mother and brother.

Believing uplifts dreams
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Cooperativity is a fundamental phenomenon in life and research. I want to thank all the people who one way or another were part of this project and without whom it would not have become a reality.

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To all, my sincere and humble thanks.
Reality does not exist until we look at it.

Quantum Physics
RESUMO

Introdução: a doença de Parkinson é uma doença neurodegenerativa motora e cognitiva prevalente. O uso de transplantes neurais de células-tronco para tratamento desta condição tem sido estudado por seu potencial neuroprotetor e restaurador; não obstante, resultados funcionais variáveis e limitações técnicas restringem o seu uso. Interessantemente, o exercício possui propriedades neuroprotetoras similares que podem atuar em sinergia para potenciar os efeitos terapêuticos. Objetivos: avaliar os efeitos da associação de exercício aeróbio e transplante de células-tronco mesenquimais humanas sobre a memória aversiva e de reconhecimento em um modelo animal de deficiência dopaminérgica nigroestriatal secundária à 6-hidroxidopamina (6-OHDA). Metodologia: Quarenta e um ratos Wistar machos foram randomicamente alocados em 5 grupos: Sham (controle sem lesão), 6-OHDA (controle com lesão), Exercício, Células-tronco e Combinado, e submetidos à injeção esquerda unilateral por estereotaxia de 6-OHDA ou salina. Após verificar a existência funcional da lesão através do desafio com metilfenidato, 2x10⁵ células-tronco (grupos Células e Combinado) ou salina foram infundidos no corpo estriado ipsilateral à lesão. Os grupos Exercício e Combinado fizeram treinamento aeróbio cinco vezes por semana em esteira elétrica a uma velocidade de 16 m/min, 30 minutos durante a primeira semana, que se incrementaram de 10 em 10 minutos nas semanas seguintes até atingir 60 minutos por dia na última semana. Ao término de 4 semanas de treino, testes comportamentais (reconhecimento de objetos e esquiva passiva) foram feitos em todos os grupos e eutanásia por perfusão foi realizada para análise imunohistoquímica da tirosina hidroxilase (TH). Resultados: todos os grupos lesionados apresentaram comportamento rotacional induzido por metilfenidato e perda assimétrica de imunoreatividade para TH na sustância nigra e VTA. Todos os grupos apresentaram memórias de reconhecimento de curto prazo e aversiva preservadas. Os dois grupos submetidos ao treino na esteira apresentaram detimento na memória de reconhecimento de longo prazo. O grupo células não teve efeito funcional no comportamento cognitivo avaliado. O grupo exercício adicionalmente apresentou respostas de medo aumentadas na esquiva passiva, enquanto que o grupo combinado paradoxalmente apresentou redução na expressão dessas. Conclusão: nesse modelo de déficit dopaminérgico mesotelencefálico não foi observado detimento marcado nas tarefas cognitivas avaliadas; entretanto, a terapia com exercício, única ou em combinação, trouxe prejuízo para a memória de reconhecimento de longo prazo assim como um efeito paroxal no comportamento de esquiva e aproximação após a aprendizagem aversiva. Esse resultado ambivalente poderia ter estado relacionado a um efeito estressor do protocolo de exercício forçado que trouxe efeitos deletérios no hipocampo, mas benéfico no corpo estriado. Mais estudos precisam ser realizados para desvendar os mecanismos envolvidos.

Palavras – chave: Doença de Parkinson, Modelo animal, Reabilitação, Implante de células-tronco mesenquimais, Exercício, Memória.
ABSTRACT

Introduction: Parkinson disease is a prevalent motor and cognitive neurodegenerative condition. Stem Cell transplantation has been studied for its restorative and neuroprotective potential in this condition; still, many technical and functional limitations have restricted its clinical use. Interestingly, aerobic exercise has similar neuroprotective properties that can interact synergically to potentiate the therapeutic effects. Objectives: To assess the effect of combining aerobic exercise and human adipose-derived mesenchymal stem cells transplants on the aversive learning and object recognition memory of an animal model of nigrostriatal dopaminergic depletion secondary to 6-hydroxydopamine (6-OHDA). Methodology: Forty one male Wistar rats were randomized to 5 groups: Sham (control without lesion), 6-OHDA (control with lesion), Exercise, Stem cells, and Combined, and received a stereotaxic left unilateral injection of 6-OHDA or saline. After verifying lesion success with methylphenidate, 2x10⁵ stem cells (Stem cells and Combined groups) or saline were infused within the striatum ipsilateral to the lesion. Only Exercise and Combined groups underwent treadmill training at 16 m/min five times a week, starting from 30 minutes on the first week, with gradual increments of 10 minutes per week, up to 60 minutes during the last week. At the end of the 4-week training period, the novel object recognition and passive avoidance tasks were carried out for all groups. Death by perfusion was employed to prepare tissue for immunohistochemical analysis of tyrosine hydroxylase (TH). Results: all injured groups had methylphenidate-induced rotational activity and asymmetry of the TH⁺ immunoreactivity in the SNc and VTA. All groups had preserved short-term recognition memory and aversive learning. Both groups submitted to the training protocol presented an impaired long-term recognition memory. Stem cells did not have a functional effect on the assessed cognitive performance. While Exercise group additionally manifested heightened aversive learning; paradoxically, this effect was not observed in the Combined group, which rather had a reduced fear response. Conclusions: In this model of dopaminergic mesotelencephalic deficit, no gross impairment was observed in assessed memory tasks. Notwithstanding, a paradoxical effect of the exercised therapy, alone or in combination, was seen in the long-term recognition memory and the avoidance-approach behavior after an aversive learning. This puzzling result may be, in effect, the result of the stressing effect of a forced exercise with regional opposite consequences on the hippocampus and the striatum. More research is due to unveil the mechanisms behind these effects.

Key words: Parkinson Disease; Disease Models, Animal; Rehabilitation; Stem Cell Transplantation; Exercise; Memory
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INTRODUCTION

Parkinson´s disease (PD) is the second most common neurodegenerative disease nowadays. Approximately 2% of the global population above 65 years is currently affected and considering that its prevalence and incidence increase in direct proportion with age (Hirsch et al., 2016; Pringsheim et al., 2014), as life expectancy extends, the number of people afflicted is expected to rise concomitantly.

PD is considered a very costly and disabling condition, source of psychological and physical morbidity and a direct cause of detriment in patients and their caregivers´ quality of life (QOL) (Shulman, 2010). It has been typically characterized as a motor disorder given its cardinal symptoms; yet, non-motor impairments (autonomic, cognitive and affective) are also present in both early and advanced stages of the disease (Balestrino and Martinez-Martin, 2017). These symptoms gradually increase as the disorder progresses in time and with age (Shulman et al., 2001) and also bring about major disruptive effects on social, personal and occupational dimensions (Song et al., 2014). Mild to moderate cognitive symptoms can be present in 20-30% of the patients at the moment of diagnosis and even have developed years before the motor symptoms appear (Bocanegra et al., 2014; Yarnall et al., 2013). Patients in whom these initial dysfunctions are present possess 4 to 6 times more risk of developing dementia later on (Aarsland and Kurz, 2010), a recognized complication of the disease with a mean prevalence of 30% and an independent contribution to mortality (Bocanegra et al., 2014; Hughes et al., 2004).

Current approved therapeutic approaches relief symptoms temporarily but seldom modify the underlying pathological mechanisms of the disease. PD possess a very complex physiopathology, where multiple cellular processes are disrupted and different systems of neurotransmission are dysfunctional to a wide range of degrees, hence the clinical heterogeneity (Johnson, 2015). Mosaic diseases call for complex therapeutic strategies; still, the most commonly used (pharmacological) come with the inherent difficulties of drug interaction, collateral effects, and variable individual responses. That is the reason why an ever-present need for disease-modifying approaches exists today and rehabilitation strategies for PD are actively researched in preclinical and clinical settings (Vale, 2008); one of such strategies is stem cell transplantation (SCT) (Yoo et al., 2013).

Stem cells have a promising potential given their proliferative capacity and the possibility of virtually replacing neurons and glial cells lost to injury or neurodegeneration. Additionally, when transplanted they secrete a myriad of neurotrophic substances, growth factors, and immunomodulators that could positively modify key host processes such as neurogenesis, brain plasticity and immune response (Oliveira et al., 2016). Specifically, Mesenchymal stem
cells (MSC) are thinly ethical-constricted and suitable for autologous transplantation (Chang et al., 2014). In animal models of PD, they have demonstrated anti-inflammatory, neurogenic and neuroprotective properties that have been linked to behavioral recovery to several degrees (Blandini et al., 2010; Schwerk et al., 2015a). Even so, a safety and bullet-proof MSCT protocol does not exist; many technical limitations, phenotypic instability, low survival rates and transplant-related variable effects still restrict their clinical use.

In rehabilitation is not uncommon to combine different strategies for better results, as complex diseases require complex approaches. Interestingly, aerobic exercise has demonstrated overlapping neuroprotective properties (Petzinger et al., 2013; Yau et al., 2012) and has proved to be beneficial in many neurological conditions. Yet, although amply studied on its own, it has been scarcely broached when combined with SCT. The interest of this master project was therefore to explore a theoretical therapeutic synergy when both strategies are used together. For this purpose, we carried out an experimental study in a rat model of PD in which motor and cognitive parameters were assessed and compared after the combined use of adipose-derived MSC transplantation and treadmill training. This dissertation will focus specifically on the results obtained in the cognitive domain.

This document is organized into three sections. The first part is a state-of-art review in which the theoretical background for the project will be presented and discussed. In the next section, an original article will describe the methods, our results, and will discuss them in the context of current knowledge. The last part will be a short concluding section where final comments and considerations will be made.
OBJECTIVES

To assess the therapeutic effect of the combined use of mesenchymal SCT and aerobic training in counteracting the mnemonic alterations of a unilateral dopaminergic mesotelencephalic deficit induced by 6-hydroxydopamine, and to compare it to that obtained with either of them alone.
LITERATURE REVIEW

PD is a long-recognized neurodegenerative disease, second most common after Alzheimer’s disease. Its prevalence is tightly related to age and therefore bound to increase given the progressively longer worldwide life expectancy. In fact, it has been projected that by 2030 the numbers would have had duplicated (Dorsey et al., 2007). In Brazil, the prevalence has been estimated in about 220 thousand patients; a number probably underestimated given the non-compulsory reporting of PD (Bovolenta and Felício, 2016). A population study in Bambuí (state of Minas Gerais) in 2006 (Barbosa et al., 2006) found a prevalence of idiopathic PD of 3.3% among a cohort of elderly people; a rate that has been similarly found in other American and European cities by means of door-to-door sampling. A startling number if we consider the over-60 population of 21 million people calculated by the “Instituto Brasileiro de Geografia e Estatística (IBGE)” in 2009.

Rarely occurring before 50 years of age, PD overall incidence rate increases steadily over time from around 3 per 100,000 person-years at age 40–49 to about 100.00 to 250.000 (sex dependent) at age 80+ (Hirsch et al., 2016). As higher numbers appear in the picture, the personal, social and monetary costs of the disease rise. PD is a very expensive and disabling condition; not only does it bring about great prejudice to the functional status and QOL of the patients and their caregivers (Shulman, 2010), but also represents an economic and technical challenge for the healthcare system, the more as the disease progresses (Rodríguez-Blázquez et al., 2015).

2.1 BRIEF PHYSIOPATHOLOGY OF NEURODEGENERATION

Historically characterized as a motor disorder (bradykinesia, tremor at rest, rigidity, speech and gait disturbances), PD is now perceived as a complex syndrome which also manifests non-motor dysfunctions. Its hallmark pathological trait is the loss of dopaminergic midbrain dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) and the ensuing loss of DA input to the striatum (caudate nucleus and putamen). It is this (the nigrostriatal pathway) the one that has been held major responsible for the motor disturbances and, as later explained, for some of the concomitants non-motor symptoms. Although the primary exact mechanism leading to this neuronal death (neurodegeneration) is still out of grasp, several biological mechanisms are believed to contribute(Jenner et al., 2013). Mutations in six specific chromosomal regions (SNCA, LRRK2, Parkin, PINK, DJ-1, and ATP13A2) account for only about 10% of the cases (monogenic familial PD) and (collectively) explain around 3-5% of the idiopathic cases. Most cases of PD are therefore
sporadic and regarded as the result of an intricated interplay between genetic and environmental risk factors (Klein and Westenberger, 2012).

Alfa-synuclein (α-Syn) protein, a presynaptic unfolded and monomeric protein believed to play a role in vesicle trafficking and neurotransmitter release (synaptic homeostasis), has been related to Lewy-body diseases including PD (Lashuel et al., 2012). Its progressive abnormal aggregation and folding lead to the formation of Lewy bodies/neurites (intracellular inclusions), a common post-mortem histological finding in the brains of PD patients. These oligomeric and fibrillar aggregates are toxic and are involved in disrupting several intracellular mechanisms including membrane and mitochondrial function (Mullin and Schapira, 2013), degradation systems (Tanik et al., 2013), calcium homeostasis (Rcom-H’cheo-Gauthier et al., 2014), protein synthesis, and axonal trafficking (Koch et al., 2015), all of special importance for cell vitality. Furthermore, recent evidence points towards a prion-like propagation of these toxic aggregates. They, once in the extracellular space following pathological release, trigger neuroinflammation and transfer to nearby neurons and glial cells, where they become the seed for further abnormal oligomerization and self-spread neurodegeneration (Olanow and Brundin, 2013; Tyson et al., 2016).

What exactly triggers this oligomerization and fibril growth in the first place is not known. Genetic polymorphisms confer risk by altering the balance between α-Syn synthesis and its degradation or its propensity to aggregate (Klein and Westenberger, 2012). Other factors such as oxidative stress, neuroinflammation, toxins, fatty acids, metal ions and oxidized Dopamine (DA) also disrupt this homeostasis (Lashuel et al., 2012). Aging is an important context here, being a clear major risk factor. Changes in the brain and its microenvironment with age may predispose and start up this imbalance. It is well known that trophic support and the efficiency of different cellular and molecular processes decrease in time; whilst oxidative stress and pro-inflammatory markers increase (Bettio et al., 2017). This faulty, less supportive and pro-inflammatory environment can then be the stage where genetic and external actors interact in the play that later on unfolds as PD. A vicious cycle is discernible; factors that trigger α-Syn misfolding are also the consequence of it, one leading to the increase of the other in an apparently headless circle of α-Syn accumulation, cell dysfunction, neuroinflammation, and ultimately neuronal death.

2.2 NEUROPATHOLOGICAL STAGES OF PD (THE BRAAK STAGING)

Lewy pathology is relentlessly progressive and imbibes different brain regions in an apparently topographic predictable way. Not all of the neuronal types appear equally susceptible to the synucleinopathy (Braak and Del Tredici, 2004), projection neurons with
long poorly or unmyelinated thin axons seem the most vulnerable, why that is, is still a matter of intense research. This unequal predisposition would mark the specific biological path that the progressive neurodegeneration would follow in PD and its probable clinical course (Braak and Del Tredici, 2017). Curiously, the disease does not start off at the SNc, the first hampered areas are the olfactory bulb and the dorsal motor nucleus of the vagus. In subsequent later stages of the disease, the compromise extends up towards the cerebral cortex, leaving a trail of continuously worsened involvement in previously affected areas (The proposed Braak stages 1 to 6 (Braak et al., 2006, 2004)).

**Stage 1**
Auerbach plexus neurons (enteric nervous system) and the long un-myelinated projections from the dorsal motor nucleus of the vagal nerve that connect with it are particularly affected. The anterior olfactory structures also have inclusion body pathology.

**Stage 2**
The reticular formation, raphe nuclei, and the Locus Coeruleus began to be affected.

**Stage 3**
Basal midbrain and forebrain structures began their involvement: SNc, the amygdala, tegmental pedunculopontine nucleus, and cholinergic magnocellular nuclei of the basal forebrain.

**Stage 4**
The disease spreads to the cerebral cortex, namely the anteromedial temporal mesocortex. This intermediate zone is deeply interconnected with the entorhinal region, hippocampal formation (CA2 inclusions specifically), and the amygdala. From here, it seems, the disease gets a go for holding other cerebral cortices. It is between these intermediate stages (3-4) that clinical PD motor symptoms begin to manifest.

**Stages 5-6**
SNc appears macroscopically bleached. CA1 and CA3 develop body inclusions. From the temporal mesocortex the neurodegeneration spreads, first to the prefrontal area and high order (multimodal) associative cortices (including the insular and cingulate cortices), then the first order (unimodal) associative cortices and premotor areas, and lastly to primary cortices, in a seemingly inverse pathway of that of myelinization maturity. Limbic, cognitive, autonomic and sensory-motor dysfunctions are unmistakable.
2.3 NEUROPSYCHIATRIC AND COGNITIVE PHENOMENOLOGY

From the previous description, we can deduce that PD is not a pure DAergic deficiency nor a mainly basal ganglia disorder. Multiple neurotransmitter signals (e.g. Noradrenaline (NA), Serotonin (5-HT) and Acetylcholine (Ach)) and processing systems are dysfunctional at mismatching levels, the degrees of which are highly determined by subtle differences in disease spreading and individual predisposition and exposures. The complex expression of this heterogeneity in a symptomatic profile reflects on the wide phenotype and clinical progression variability among different populations and patients (Selikhova et al., 2009; Van Den Eeden et al., 2003).

PD generally has a prolonged motor prodromal phase (stages 1-3), years can pass before the first motor manifestations make the patients seek medical care. It has been determined that roughly a neuronal loss of at least 30-50% in the SNc (and greater loss (round 50-70%) of striatal or putaminal dopaminergic markers) need to occur before the compensatory mechanisms give in and motor disturbances appear (Cheng et al., 2010), an event occurring well into the stage 3 of the disease (Dijkstra et al., 2014).

Notwithstanding, prior to the appearance of motor complaints, non-motor symptoms can hint towards the underlying pathological process (Chen et al., 2013). The not spared olfactory bulb and brainstem nuclei can reveal themselves by means of non-specific symptoms such as anosmia, constipation and sleep disturbances (confounding, as all are very prevalent among the elderly population regardless of Lewy body pathology). Anxiety, apathetic personality and depression can also precede motor symptoms, being them a possible consequence of disturbances in DA, NA and 5-HT regulation (a sign of midbrain synucleinopathy). Cognitive deficits begin to appear early in the course of the disorder, mild to moderate symptoms are already present in one-third of the patients at the moment of diagnosis and augment over the next 5 years (M. Broeders et al., 2013; Mark Broeders et al., 2013). Their presence supposes an increase in the risk of PD-associated dementia (PD-D) of 4-6 times that of the general population (Aarsland and Kurz, 2010), which can have a cumulative prevalence of 75–90% after 10 years or more of disease progression (Buter et al., 2008).

Considering the frequency of these symptoms among the old age population makes one question their specificity regarding PD; in fact, the significance of these prodromal signs as markers of future development of PD in high-risk populations and disease evolution is still under scrutiny (Heinzel et al., 2016). Their rate of co-occurrence might be of greater value in this respect but has been underexplored so far (Chen et al., 2013). It is undeniable, though, that their recognition and further study will cast light on obscure or poorly understood aspects of the disorder. What is more, in the future, their understanding and diagnoses can be of
great help in halting the pathological process (Chen et al., 2013). By aiding in catching the
disease in its juvenile phases, a time where the process is not wide-spread and some
compensatory mechanisms are still spared, they can represent the entry door for disease-
modifying therapeutic tactics that might stop, or even reverse, the entire process before
greater impairment ensues. These non-motor complaints become increasingly prevalent over
the course of the illness (21% at diagnosis vs 88% after 7 years of progression) and are a
major determinant of QOL and disability; some are at least as debilitating as the motor
symptoms themselves (Storch et al., 2013). Progressive cognitive decline and dementia are
a feature of late-stage PD, as well as anxiety, depression and impulsive/compulsive
behavior. Despite all that, they are still under-recognized and their management remains
mostly ineffective (Seppi et al., 2011).

2.3.1 Cognitive Physiopathology

Although very heterogeneous, frequent cognitive complaints in PD encompass progressive
difficulties in concentrating, planning, and retaining information. This dysexecutive syndrome
is seen in prodromal and early PD and can perceptively intensify in time (Goldman et al.,
2014). Its manifestation has been mainly associated with frontostriatal dysfunction, at least
during the first stages of the disease.

Subcortical breakage

Dopamine-dependent circuitry

Segregated cortico–basal ganglia circuits serve different but inter-related functions for
purposeful behavior (limbic, prefrontal-associative, oculomotor and motor).
Within the limbic and associative loops, the prefrontal cortex (PFC) highly interconnects with
the striatum and depends on an adequate striatal performance for its own effective
functioning. DP pathology leads to dysregulated fluctuating levels of DA within the striatum
and its interconnected circuits (Kehagia et al., 2013). Remembering the inverted ‘U-shaped’
curve linking DA levels to cognitive performance, we know that either high quantities or low
quantities prejudice cognition, requiring an intermediate level for optimal functioning (Cools
and D'Esposito, 2011). With that in mind, PD associated uneven affectation of frontostriatal
reciprocal connections (dorsolateral PFC-dorsal striatum(DS) vs. orbitofrontal cortex- ventral
striatum(VS)) and subsequent uneven and unstable DA signalling (in addition to early up-
regulatory compensation of extrastriatal DA levels (Kaasinen et al., 2001; Rakshi et al.,
1999)) can manifest as ineffectual executive control. This also can explain the contradictory
therapeutic results of DA replacing therapies. According to the DA overdose hypothesis
(Cools et al., 2001), DA therapies bring benefits to the more severely impaired dorsal circuits (restoring DA levels), while being detrimental to the ventral circuits (less affected) by overdosing them.

A second component of the DAergic system, the mesocortical network, also participates in the executive decay. The ventral tegmental area (VTA) and its diffuse cortical projections (particularly to the PFC, VS and insular cortices) play an important role in cognitive flexibility by recruiting additional cognitive circuits such as the posterior parietal cortex. Evidence suggests that initial malfunction of the frontostriatal network can be compensated for by an hyperactivation of this mesocortical network, which once compromised heralds major impairment (Cools, 2006).

Additionally, the mesolimbic network implicated in reward processing, learning and memory can be interfered with too. The entorhinal region, the hippocampus, and the amygdala are functionally coupled and send important projections to the VS (accumbens nucleus/limbic putamen). Ventral DA levels variations can alter this circuit, probably accounting for changes in reward sensitivity, motivation and impulsive behavior in some medicated patients and with disease progression (Bostwick et al., 2009; Czernecki et al., 2002).

Non-DAergic circuitry
According to Braak disease progression, NAergic and cholinergic involvement occur along with DAergic dysfunction; both with recognized roles in cognition. NAergic modulation is important for cognitive flexibility, attention and emotional memory (Chamberlain and Robbins, 2013), whereas cholinergic inputs maintain adequate attention during cognitive tasks and participate of memory encoding. Attentional, visuospatial and explicit mnemonic deficits are present early in PD (Muslimovic et al., 2005). Voluntary directed attention requires “top-down” modulation, while stimulus-driven attention requires “bottom-up”; twain processes highly dependent on the frontoparietal network (PFC and posterior parietal cortices) and its NAergic and cholinergic modulation (Chamberlain and Robbins, 2013; Katsuki and Constantinidis, 2014; Sarter et al., 2005). As the disorder progresses from stage 2 on, pronounced destruction begins to take place in these centers drawing on ever more flawed regulation within their target subcortical and cortical structures.

Cortical breakage

The first vulnerable cortical structure is the anteromedial temporal lobe, a transitional zone of convergence, where exteroceptive (neocortical) and interoceptive (subcortical structures and brainstem) information is jointly processed within the limbic loops, and that partakes in episodic memory and reward and fear learning (Strange and Dolan, 2006). The conforming
structures (amygdala, hippocampus, parahippocampus, entorhinal and perirhinal cortices) are differently distressed within PD course, and can, temporarily speaking, add to the mnemonic deficits as their compromise deepens (Braak et al., 2004). Memory is not a single process; it requires multiple well-functioning cognitive systems for its encoding, storing, and retrieval. In PD, deficits are multifactorial (Gratwicke et al., 2015): initial executive dysfunction contributes to the impaired free recall of early phases; similarly, attentional (ACh-mediated) deficits weaken the encoding of new information; as the disease advances and cortical damage ensues, storage mechanisms begin to fail and deficient recognition and semantic memory appears.

Cortical hypometabolism and posterior atrophy have been seen in early and late PD stages within the neocortex, respectively. Frontal, medial temporal, and parietooccipital cortices atrophies have been correlated with cognitive decline and dementia (González-Redondo et al., 2014; Pan et al., 2013). Moreover, these structural changes have been related to visuoperceptual alterations, first with subtle shortfalls in spatial perception and recognition of objects and, later on, in the formation of dementia-related complex hallucinations (Gratwicke et al., 2015).

2.3.2 Affectivity Partake

Mood fluctuations are an important issue to bear in mind when assessing cognitive function, as the former have a strong influence on the latter and their neural basis is highly intertwined. In PD, affective and psychiatric symptoms are prevalent but variable: depressive disorders (~20%), dysthymia (13%), Anxiety and apathy (~30-40%), impulse control disorders (less than 20%), and psychotic symptoms and hallucination, which vary greatly with reports of less than 20% up 75% (Balestrino and Martinez-Martin, 2017). Depression, flat affect, lack of motivation, and anxiety have a negative impact, as patients reporting these affective disorders have performed worse on cognitive tests assessing executive function and memory (Poletti et al., 2012). What is more, the severity of the apathy was correlated with the level of executive dysfunction, self-report depression (Zgaljardic et al., 2007) and, in the long run, with dementia (Dujardin et al., 2009).

Physiopathologically, their emergence can be multifactorial and related to the explained dysregulation of DA, 5-HT, and NA, in vulnerable structures and processing hubs previously considered (Beucke et al., 2010; Drui et al., 2014; Loued-Khenissi and Preuschoff, 2015). Being generally comorbid, its influence on cognitive and motor symptoms, and likewise, the burden of these symptoms on the psyche should be considered and studied when planning diagnostic tools and therapeutic approaches.
In summary, PD is a very complex syndrome, not restricted to the motor domain, but also characterized by executive, attentional, mnemonic, visuospatial, and mood disturbances. Functional and structural disconnection within different neural networks underlies its physiopathology. Progressive pervasive neurodegeneration relentlessly compromise diffusely distributed, yet interrelated, neurotransmission systems and brain structures, causing fluctuating but escalating symptoms along the disease evolution (Goldman et al., 2014; Gratwicke et al., 2015; Kehagia et al., 2013). Cognitive dysfunctions and mood swings are also a prominent component of the disease that influence patients’ QOL and that of their caregivers and family. More so if we consider that besides motor symptoms, mild cognitive impairment is a established risk factor for developing late-stage dementia, of high impact on QOL and source of mortality.

2.4 6-OHDA ANIMAL MODEL: COGNITIVE PERSPECTIVE

Animal models have been very valuable for scientific progress; although imperfect, they have been a crucial tool in the quest for understanding several neurological conditions and developing restorative approaches. PD models encircle three types: genetic, neurotoxin-induced and chemical-induced (pesticides). Within the second category, the 6-hydroxydopamine (6-OHDA) model has been widely used and validated for motor symptoms and has recently begun to be assessed more consistently in the non-motor domain. The catecholamine toxin 6-OHDA, once taken up by DAergic and NAergic cells via membrane transporter, causes mitochondrial dysfunction by way of respiratory chain inhibition and oxidative stress. The following cellular dysfunction leads to nerve degeneration and ultimately cell death (Blum et al., 2001). By injecting it intracerebrally along the nigrostriatal pathway, nigral degeneration is achieved. Particularly, when injected unilaterally in the medial forebrain bundle (nigral axonal projections), it can cause almost total destruction of SNc and VTA neurons, inducing high degrees of DA depletion and a supersensitivity of the postsynaptic DA receptors within the ipsilateral caudate-putamen complex (CPu) but without causing impairment in feeding behavior. These former characteristics allow the confirmation of the injury through the identification of a postural bias and the active rotational activity that follows the systemic administration of DA agonists. Ipsilateral rotations are present when DA-releasing agents like amphetamine or methylphenidate are given, and contralateral with post-synaptic agonists like apomorphine (Deumens et al., 2002). An induced turning behavior that corresponds roughly to 75%/90% of striatal DA content loss and 50% of nigral cell loss, respectively (Hudson et al., 1993). The nigral loss and DA reduction achieved with this model can mimic then moderate to severe stages of nigrostriatal dysfunction (Truong et al., 2006).
Although there is still a lack of consensus about the best behavioral tests to use, especially when considering cognitive symptoms, some familiar tests (such as the passive avoidance, Y-maze, the Water Morris Maze (WMM), and the novel object recognition test) have been used in an attempt to characterize the cognitive profile and test therapeutic recovery. Still, it is important to bring the attention to two important facts. First, as per model construct, cognitive, psychiatric, and other non-motor traits found with this model are mainly due to the regulation of catecholaminergic activity, both within and beyond the nigrostriatal system. Second, since this disease manifests motor impairment and, in learning and memory tasks, memory is articulated through a motor response, interpreting results can be challenging.

The integrity of the mesotelencephalic DAergic system is relevant for the processing and storage of information. The PFC and its rich interconnections with the basal ganglia, mesencephalic structures and mnemonic areas play important roles in memory and cognition. Correspondingly, different 6-OHDA concentrations and injection sites have shown cognitive deficits in a somewhat simile of PD cognitive profile. In a bilateral model of mild striatal toxicity (36% DA loss), a depression-like behavior accompanied by a less anxious tendency, but not cognitive deficits (WMM), were found (Branchi et al., 2008). These behaviors were found earlier than the reduction of locomotor activity, in a simile of the premotor phase of the disease. In a similar study (striatal DA loss of 59%) by Tadaiesky and colleagues, an anhedonic-depressive-like effect was seen as well; but this time it was accompanied by anxiety and procedural learning impairments in the MWM and social recognition tests 3-weeks post lesion. The effects were related to striatal, prefrontal and SNc reductions of DA levels (Tadaiesky et al., 2008). Around 60% of DA depletion was enough to cause impairment in habit learning and spatial working memory (Ferro et al., 2005). Similarly, a higher number of arm entries and decreased spontaneous alternation were observed in the Y maze after 6-OHDA within the substantia nigra and VTA (Hefco et al., 2003; Zhou et al., 2017). Left unilateral DA striatal depletions of 70% caused impaired spatial working memory but not spontaneous rotational activity (BELLISSIMO et al., 2004). With further bilateral DA striatal depletion (77%), motor and cognitive symptoms (swim maze task) were present simultaneously (Lindner et al., 1999). Likewise, with 74% DA loss, rats showed a bad performance in a conditioned reaction time task (release a lever after a visual cue within a specific frame of time). Interestingly, bilateral large lesions reaching the ventral striatum resulted in both anticipated and delayed lever release, whereas smaller lesions confined to the dorsolateral striatum resulted in an increase in delayed responses only (Amalric et al., 1995). This goes in line with the participation of the dorsal striatum in movement initiation and of the ventral striatum in attentional inhibition of a motor response. Thus, the cognitive processes governing the stimulus-response association are impaired by substantial striatal DA depletion. Indeed, the cue (striatum dependent) and place version (hippocampal
dependent) of the MWM task were affected when 99% of DA content was caused (Ma et al., 2014; Whishaw and Dunnett, 1985), and the performance in active and passive avoidance tasks (associative learning of aversive stimuli) is also compromised (Blurton-Jones et al., 2009; Kopalli et al., 2013; Mansouri et al., 2013; Rajendra Kopalli et al., 2012).

DA also participates in the encoding and retrieval of object recognition memory (Pezze et al., 2015). Consistently, deficits in recognition memory in the novel object recognition test have been observed after both unilateral and bilateral 6-OHDA lesions (Chao et al., 2013; Nezhadi et al., 2016; Zhou et al., 2017). While there are confounding results regarding the effect of short or long retention times (Bonito-Oliva et al., 2014; Goes et al., 2014), other models of PD support the effects of DA depletion in this type of memory (Ho et al., 2011; Magen et al., 2012; Santos et al., 2013; Sardi et al., 2013).

In summary, both affective and striatal and hippocampal mnemonic deficits have been documented following the administration of 6-OHDA at different levels of the DAergic mesotelencephalic system.

2.5 STEM CELLS TRANSPLANTATION

Stem cells (SC) as a therapeutic approach for the treatment of neurodegenerative disorders have been an imperative area of research. SC are a special type of cell with self-renewal capacity and potential to mature into different cell lineages, including those of the central nervous system (CNS). This flexible capability of differentiating brought initial attention to their promising use as cell replacements of neurons lost to different insults. Notwithstanding, research in the last decades has shade light on additional outstanding properties that have made them even more attractive for neural therapy in the field of neurorehabilitation (Yoo et al., 2013).

Different sources of SC are actively researched nowadays, each with specific pros and cons. Embryonic stem cells (blastocyst and embryonic CNS-derived) come with ethical and technical restrictions that have highly limited their use. Moreover, their tendency to form teratomas and suffer immunological rejection are challenges still unresolved (Robertson et al., 2008). Adult Neural SC (from adult neurogenic niches) have a limited availability and therefore are difficult to obtain in sufficient numbers for clinical application. Somatic adult cells reprogrammed to be pluripotent stem cells (iPSC), as promising as they are, they also come with genotypic and phenotypic risks inherent to the technique (Zhao et al., 2014). Mesenchymal stem cells (MSC) represent a less ethical-constricted source that also overcomes the immunological and teratogenic drawbacks of embryonic SC. They can be obtained from several adult tissues (blood, fat, skin, and bone marrow) allowing for
autologous transplantation. Being multipotent, they have displayed good expansion and neuronal differentiation under several in vitro protocols. And last but not least, by sharing many of the neuroprotective mechanisms exhibited by other SC, they have been increasingly researched in many preclinical and clinical studies (Volkman and Offen, 2017).

2.5.1 Mechanisms of Action of SC grafts

The mechanisms involved in a successful neural transplantation are multivariate; exogenous and endogenous factors interplay in manifold ways to bring recovery. The host-graft interaction that ensues after transplantation is that of a reciprocal modulation that, under optimal circumstances, culminates in gross neuroprotective effects in immune response, neuroplasticity, neurogenesis, and neurotransmission (Figure 1) (Oliveira et al., 2016).

Exogenous factors

The exogenous factors are those intrinsic to the SC and whereby the injected cells influence the underlying microenvironment and, maybe, become integrated into the host’s neuronal networks. The paracrine secretion of different immunomodulatory and trophic factors influence immune behavior and repairing systems (Oliveira et al., 2016).

Transplanted cells have shown to provoke a shift in the local cytokine profile and inflammatory cell repertoire; an immunosuppressive effect wherein remyelination processes are also enhanced (Yang et al., 2009). MSC are weakly immunogenic and exert immunosuppressive effects on T and B-cells, dendritic cells, natural killer cells, and neutrophils through several mechanisms (Wada et al., 2013); they also decrease microglial activation, deviate their cytokine production towards an anti-inflammatory profile and protect the BBB integrity by modulating reactive astrocytes (Ooi et al., 2014; Park et al., 2015). Taking into account that neurodegeneration is a proinflammatory state, and immune-related factors impair neuronal survival and inhibit regeneration, this anti-inflammatory property emerges as a potential disease-modifying effect.

Additionally to immune modulation, trophic production (e.g. glial cell line–derived neurotrophic factor GDNF, nerve growth factor(NGF), Brain-derived neurotrophic factor (BDNF), Vascular endothelial growth factor (VEFG), thrombospondins (TSP) 1 and 2, and Neurotrophin 3) promotes axonal outgrowth and protects against glutamate-mediated excitotoxic and neuronal death. That and the differentiation towards the neuronal lineage, are believed to contribute to the reshaping of surviving circuits (Oliveira et al., 2016).

What is more, neurodegenerative processes have detrimental effects in neurogenesis, rendering endogenous repairing mechanisms insufficient for balancing the damage (Winner
et al., 2011). Trophic and modulatory mechanisms may not only occur at injured sites, they can reach neurogenic niches to exert regulation. In fact, a stimulating effect on endogenous neurogenesis has been seen in animal models of Alzheimer's disease, spinal cord injury, stroke and PD (Berg et al., 2015; Kan et al., 2011; Yoo et al., 2013). Thusly, paracrine tuning from MSCs can result in enhanced neurogenic and reconstructive capacity, a protective environment, and subsequently, functional improvement.

Endogenous factors

The host response towards the introduction of SC has an influence per se on the stem cells behavior. Neurogenic niches actively participate in stem cell maintenance, activation and differentiation. Niches facilitate dynamic interactions to occur within; cell to cell communication and diffused or embedded signals and molecules co-act spatially and temporally to govern stem cell survival and fate (Oliveira et al., 2016). Independently of the site of origin, the phenotype of derived mature cells mirrors that of local neurons. For example, forebrain stem cells gave origin to glial and neuronal cells with morphologic features of the implanted zones (striatum, hippocampus or cortex)(Eriksson et al., 2003). Once differentiated, the axonal projection and connectivity of newborn cells are also driven by regional factors in the developing brain, which is important for establishing functional synapses within the host circuitry (Espuny-Camacho et al., 2013). Stem cells also migrate parenchymally across great distances to places of injury (Steward et al., 2014). This valuable quality is related to the chemoattraction exerted by places where neuroinflammation and reactive gliosis are occurring. Immune cells release factors that act on Toll-like receptors expressed on SC; by means of that, a mutual regulation occur and, both, prejudicial and favorable consequences can manifest at different stages of response. Different patterns of cell activation and their cross-talk can lead to multitudinous SC behaviors and modify their self-renewal capacity, phenotyping and progeny survival (Kokaia et al., 2012).

In summary, the long-held prime mechanism of transplanted SC (namely, the cell-turnover and host-circuitry reconstructive capacity) is now considered outdated. Multiple bystander mechanisms are on the table today and should be considered whenever a graft is at test; as sundry as they are, so are their complex interaction and final result.
2.5.2 SCT and Cognitive Deficit in PD

Although PD is one of the most studied conditions in which these type of transplants have been tested; few have evaluated their impact on the non-motor symptoms of the disease. Insomuch as PD shares mechanisms of degeneration with similar conditions, we can reason that results obtained in other models of neurodegeneration can be extrapolated to some extent to PD.

Stem cell-based therapy has shown a potential therapeutic benefit for memory dysfunction and neuronal loss. In a model of hippocampal neuronal loss, NSC improved the performance in short-term memory tasks involving object and spatial recognition (Yamasaki et al., 2007). The positive results were mainly attributed to the regeneration of hippocampal neurons and an increased production of BDNF. Similarly, NCS grafted within the hippocampus of a senescence-accelerated mice model also brought about a better performance at the MWM test through neuronal differentiation and synaptic upregulation of NMDA receptor (Zhang et al., 2004). This benefit has also been seen in aged transgenic mice that express pathogenic forms of the amyloid precursor protein, presenilin, and tau; NSC transplanted intrahippocampally improved memory deficits on the MWM and context-dependent novel object recognition tests by enhancing synaptic BDNF-mediated plasticity without affecting the proteinopathy (Blurton-Jones et al., 2009). MSC have accomplished cognitive improvements in models of neurodegeneration as well. The intravenous or intraventricular transplantation of MSC derived from human adipose tissue (hAMSC) improved the cognitive and motor functions of aged mice (Park et al., 2013). Passive avoidance results and WMW maze
performance were better after one single injection of these cells, and even better with subsequent injections 2 weeks apart. These effects were partially due to increased differentiation towards cholinergic neurons, along with supportive cell lineages, and restoration of choline acetyltransferase (ChAT) activity and Ach levels. Furthermore, MSC therapy rescued spatial learning acquisition, reduced microglial and astrocytic activation, increased hippocampal neurogenesis, and lowered the degree of oxidative stress in Aβ-inoculated AD models (Chang et al., 2014; Oh et al., 2015).

In regards to PD, MSC have been mainly studied for their potential to alleviate motor symptoms and pathological mechanisms; behavioral cognitive effects have been scarcely studied in animal models of PD. Regardless of the model, route of delivery, dose, site or time of implantation; all studies have attested favorable changes on trophic support, immune response, neuronal preservation, α-synuclein clearance and endogenous neurogenesis. Some reporting motor improvements by assessing induced rotational activity post-transplantation. Only three studies among the ones reviewed assessed a cognitive endpoint (Table 1). Adipose-derived MSC grafts within the SN have shown to improve working memory (eight-arm radial maze test), by bringing the short-term and reference memory performance over time to a level similar to that of the non-lesioned group (Schwerk et al., 2015a). This improvement was concomitant with higher levels of hippocampal and subventricular neurogenesis, higher density of TH (tyrosine hydroxylase) positive cells, and increased production of neuroprotective cytokines/growth factors like IL-10 or BDNF. Since hippocampal and subventricular neurogenesis are affected in PD as a consequence of dopaminergic denervation, the boosting effect seen after stem cell transplantation might help repair these dysfunctional loops, and with them, the cognitive related symptoms. In a similar study, although no deleterious effect on working and reference memory was found after 6-OHDA lesion, similar mechanisms were found to be related to motor recovery (Berg et al., 2015). Growth factors, behaving as triggers of endogenous repairing mechanisms, promote the survival of the graft and mediate the cellular interlinking processes within the host. Indeed, NSC in which the secretome of MSC was applied were better than untreated NSC at: differentiating into dopaminergic neurons in the previously injured VTA and MFB, lesion-guided migration, reducing rotational activity and improving spatial learning (Yao et al., 2016).

In summary, while few studies have assessed the therapeutic value of stem cell transplantation in restoring the cognitive function in PD. Their so-far proved restorative properties, and positive results in other degenerative conditions, give support to the potential they hold for aiding in reinstalling a functional dopaminergic tone and, with that, cognitive processing.
<table>
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<tr>
<th>CELL SOURCE</th>
<th>QUANTITY/SITE</th>
<th>TIME</th>
<th>PD MODEL</th>
<th>CELLULAR MECHANISMS</th>
<th>BEHAVIORAL MODIFICATION</th>
<th>AUTHORS</th>
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</thead>
<tbody>
<tr>
<td>Rat bone marrow</td>
<td>180,000 /Striatum</td>
<td>2 weeks post-lesion</td>
<td>Unilateral Striatal 6-OHDA / Sprague-Dawly</td>
<td>Higher density of dopaminergic markers and vesicular striatal pool of DA</td>
<td>Reduced amphetamine-induced rotations</td>
<td>(Bouchez et al., 2008)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>MSC or NTF-SC 150,000 or 450,000 cells /Striatum</td>
<td>Day of lesion</td>
<td>Unilateral Striatal 6-OHDA / Sprague-Dawly</td>
<td>BDNF and GDNF secretion, migration towards the lesion, and reduced dopamine striatal depletion.</td>
<td>Reduced amphetamine-induced rotations. Not statistical effect on hypoactivity in the open field</td>
<td>(Sadan et al., 2009)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>200,000 -300,000 /SN</td>
<td>4-6 Weeks post-lesion</td>
<td>Unilateral nigral 6-OHDA / Sprague-Dawly</td>
<td>Differentiation towards neuronal lineage</td>
<td>Reduced apomorphine-induced rotations</td>
<td>(Shetty et al., 2009)</td>
</tr>
<tr>
<td>Commercial hMSCs (Bone-marrow)</td>
<td>32,000 or 180,000 / Striatum</td>
<td>5 Days post-lesion</td>
<td>Unilateral Striatal 6-OHDA / Sprague-Dawly</td>
<td>Phenotypic shift towards the glial lineage. GDNF secretion. Dopaminergic neurons survival in striatum/SNpc and enhanced SVZ neurogenesis</td>
<td>Reduction of apomorphine-induced rotations</td>
<td>(Blandini et al., 2010; Cova et al., 2010)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>BDNF-secreting hMSC 50,000 /SN</td>
<td>1 week post-lesion</td>
<td>Unilateral nigral 6-OHDA / Sprague-Dawly</td>
<td>Integration into local circuitry, trophic production, higher density of TH+ -cells.</td>
<td>Stabilization of amphetamine-induced rotation</td>
<td>(Shetty et al., 2009)</td>
</tr>
<tr>
<td>Rat bone marrow</td>
<td>300,000 or 500,000 / intranasal</td>
<td>Not specified</td>
<td>Unilateral MFB 6-OHDA / Sprague-Dawly</td>
<td>Widespread migration, higher density of TH+ -cells, and decreased inflammatory cytokines concentration</td>
<td>Reduced amphetamine-induced rotations and better forepaw motor control</td>
<td>(Danielyan et al., 2011)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>1,000,000 / Tail vein</td>
<td>4 Days post-lesion</td>
<td>MPTP /C57BL/6 mice</td>
<td>Enhanced neurogenesis in SVZ and SN. Increased differentiation of NPCs into dopaminergic neurons in SN.</td>
<td>Not tested</td>
<td>(Park et al., 2012)</td>
</tr>
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</table>

Table 1. PD animal studies assessing the effect of MSC transplantation on cellular and behavioral endpoints.
Abbreviations: 6-OHDA (6-hydroxydopamine), DA (Dopamine), hMSC (Human Mesenchymal Stem Cells), NTF-SC (Neurotrophic Factors-Secreting Cells), BDNF (Brain-derived neurotrophic factor), GDNF (Glia lcell-derived neurotrophic factor), SN (substantia nigra), SVZ (Subventricular zone), TH (Tyroxine Hydroxylase), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).
<table>
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<tr>
<th>CELL SOURCE</th>
<th>QUANTITY/SITE</th>
<th>TIME</th>
<th>PD MODEL</th>
<th>CELLULAR MECHANISMS</th>
<th>BEHAVIORAL MODIFICATION</th>
<th>AUTHORS</th>
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<tbody>
<tr>
<td>Rat bone marrow</td>
<td>1,000,000 / Jugular vein</td>
<td>Immediately after MPTP</td>
<td>MPTP/Wistar Rats</td>
<td>Prevention of neuronal TH+ loss</td>
<td>No improvement on immobility time or decreased rearing.</td>
<td>(Capitelli et al., 2014)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>1,000,000 / Tail vein</td>
<td>1 Day post-lesion</td>
<td>MPTP/C57BL/6 mice</td>
<td>Increased cellular viability, attenuated expression of α- synuclein, enhanced autophagy</td>
<td>Not tested</td>
<td>(Park et al., 2014)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>10,000,000 / Femoral vein</td>
<td>2 weeks post-lesion</td>
<td>Unilateral Striatal 6-OHDA / Sprague-Dawly</td>
<td>Preserved TH+ neurons in the SN and lower microglial reactivity</td>
<td>Reduced amphetamine-induced rotations</td>
<td>(Suzuki et al., 2015)</td>
</tr>
<tr>
<td>Human Bone Marrow</td>
<td>1,000,000 / Tail vein</td>
<td>at time of α-Syn</td>
<td>α-Syn inoculated C57BL/6 mice</td>
<td>Phagocytic clearance of extracellular α-syn by inducing M2 polarization of microglia.</td>
<td>Not tested</td>
<td>(Park et al., 2016)</td>
</tr>
<tr>
<td>Institutional hMSCs</td>
<td>1,000,000 / Tail vein</td>
<td>3 Days post-lesion or at day 1 of α-Syn</td>
<td>MPTP or α-Syn inoculated C57BL/6 mice</td>
<td>MMP-2-related proteolysis of aggregated α-Syn. Microtubule stability by controlling α-Syn-induced tau phosphorylation. Inhibition of extracellular α-synuclein transmission</td>
<td>Not tested</td>
<td>(Oh et al., 2017a, 2017b, 2016)</td>
</tr>
<tr>
<td>Human adipose tissue</td>
<td>300,000 / SN</td>
<td>1 week post-lesion</td>
<td>Unilateral MFB 6-OHDA / Wistar rats</td>
<td>Enhanced SVZ and SGZ neurogenesis, trophic secretion, increased TH and anti-inflammatory cytokines levels</td>
<td>No change on D-amphetamine-induced rotations. Better working memory performance</td>
<td>(Schwerk et al., 2015a, 2015b)</td>
</tr>
<tr>
<td>Human adipose tissue</td>
<td>300,000 / SN</td>
<td>8 days post-lesion</td>
<td>Unilateral MFB 6-OHDA / Wistar rats</td>
<td>Increased TH+ cells, BDNF, and GFAP</td>
<td>Reduced amphetamine-induced rotations, no differences in working memory across groups</td>
<td>(Berg et al., 2015)</td>
</tr>
</tbody>
</table>

Table 1. PD animal studies assessing the effect of MSC transplantation on cellular and behavioral endpoints (continued).

Abbreviations: 6-OHDA (6-hydroxydopamine), α-Syn (α-Synuclein), hMSC (Human Mesenchymal Stem Cells), MMP-2 (Metalloproteinase -2), BDNF (Brain-derived neurotrophic factor), GFAP (Glial fibrillary acidic protein), SN (substantia nigra), SVZ (Subventricular zone), SGZ (Subgranular zone), TH (Tyroxine Hydroxylase), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).
2.6 EXERCISE AS A POTENTIAL ADJUVANT THERAPY

Exercise is a notable neuroprotector and modulator of neuroplasticity with undeniable benefits for brain health and cognitive function in late ages (Aguiar et al., 2016). Given its marvelous qualities, its use as a therapy for many neurological conditions, including those associated with progressive neurodegeneration, does not come unexpectedly. Despite the lack of a full understanding of the mechanisms through which it begets such protection, clinical and preclinical studies have assessed its merit as a disease-modifying intervention.

2.6.1 Mechanisms of Action

Being an active repetitive task, it engages motor and cognitive processing networks, favoring their plastic remodeling and possibly bringing skill recovery in complex syndromes such as PD (Petzinger et al., 2013). Besides this motor re-learning advantage, and in a similar fashion to SCT, supplementary innards provide additional perks. Aerobic exercise is a proven booster of adult neurogenesis, which augments the number of newborn cells and foster their integration into established circuits (Abrous et al., 2005). DA depletion and neurodegenerative processes are related to impaired neurogenesis(Winner et al., 2011), by mending this endogenous restorative mechanism, benefits in learning and other complex cognitive task can be brought about; like that, not only motor, but also non-motor symptoms can be tackled down (Lamm et al., 2014). Adequate blood supply is critical for normal brain function, prove to that is that age–related hypoperfusion has been associated with neurodegeneration, cortical atrophy and cognitive decline. Exercise increases blood flow and promotes VEGF synthesis and angiogenesis (Nishijima et al., 2016), thus, better metabolic and signaling molecules deliver can have an effect on synaptic function and synaptogenesis in different motor and cognitive-related areas affected in PD (Petzinger et al., 2013). De facto, exercise is known to improve plastic adjustments such as long-term potentiation and depression (LTP, LTD, respectively)(Patten et al., 2013), increase dendritic spine density and arborization (Redila and Christie, 2006), and preserve gray matter volume in brain regions engaged in cognition (Erickson et al., 2014). Such functional upgrades are thought to be, at least partially, related to higher quantities of neurotrophins and growth factors. Elevation of BDNF and Insulin-like growth factor I (IGF-I) (among other trophic factors) have been consistently associated with regular exercise, both molecules serving neurogenic and neuroprotective functions (Campos et al., 2016). On top of that, immune and oxidative regulation also partake of exercise assets. A Rise in systemic anti-
inflammatory mediators and regulatory T-cells, balance in the myeloid cell lineage activity, including the resident microglia, and upregulation of anti-oxidative systems function and related repairing proteins occur after regular physical activity (Di Benedetto et al., 2017). Undoubtedly, exercise sounds like a formidable and inexpensive therapeutic tool for many neurological conditions. For PD, goal based and aerobic training have demonstrated motor improvements by facilitating automatic re-learning and cognitive engagement (Petzinger et al., 2013). What is more, its multi-varied neuroprotective mechanisms and mean effect on brain function and plasticity can potentially halt, or even reverse, defective cellular and molecular events leading to neuronal loss, unbeneﬁcial inﬂammation and dysfunctional connectivity (Table 2). In a PD model, long-term exercise, partially reverted the faulty communication of specific motor pathways, reintegrating the striatum signaling into the motor network hubs (Wang et al., 2015b). Additionally, dopamine levels have been normalized by calcium-mediated enhanced synthesis following physical activity (Sutoo and Akiyama, 2003). Treadmill training for four weeks in a 6-OHDA striatal injured model improved motor function, preserved DA terminals and favored trophic production and regenerative processes within the striatum (Tajiri et al., 2010). A new exercise paradigm of both skilled and aerobic nature brought motor recovery through enhanced functional connectivity between the prefrontal cortex and motor areas (Wang et al., 2015a). In PD 6-OHDA mice model, a 4-week swimming training, not only brought motor recovery, but also, attenuated inﬂammation, depressive like behaviors and improved long term recognition memory (Goes et al., 2014). Thus, not only motor, but associated non-motor complaints of the disease can be targeted. Current literature on the topic is very scarce though. In clinical settings, cognitive restoring have not been fully proven (Kalron and Zeilig, 2015). Yet, its well-known positive effect on complex functions such as mood regulation (Adamson et al., 2015) and the already aforementioned neuroplastic changes provide a window of opportunity that deserves being looked from.

2.6.2 Exercise and SCT

Overlapping restorative mechanisms between physical exercise and stem cell transplants can be immediately pointed out. This overlaying makes a synergistic interaction feasible, thereby larger beneﬁcial effects might be induced. Neurobiological mechanisms triggered by exercise and stem cells would cooperate to shape up the brain microenvironment, to induce cerebrovascular plastic changes and to increase the potential for neurogenesis and synaptogenesis.
Studies assessing the effect of exercise on endogenous hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells have shown that exercise greatly influences the mobilization and function of these cells, an adaptative measure to repair tissue damage and promote vascular health (Boppart et al., 2015). Thereupon, a potential exercise-directed modulation of stem cell performance is also probable. Not just the host, but grafts can also be sensitive to external influences (Döbrössy and Nikkhah, 2012).

To my knowledge, no study has investigated this duple therapy for Parkinson disease so far. In the few studies carried out in other neurological conditions, such as stroke or spinal cord/nerve injury, the combined use of SC transplantation and training have resulted in somewhat contradictory outcomes. In rodent cerebral infarct models, embryonic stem cell-derived neuronal precursors implanted intrastriatally were not potentiated by a 4-week treadmill training, and in fact, exercise-only brought better motor recovery and higher reduction of infarct size (Kim et al., 2007). On the opposite side, intravenous infusion of MSC plus treadmill rehabilitation reduced lesion volume, induced synaptogenesis, inhibited the apoptosis of transplanted MSC, and elicited motor, sensitivity and balance functional improvement to a greater extent than each strategy alone (Sasaki et al., 2016; Zhang et al., 2015). After traumatic spinal injury and dental pulp stem cells transplantation, 6-week treadmill training caused no functional locomotor improvement; while cells alone were neuroprotective by reducing TNF-α levels and scar formation (Nicola et al., 2016). On the contrary, a 6-week swimming training resulted in significant motor recovery (Carvalho et al., 2008). In models of sciatic lesion, the combined therapy (MSC + 1-week swimming) have brought, both, promoted or halted recovery, being the temperature of the water one possible factor causing the difference (Wang et al., 2010; Yang et al., 2015).

No study assessing this joined approach on neurodegenerative conditions or cognitive endpoints was found. Neurological conditions in which it has been implemented have an important motor component, and the reason why exercise was thought as a complementary tool; however, given its appended properties, its potential influence in other conditions in which neuronal loss and dysfunction beyond the motor domain are also chiefly present (like PD) warrant proper scrutiny. Thus and so, this area of study is not but in its fetal state; many gaps remain in already studied conditions and a whole world beyond the current bubble of knowledge awaits to be discovered. Further studies are required to better understand key mechanisms behind graft plasticity and how training protocols can influence graft-host interaction and functional recovery. This project is therefore an attempt to wander into foreign lands.
<table>
<thead>
<tr>
<th>MODALITY</th>
<th>TIME</th>
<th>PD MODEL</th>
<th>CELLULAR MECHANISMS</th>
<th>BEHAVIORAL MODIFICATION</th>
<th>AUTHORS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treadmill / non-noxious stimulus - 5 m/min</td>
<td>60 min-5d/week-28 days</td>
<td>MPTP/ C57BL/6J mice</td>
<td>Increase in stimulus-evoked dopamine release, most pronounced in the dorsolateral striatum. No differences in total striatal dopamine</td>
<td>Improved motor and balance</td>
<td>(Petzinger et al., 2007)</td>
</tr>
<tr>
<td>Treadmill-3m/min</td>
<td>30min/d-14 days</td>
<td>Unilateral Striatal 6-OHDA / Sprague-Dawly</td>
<td>Enhanced survival of dopaminergic neurons in the SN</td>
<td>Reduced apomorphine-induced rotations</td>
<td>(Yoon et al., 2007)</td>
</tr>
<tr>
<td>Treadmill - 18m/min</td>
<td>40 min-5d/week- 4 weeks</td>
<td>MPTP/ C57BL/6J mice</td>
<td>Physical endurance. Increased angiogenesis in the striatum. No change in nigrostriatal DAergic markers</td>
<td>Reduced amphetamine-induced rotations</td>
<td>(Al-Jarrah et al., 2010, 2007)</td>
</tr>
<tr>
<td>Treadmill – up to 15m/min</td>
<td>40 min-5d/week- 18 weeks</td>
<td>MPTP/ C57BL/6J mice</td>
<td>No change in neuronal loss.</td>
<td>Better gait pattern, spontaneous ambulatory movement, and balance. No change in habit learning, motor coordination, or amphetamine-induced rotations</td>
<td>(Pothakos et al., 2009)</td>
</tr>
<tr>
<td>Treadmill - 11m/min</td>
<td>30 min-5d/week- 4 weeks</td>
<td>Unilateral striatal 6-OHDA / Sprague-Dawly</td>
<td>Preserved TH+ striatal and nigral cells, enhanced neuronal migration and upregulation of BDNF and GDNF</td>
<td>Better balance, reduced amphetamine-induced rotations,</td>
<td>(Tajiri et al., 2010)</td>
</tr>
<tr>
<td>Voluntary wheel running</td>
<td>3 weeks</td>
<td>Unilateral MFB 6-OHDA / Sprague-Dawly</td>
<td>Reversed molecular changes in the hippocampus induced by maternal separation</td>
<td>Not tested</td>
<td>(Dimatelis et al., 2013)</td>
</tr>
<tr>
<td>Treadmill 10m/min</td>
<td>40 min-3/week-4 weeks</td>
<td>Unilateral striatal 6-OHDA / Wistar</td>
<td>Preserved TH+ striatal and nigral levels via BDNF production</td>
<td>Reduced apomorphine-induced rotations</td>
<td>(Real et al., 2013)</td>
</tr>
</tbody>
</table>

Table 2. PD animal studies assessing the effect of aerobic exercise on cellular and behavioral endpoints.
Abbreviations: 6-OHDA (6-hydroxydopamine), DA (Dopamine), BDNF (Brain-derived neurotrophic factor), GDNF (Glial cell-derived neurotrophic factor), SN (substantia nigra), TH (Tyroxine Hydroxylase), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), MFB (Middle forebrain bundle).
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</tr>
</thead>
<tbody>
<tr>
<td>Swimming training</td>
<td>5d/week-4 weeks</td>
<td>Unilateral Striatal 6-OHDA / C57BL/6J mice</td>
<td>Protection against oxidative stress, lowered IL-1β and preserved DA levels</td>
<td>Less depressive-like behavior, better balance, improved long-term memory object recognition</td>
<td>(Goes et al., 2014)</td>
</tr>
<tr>
<td>Treadmill-15m/min</td>
<td>30 min-5d/week- 4 weeks</td>
<td>Unilateral MFB 6-OHDA / Long Evans</td>
<td>Not tested</td>
<td>No change in apomorphine-induced rotations, forelimb placement asymmetry, or exploratory rearing</td>
<td>(Landers et al., 2014)</td>
</tr>
<tr>
<td>Treadmill / non-noxious stimulus – 10- 24 m/min</td>
<td>5d/week-6 weeks</td>
<td>MPTP/ C57BL/6J mice</td>
<td>Increased dendritic spine density and arborization of striatal neurons, increased expression of synaptic proteins PSD-95 and synaptophysin.</td>
<td>Improvement in running velocity</td>
<td>(Toy et al., 2014)</td>
</tr>
<tr>
<td>Treadmill-12m/min</td>
<td>30 min-5d/week- 4 weeks</td>
<td>MPTP/ C57BL/6J mice</td>
<td>Increased expression of synaptophysin, PSD-95, TH, and dendritic spines in nigrostriatal neurons.</td>
<td>Improved motor coordination</td>
<td>(Shin et al., 2016)</td>
</tr>
</tbody>
</table>

**Table 2. PD animal studies assessing the effect of aerobic exercise on cellular and behavioral endpoints (continued).**

Abbreviations: 6-OHDA (6-hydroxydopamine), DA (Dopamine), TH (Tyroxine Hydroxylase), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), MFB (Middle forebrain bundle).
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Mesenchymal stem cells and treadmill training yielded paradoxical results in long-term recognition memory and aversive learning in a 6-OHDA model of Parkinson disease: compartmentalized effects.

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**Key Words:** Parkinson Disease; Disease Models, Animal; Cognition; Stem Cell Transplantation; Exercise; Rehabilitation.

**Highlights:**

1. No gross mnemonic impairment was present at 7 weeks post-lesion.
2. Forced treadmill training was related to long-term recognition memory impairment.
3. Stem cell transplantation had minimal effect on assessed cognitive performance.
4. Combined therapy had paradoxical effects on recognition memory and aversive learning.
Abstract
Cognitive deficits are also present in Parkinson disease (PD) since early phases of disease progression. Disease modifying strategies such as stem cell transplantation (SCT) and exercise have shown highly overlapping neuroprotective and pro-cognitive properties; still no studies have assessed potential synergy in neurodegenerative conditions such as PD. The aim of this study was to assess the efficacy of combining mesenchymal SCT and treadmill training for counteracting cognitive alterations induced by 6-hydroxydopamine (6-OHDA). To assess this, male Wistar 6-OHDA-injured rats were randomized to receive either none or one of three treatments: 4-week treadmill training, $2 \times 10^5$ human adipose-derived mesenchymal stem cells transplanted within the striatum ipsilateral to the lesion, or both. At the end, short and long-term object recognition memory and aversive learning were evaluated and compared. No outright deficit in either cognitive task was found after unilateral mesotelencephalic dopaminergic deficit; however, while SCT alone had minimal effect on task performance, treadmill training was associated with impaired long-term recognition memory, anxiety-like behavior and enhanced fear response to an aversive memory. Paradoxically, combining both strategies had regionalized effects; whereas the negative effect on long-term memory persisted and even deepened, the previously enhanced avoiding and anxious behavior reverted. Based on our results and in view of current literature, it is possible that the stressing effect of a shock-motivated treadmill training would have canceled any beneficial effect of the combined therapy in areas vulnerable to stress but would have had potentiating effects in activity-engaged basal ganglia circuitry.

Abbreviations

5-HT Serotonin
6-OHDA 6-hydroxydopamine
ACh Acetylcholine
DA Dopamine
daergic Dopaminergic
DS Dorsal striatum
hAMSC Human Adipose-derived Mesenchymal Stem Cells
MCS Mild chronic stress
MSC Mesenchymal Stem Cells
NA Noradrenaline
NOR Novel Object Recognition task
PA Passive Avoidance task
PD Parkinson’s Disease
PD-D PD-associated Dementia
1. Introduction

Neurodegenerative diseases are a prevalent ailment nowadays; as life expectancy has extended in the last decades, the number of people afflicted by these disorders has risen proportionally. At present, Parkinson’s disease (PD), a very costly and disabling condition, is the second most common degenerative disease after Alzheimer’s disease (Pringsheim et al., 2014). Although typically characterized as a motor disorder, it is now recognized as a complex syndrome in which non-motor impairments (autonomic, cognitive and affective) are also present in both early and advanced stages of the disease. Particularly, mid to late stages of the disease, besides manifesting the well-known motor disturbances; are also characterized by progressive cognitive decline, dementia, anxiety, depression and impulsive/compulsive behavior (Balestrino and Martinez-Martin, 2017). Mild to moderate executive, attentional, mnemonic, and visuospatial disturbances may appear even before motor disturbances materialize, and can be present in around one-third of the patients at the moment of diagnosis (Mark Broeders et al., 2013). Moreover, over the course of the illness, these cognitive complaints become increasingly prevalent (21% at diagnosis vs 88% after 7 years of progression), and turn into a major determinant of the quality of life (QOL) and degree of disability of the patients; some coming to be at least as debilitating as the motor symptoms themselves (Storch et al., 2013). They represent a recognized risk factor for PD-related dementia (PD-D) (Aarsland and Kurz, 2010), and a therapeutic challenge. Current approved therapeutic approaches relief symptoms temporarily but seldom modify the underlying pathological mechanisms of the disease, which will continue to progress relentlessly. What is more, their clinical effectiveness is less consistent when cognitive and affective symptoms come into the picture (Jenner et al., 2013). This is the reason why an ever-present search and test of potential disease-modifying approaches that halt or reverse PD pathological processes, and which are not just palliative in nature, exist today. One of such strategies is stem cell transplantation (SCT).
Among the multiple types of stem cells available for transplantation today, Mesenchymal Stem Cells (MSC), self-renewed multipotent cells with attributed proliferative, restorative, neuroprotective and immunomodulatory properties, are a very attractive option. Their suitability for autologous transplantation (Volkman and Offen, 2017) and their link to cellular and behavioral recovery in experimental studies of neurological conditions, including those of neurodegenerative nature such as PD (Blandini et al., 2010; Schwerk et al., 2015a) have brought scientist’s attention to them. Despite this, many technical limitations, phenotypic instability, low survival rates and transplant-related variable effects still restrict their clinical use (Ikehara, 2013). Ever-more sophisticated engineering techniques have tried to overcome these drawbacks by genetically gearing them up. Yet, no flawless method has been found. We hypothesized that a much simpler and less expensive way to achieve this could be aerobic training. Exercise, on its own, stands high as a promoter of brain health throughout life; when routinely practiced, it preserves brain function, lessens the normal age-related cognitive decline, and protects against neurodegeneration (Aguiar et al., 2016). Neuroplastic, trophic and angio-immunomodulatory roles are thought to mediate these functional upgrades (Raichlen and Alexander, 2017), all properties of great interest for neurorehabilitation and that have validated its use as a promising therapy for many neurological conditions, including PD (Petzinger et al., 2013). What draws even more attention, though, is the fact that these two approaches (STC and exercise) possess highly overlapping mechanisms (Oliveira et al., 2016). This super-imposition leads us to reason that a synergistically interaction is feasible whereby neurobiological mechanisms triggered by exercise and stem cells implants would interact positively to shape up the pathological microenvironment, favor cerebrovascular plasticity, and ultimately bring functional results to a greater extent. To our knowledge, no study has investigated this duple therapy for PD so far. In the few studies carried out in stroke or spinal cord/nerve injury, the combined use of SC transplantation and physical training have yielded somewhat contradictory outcomes; some showing potentiation (Sasaki et al., 2016; Yang et al., 2015; Zhang et al., 2015), others showing none (Kim et al., 2007; Nicola et al., 2016; Wang et al., 2010). It is important to highlight that since these conditions have an important motor component, all studies only evaluated motor endpoints. Exercise being an active repetitive task that favors the plastic remodeling of motor networks represents a logic complementary tool for motor impairments. However, given its appended properties, its potential use as a supplementary approach to SCT for dysfunctions beyond the motor domain warrant proper scrutiny. PD is both motor and non-motor in nature, and therefore a convenient model to study this hypothesized synergic interaction. 6-OHDA is a catecholaminergic toxin that once injected within the left middle
forebrain bundle (MFB) causes almost total destruction of dopaminergic neurons within the substantia nigra (SNc) and the ventral tegmental (VTA), wherefore mimicking moderate to severe stages of the nigrostriatal dysfunction seen in PD (Truong et al., 2006). Thus and so, the aim of this study was to determine whether the combined use of mesenchymal SCT and aerobic training is capable of counteracting the cognitive alterations induced by 6-hydroxydopamine (6-OHDA), largely than either of them alone, on two cognitive tasks (the novel object recognition and passive avoidance) previously proven dependent on the DAergic telencephalic modulating system.

2. Methods

2.1 Animals

Adult male Wistar rats (200-250g) were housed at the Animal Breeding Unit of the Universidade Federal de Ciências da Saúde de Porto Alegre, under a 12 h/12 h light/dark cycle, controlled temperature (22°C±2), and ad libitum access to water and food pellets. All experimental procedures were performed during daytime and according to the National Institutes of Health guidelines for ethical care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and the Brazilian law for the use of experimental animals (law 11.794, 2008). The institutional Ethics and Research Committee gave its approval for all procedures (protocol no.176/15).

2.2 Experimental Design

All animals were habituated to handling for one week and randomly allocated to five groups: “Sham” (n=9), “6-OHDA” (n=8), “Exercise” (n=8), “Stem cells” (n=8), and “Combined” (n=8). A single stereotaxic 6-OHDA (or saline for Sham group) injection was performed within the left middle forebrain bundle (MFB). Two weeks after the surgery, the success of the lesion was confirmed through the assessment of methylphenidate-induced rotational activity. Once again, a stereotaxic surgery was carried out to give either, a striatal cell graft of hAMSC (Stem cells and Combined, n=16) or a striatal NaCl injection (6-OHDA and Exercise, n=16). After allowing for 3 days of recovery, the Exercise and Combined groups underwent aerobic training for 4 weeks, at the end of which all groups were tested in two cognitive tasks: the novel object recognition and the passive avoidance tests. At the end, all animals were transcardially perfused and the brains were prepared for histological characterization (Figure 1).
Figure 1. Study timeline. The numbers represent the days since the start of the experiment and the symbols the experimental conditions. Sham is not represented. For clarity purposes, Sham received a NaCl lesion/graft within the same stereotaxic coordinates but did not receive any training. Similarly, 6-OHDA received a NaCl graft and did not perform any training. All groups underwent identical behavioral tests at the same time points. Abbreviations: 6-OHDA (6-hydroxydopamine), SCT (stem cell transplantation), NOR (novel object recognition test), PA (passive avoidance test).

2.3 6-OHDA Lesion and Drug-induced rotational activity

After one week of habituation to handling, a stereotaxic surgery (Torres and Dunnett, 2011) (Kopf Instruments, USA) was carried out under anesthesia with xylazine (10mg/kg i.p.) and isofluorane (2%, 100% oxygen at 0.5-1 L/min). Animals were fixed on the apparatus and a medial incision was made to expose the scalp. Left MFB coordinates were calculated (from bregma, anteroposterior (AP): −4.0mm, mediolateral (ML): 1.3mm, and below the dura matter, dorsoventral (DV): −7.0mm). Using a 10µL microsyringe (Hamilton, 701N) and a syringe pump (Harvard 11plus, USA), 12 µg of 6-OHDA (Sigma-Aldrich, USA) (3µL of a solution containing 4µg/µL of 6-OHDA dissolved in 0.9% saline and 0.1% ascorbic acid (Sigma-Aldrich, USA)) were injected unilaterally at a rate of 1µL/min. To optimize toxin diffusion, the needle was left at the injected site for 3 min before slow withdrawal. Sham animals received the same volume of saline.

On the 14th day after surgery, following a 30 min room-habituation period, a challenge with Ritalin® (methylphenidate, 40 mg/kg i.p, Novartis, Brazil) was performed (Silvestrin et al., 2009). After injection, the animals were immediately placed in a 90 cm diameter open field arena and the number of left (ipsilateral to lesion) 360-degree rotations were counted for 30 min. Animals averagely showing more than five turns/min were considered injured and continued on the experiment.
2.4 hAMSC preparation

2.4.1 Isolation & cultivation
Human adipose-derived mesenchymal stem cells (hAMSC) were extracted from the abdominal adipose tissue of a 31 y.o female healthy donor with normal body mass index. The Patient’s consent was obtained before liposuction. All protocols were approved by the Committee of Ethics in Research of the Hospital Santa Casa de Misericórdia de Porto Alegre (REC-ISCMMPA n°882968). The fat tissue was washed extensively with phosphate-buffered saline (PBS) to remove blood. Fat fragments were relocated to a Falcon tube containing collagenase solution (Type I, 3 mg/mL solubilized in Dulbecco’s modified Eagle’s medium (DMEM)) and incubated at 37°C for 30 minutes (vortexed every 10 min). The collagenase was then inactivated with a solution of DMEM with low glucose, supplemented with 10 mM HEPES, 23.8 mM NaHCO3, 100.000 U/mL penicillin, 100 mg/mL streptomycin (Sigma, USA), and 10% (v/v) fetal bovine serum (FBS) (Cultilab, Brazil). The cells were centrifuged at 600×g for 10 minutes, and after discarding the supernatant, were resuspended in an erythrocyte lysis solution (150 mM NH4Cl, 10 mM NaHCO3 and 1 mM EDTA diluted in Milli-q water 1:1(v:v)) and incubated for 15 minutes at room temperature under mechanical shaking. After a second cycle of centrifugation, the cells were resuspended in DMEM in 10% FBS and counted using the trypan blue exclusion test in a Neubauer chamber. Viable cells were seeded (5.2×10³ cells/cm²) in a six-well plate and maintained in a humidified incubator at 37 °C with 5% CO2. The medium was replaced 48 hours later and every 4 days from then on until preconfluence was reached. After 90% of confluence, the primary cultures were washed in PBS without Ca2+ and Mg2+ (Sigma) and routinely passaged using 0.25% trypsin and 0.01% EDTA (Invitrogen) at 37°C.

2.4.2 Differentiation in vitro
The differentiation of the MSCs was performed between passages 4 to 7 at 2.2×10⁵ cells/cm² using specific mediums for chondrogenic, adipogenic and osteogenic induction (Gibco, USA). The cells were cultivated for 4 weeks and the medium changed every 3 days. After this period, the cells were washed once with PBS and fixed with 4%(v/v) paraformaldehyde in PBS for 30 minutes at 2-8 °C. For the chondrogenic assay, cultures were stained with 1% Alcian Blue in 0.1N HCL for 30 minutes at room temperature; for the adipogenic assay with 3.4% Oil Red O in isopropyl alcohol for 5 minutes at room temperature; and for the osteogenic assay with 2% Alizarin Red S in water (pH 4.2) for 2-3 minutes at room temperature. Stained cultures were
compared to undifferentiated controls using a BX-50 Olympus microscope with optical lens (10X/0.30 Ph1-UplanFl), coupled to a camera Motion 2500 (Olympus, Germany).

2.4.3 Characterization of hAMSC
Analyses for the expression of human CD14, CD34, CD44, CD45 and CD105 were performed. After dissociation with trypsin, the cells were centrifuged and incubated for 30 minutes at 4°C with phycoerythrin (PE) or fluorescein isothiocyanate (FITC) conjugated antibodies against the markers of interest (Invitrogen, USA). The analyses were performed using a FACSCalibur flow cytometer equipped with 488 nm argon laser (Becton-Dickinson, USA) with CellQuest software. At least 10,000 events were collected.

2.5 hAMSC transplantation
MSCs between passages 4 to 7 were washed twice with PBS and detached using 0.25% trypsin for 10 minutes at 37°C. Inactivation occurred using DMEM in 10% FBS. After centrifugation at 600×g for 10 minutes, the cells were resuspended in PBS and counted using the trypan blue exclusion test. 2x10⁵ viable cells were once again centrifuged (600×g, 10 minutes), resuspended in 6 µL PBS, and maintained under low temperature (2-8 °C) until transplantation (for up to 2 hours). On the 25th day of the experiment, under Ketamine (90mg/kg i.p.)/Xylasine (10mg/kg i.p.) anesthesia and using a stereotaxic apparatus (Kopf Instruments, USA), the cells were injected into the left striatum at coordinates AP 0,5mm, ML 3,2mm, and DV-4,5mm (Paxinos and Watson, 2007). Two hundred thousand cells were infused with a 10-gauge microsyringe (Hamilton, 701N) at 1µL/min. The needle was left in place for 5 min and then retracted slowly. Animals belonging to the Sham and Exercise groups received the same volume of saline (6 µL).

2.6 Treadmill Training Protocol
The animals were adapted to the 8-lane treadmill of adjustable speed and a shock grid (0,5mA) (Projetos ABS, Brazil) by running for 10 min during three consecutive days (9 m/min), at the beginning of the experiment, during the habituation week. After 3 days of post-surgical recovery, Exercise and Combined groups underwent a 4-week aerobic training of running at a speed set at 16 m/min, which corresponds to an intensity of 55% VO2max, as previously described (Gava et al., 1995; Nunes et al., 2013). The sessions were 5 times per week and were progressively
longer, from 30 min during the first week to 60 minutes during the last week (10-minute increment per week).

2.7 Behavioral tests

All experiments were carried through during the morning hours, between 9:00 hrs and 12:00 m.

2.7.1 Novel Object Recognition (NOR)
The ability of rodents to discriminate a familiar from a new object (memory of a previously encountered object) is assessed by this test. The test consisted of four phases: habituation, acquisition, short-term retention, and long-term retention. For each phase, the animals were brought to the testing room and allowed a 30-minute adjusting time. During the habituation phase, the rats were individually familiarized to the squared Plexiglas test chamber (33 x 41 x 24 cm) for 10 minutes. For the acquisition phase, two identical objects were placed symmetrically, and equidistantly, in the center of the arena; the subjects were subsequently released within, with its back to the objects, and allowed to explore them for 10 min. One hour and 24 hours after the acquisition phase, one of the objects was replaced by a novel object (different each time) and an exploration time of 5 min was given (short-term (ST) and long-term (LT) retention, respectively). The novel objects were side-counterbalanced across animals. After each individual session, the objects and the arena were thoroughly cleaned with 75% ethanol to prevent odor recognition. All sessions, except for habituation, were recorded by a camera located above the arena and manually analyzed on replay.

Exploration was defined as the orientation of animal’s snout towards the object, sniffing or touching it; running around, sitting or climbing on the object was not considered as exploration. Main variables compared across groups were the Difference Score (Novel object exploration time - Familiar object exploration time) and the Discrimination Index (Novel object exploration time/ Total exploration time) for each of the three experimental moments. Successful recognition of a previously explored object was considered when a positive DS or a DI above 0.5 were obtained. Supplementary variables compared among groups were the total exploration time and the number of rearings (forepaw free-standing or against the walls).

2.7.2 Step-through passive avoidance

An acrylic box (31 x 33 x 54 cm) with a floor of stainless steel rods, divided into two compartments, and connected to an electronic stimulator (Insight EP-111, Brazil) was adapted to
perform the task. A door remotely controlled separated the two compartments, one had a light on and the other was in complete darkness. On the training trial, the subject was placed in the lighted compartment and allowed a habituation of 30 seconds. After this period, the door was opened and the time to enter (including hindlimbs) the dark compartment was counted. The door was closed immediately and an inescapable electric shock (0.8 mA for 3 s) was delivered from the grid floor; after 10 seconds, the rat was removed. 24 hours later, the same procedure (retention trial), except for the delivery of the shock, was performed. The time to enter the dark side was once again timed, with a cut-off of 600 seconds (10 minutes). After each individual session, the floor and walls were thoroughly cleaned with 75% ethanol. All sessions, except for habituation, were recorded and manually analyzed on replay. The main variable explored was the time to enter the dark side on both, the training and retention trials. Supplementary variables were the number of rearings (free-standing or against walls) on training trial, the time exploring the dark side (defined as an active exploration of the dark side without completely crossing), the latency (in seconds) to the first moment of the subject explored the dark side, and the percentage of time spent in minimal activity (breathing and light whiskers movements) during the retention trial.

2.8 Perfusion and immunofluorescence staining

On the 67th day, all animals were deeply anaesthetized with Ketamine/Xylazine (100mg/kg; 10mg/kg i.p.). Following a single cardiac injection of heparin (1000 IU), the animals were transcardially perfused with 350ml of 4% paraformaldehyde (Dinâmica, Brazil) in 0.1 M PBS (pH 7.4) (Tao-Cheng et al., 2007; Zancan et al., 2017). The brains were removed, postfixed in 4% PFA/0.1M PBS overnight at 4°C and subsequently dehydrated in 30% sucrose/0.1M PBS for 72 hrs. After checking that the samples had sunk, they were wrapped in foil, frozen in liquid nitrogen, and kept at −80°C until histological processing. Brains were cut with a cryostat (CM3050s, Leica, USA) at −23°C into 50 μm coronal sections and stored at −20 °C in 30 % sucrose/0.1 M PBS. For tyrosine hydroxylase (TH) and after a PBS wash, free-floating sections were preincubated with blocking solution (1% Bovine Serum Albumin and 0.025% Triton X-100 in 0.1 M PBS) for 30 min at room temperature. Sections were then incubated with rabbit α-TH antibody (1:500, Life Technology, cat# P21962) diluted in blocking solution for 24 hours at 4°C. After this, the slices were washed in PBS for 40 min and incubated for 90 min at room temperature with donkey α-rabbit-Alexafluor 568 (1:400; Invitrogen, cat# A10042) diluted in
blocking solution. Finally, several rinses of PBS (40 min) were done and the sections were mounted and coverslipped with Vectashield (Vector Labs). Fluorescence was detected in an Olympus IX51 U-RFLT Inverted Microscope (Olympus Corporation, USA). The 510-550 wavelength laser was employed to excite the TH samples. The Olympus DP controller 3.3.1292 software was used for photographic capture. Two set of photos were taken, at 4x and 20x objectives. All technical parameters were maintained for shooting different samples.

2.9 Statistical Analysis

The statistical analysis was performed using IBM SPSS version 22. All variables were numerical. The Shapiro-Wilk test was used to assess normality, followed by the Levene test to assess homoscedasticity. For intergroup comparisons of parametric data (Mean (SD)), the one-way analysis of variance (ANOVA) followed by Tukey HSD post hoc test was used. For within group comparisons, a repeated-measures One-way ANOVA (rmANOVA) was executed after checking for sphericity (Mauchly’s test). Least significant difference (LSD) post hoc analysis was effectuated for multiple pairwise comparisons. For non-parametric variables (median [IQR]), Kruskal-Wallis H test followed by Dunn’s tests were employed for multiple group comparisons. Friedman and Wilcoxon Signed Rank Test were employed for within group comparisons. The α level was set at p=0.05 for all variables.

3. Results

3.1 Human Adipose Mesenchymal stem cells (hAMSC) characterization

hAMSC exhibited elongated fusiform morphology (fibroblast-like), self-renewal potential and plastic adherence (Figure 2A). After incubating the cells in media promoting differentiation into the chondrogenic, adipogenic or the osteogenic lineage, the cells showed multipotency. Blue staining indicates the synthesis of proteoglycans by chondrocytes (Figure 2B); yellow staining evidence the presence of intracellular lipid droplets (adipocytes) (Figure 2C), and the red staining, calcium-rich mineralized matrix deposits by osteoblasts (Figure 2D). The analysis of phenotype by flow cytometry showed that the hAMSC expressed CD44 and CD105 but were negative for the hematopoietic markers CD14, CD34, and CD45 (Figure 3).
Figure 2: Characterization of adipose-derived MSC. **A.** Primary hAMSC cultures had a fibroblast-like shape and demonstrated plastic adherent proprieties. MSC underwent differentiation towards: **B** chondrocytes stained by Alcian blue; **C** adipocytes showing lipid vesicles stained by Oil red O; and **D** osteoblasts with calcium phosphate deposits shown by Alizarin Red S.

Figure 3. Flow Cytometry (FACS) analysis. Human Adipose-derived MSC were homogeneously positive for mesenchymal markers CD44, and CD105 but negative for hematopoietic markers CD14, CD34, and CD45. Abbreviations: phycoerythrin (PE); fluorescein isothiocyanate (FITC).
3.2 Rotational behavior following 6-OHDA infusion

Two weeks after the stereotaxic injection of 6-OHDA, all groups, with the exception of Sham, presented methylphenidate-induced ipsilateral rotations. While 6-OHDA and the treatment groups were significantly different from Sham, no significant difference was found among them (Table 1). Visual confirmation of the unilateral depletion of nigral/VTA TH+ immunoreactivity was obtained from a sub-sample of each group (Figure 4).

Table 1.
Methylphenidate-induced ipsilateral rotational activity

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>M</th>
<th>SD</th>
<th>95% CI</th>
<th>p-value*</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>[0, 0]</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>6-OHDA</td>
<td>8</td>
<td>254.3</td>
<td>50.79</td>
<td>[211.8, 296.7]</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Exercise</td>
<td>8</td>
<td>285.5</td>
<td>125.2</td>
<td>[180.9, 390.1]</td>
<td>&lt;0.0001</td>
<td>0.942</td>
</tr>
<tr>
<td>Stem Cells</td>
<td>8</td>
<td>284.4</td>
<td>117</td>
<td>[186.6, 382.2]</td>
<td>&lt;0.0001</td>
<td>0.949</td>
</tr>
<tr>
<td>Combined</td>
<td>8</td>
<td>245.90</td>
<td>59.44</td>
<td>[196.2, 295.6]</td>
<td>&lt;0.0001</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Drug-induced left rotations were counted for 30 minutes 2 weeks after the 6-OHDA left MFB-stereotaxic injection. Group comparisons were effectuated by one-way ANOVA followed by Tukey HDS post hoc. A p-value <0.05 was considered significant. *p-value of comparison to Sham. †p-value of comparison to 6-OHDA. Abbreviations: M (Mean), SD (Standard Deviation), 6-OHDA (6-hydroxydopamine), MFB (Middle Forebrain Bundle).

3.3 Performance in the NOR test

All groups presented positive Difference Scores (DS) and Discrimination Indexes (DI) above 0.5 at Short-term (ST) and Long-term (LT) phases. When DS was compared across groups at each time point (Kruskal Wallis test), no statistically significant difference was found at either Ac ((H(4) =6.935, p=0.13) or ST (H(4)=4.978, p=0.29). All groups showed a positive shift of the difference Score at ST, indicating more time spent with the new object at this time point. 24 hours later (LT Retention), although a positive difference was still maintained, a dropping in the value was observed for all groups, more distinctly in 6-OHDA and treatment groups. Notwithstanding, only the Combined group was significantly different from Sham at this moment (F(4,36)=3.603, p=0.014; Tukey’s HSD, p=0.007). For within group comparisons across the three phases of the test, nonparametric Friedman tests of differences among repeated measures were conducted. Only the Sham group rendered a significant chi-square value of 11.486 (p=0.003). Post hoc pairwise comparisons by Wilcoxon Signed Rank Test showed that both the Short (Z=-2.547,
p=0.01) and Long-term DS (Z=-2.670, P=0.008) were significantly higher than that of Ac (Figure 5A).

![Image of brain regions labeled VTA, SNc, and 6-OHDA, Exercise, and Combined]

**Figure 4. Tyroxine Hydroxylase (TH+) Immunofluorescence** of the Substantia Nigra pars compacta (SNc) and Ventral Tegmental Area (VTA) in a subsample of each experimental group. The same subject served as its own positive control. A visual disparity between the left (L) and right side of each group is easily appreciable.

There was no statistically significant difference across the DI means (one-way ANOVA) in all three phases (Table 2). During the Acquisition (Ac) time, although all groups, except for Sham, presented a slight preference for one of the identical objects, this was not statistically significantly different from Sham (F(4,34)=1.147, p=0.23). Within group analysis (mrANOVA) showed a statistically significant difference in the DI in all (but exercise) groups when all three time points were contrasted (Table 2). LSD post hoc analysis revealed that a significant bias of exploration towards the novel object occurred in all groups when one of the familiar objects was replaced one hour after the object familiarization (ST phase). Only in the exercise group, this preference (DI) for the new object was not statistically significantly different from the score obtained during the Ac time. After 24 hours of retention time, this biased interaction was not maintained in the Combined and Exercise groups, as seen by the significant dropping of the DI at this time point. In fact, only the DI score of Sham and 6-OHDA groups was significantly
different from that obtained during the acquisition time (Figure 5B). The Stem Cells group also showed a falling, but it was not enough to be significantly different (p=0.055); yet, the DI this group obtained was not statistically different from that of the Ac phase, when both objects were identical and unfamiliar (p=0.578).

Table 2.
**Discrimination Index at the three Novel Object Recognition Test phases**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Sham</th>
<th>6-OHDA</th>
<th>Exercise</th>
<th>Stem Cells</th>
<th>Combined</th>
<th>ANOVA</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>M(SD)</td>
<td>F-value*</td>
<td>p-value*</td>
<td></td>
</tr>
<tr>
<td>Acquisition</td>
<td>0.50 (0.06)</td>
<td>0.54 (0.10)</td>
<td>0.60 (0.07)</td>
<td>0.59 (0.04)</td>
<td>0.54 (0.13)</td>
<td>1.147</td>
<td>0.233</td>
<td></td>
</tr>
<tr>
<td>Short-term</td>
<td>0.81 (0.11)</td>
<td>0.75 (0.14)</td>
<td>0.77 (0.22)</td>
<td>0.75 (0.10)</td>
<td>0.76 (0.12)</td>
<td>0.5</td>
<td>0.736</td>
<td></td>
</tr>
<tr>
<td>Long-term</td>
<td>0.75 (0.10)</td>
<td>0.70 (0.12)</td>
<td>0.59 (0.28)</td>
<td>0.61 (0.14)</td>
<td>0.52 (0.19)</td>
<td>2.231</td>
<td>0.082</td>
<td></td>
</tr>
<tr>
<td>mrANOVA</td>
<td>F-value*</td>
<td>36.568</td>
<td>7.752</td>
<td>2.563</td>
<td>5.595</td>
<td>5.707</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Value*</td>
<td>&lt;0.0001</td>
<td>0.005</td>
<td>0.126</td>
<td>0.016</td>
<td>0.015</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** The discrimination index (Novel object exploration time/Total exploration time) was calculated for each phase of the test. Comparison of group means was carried out by one-way ANOVA*. Within groups comparison across the three phases were done by mrANOVA*. A p-value <0.05 was considered significant. Abbreviations: M (Mean), SD (Standard Deviation), 6-OHDA (6-hydroxydopamine), ANOVA (Analysis of Variance), rmANOVA (Repeated-measures ANOVA).

By one-way ANOVA, no significant differences were found in the total exploration time at Ac (F(4,34)=0.804, p=0.23) or ST (F(4,35)=1.344, p=0.27) phases across groups. Combined had the lowest exploration time at ST, but not significantly lower than any other group. After 24 hours (F(4,36)=5.520, p=0.001), the 6-OHDA and Combined groups explored the objects significantly less than the Sham group. Paradoxically, the Stem Cells group explored the objects as much as the Sham group (p=0.999), being significantly different from the 6-OHDA (p=0.018) and Combined (p=0.015) group. Exercise did not significantly differ from any group (Figure 5C). Lastly, all groups had a similar number of rearings at the three times points; whilst the Combined group had the lowest number of rearings at all phases, this difference was not statistically significant. (F(4,36)=2.354, p=0.0723 for Ac, F(4,36)=1.656, p=0.181 for ST, and F(4,36)=1.261, p=0.3 for LT). Data not shown.
Figure 5. Novel object recognition test. A. DS data in Median [IQR]. All experimental groups presented a positive DS score at ST and LT points. No statistically significant differences were found between the groups at either Ac (p=0.13) or ST (p=0.29). At LT, only the Combined group had a significantly lower DS score (-5[8.5]) compared to Sham (21[21,50]) (p=0.007). After within group comparisons, only Sham had a significant change in the DS between the Ac (-1[10]) and the retention times (ST 33[19.50], p=0.01; LT 21[21.50], p=0.008). B. DI means are shown. Familiar and novel object interaction were similar during Ac (p=0.23) and ST (p=0.73) for all groups. When comparing novelty preference during the two retention times to Ac, all groups (**p<0.01), except for exercise (p=0.17), showed a statistically significant biased interaction with the new object at ST; 24 hours later, only the DI score of Sham (**p<0.001) and 6-OHDA (*p= 0.025) were significantly different from that obtained during at the Ac phase. Exercise (p=0.774), Stem Cells (p=0.578), and Combined (p=0.863) groups were not. When comparing the two retention times, Exercise (p=0.028) and Combined (p=0.029) groups showed a significant drop at LT. C. Total exploration time at each test phase. Data in mean (SD). No difference was found at either Ac (p=0.23) or ST (p=0.27). At LT, a significant reduction in the time exploring the objects occurred in 6-OHDA and Combined groups when compared to Sham (p=0.026 and p=0.024, respectively) and Stem Cells (p=0.018 and p=0.015, respectively). A p-value <0.05 was considered significant. Abbreviations: DS (difference score), IQR (interquartile range), Ac (Acquisition phase), ST (Short -term retention time), LT (long-term retention time), SD (Standard Deviation), 6-OHDA (6-hydroxydopamine).
3.4 Performance in the step-through passive avoidance test

The time to enter the dark side on training and retention days was not significantly different across groups. However, during the training trial, 6-OHDA and exercise groups had comparatively shorter and longer latency to enter the dark side (respectively). Besides, at this time, the number of rearings was different among groups (H(4) =12.705, p = 0.013), the 6-OHDA and treatment groups presented fewer comparable rearings than Sham, but only the 6-OHDA (p=0.027) and Stem Cells (p=0.040) groups reached statistical significant difference (Data not shown). When performing inter-day analysis, all groups took longer to enter the dark compartment 24 hours after receiving the shock, the combined group showed the highest variation on this variable, showing shorter latencies than any other group (Table 3).

Table 3. Step through Passive Avoidance test

<table>
<thead>
<tr>
<th>Time to enter the dark side</th>
<th>Sham</th>
<th>6-OHDA</th>
<th>Exercise</th>
<th>Stem Cells</th>
<th>Combined</th>
<th>Kruskal Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trial</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>H-value</td>
</tr>
<tr>
<td>Training</td>
<td>25 (23,50)</td>
<td>15 (17)</td>
<td>54 (93,75)</td>
<td>27 (25)</td>
<td>29 (32,25)</td>
<td>7,593</td>
</tr>
<tr>
<td>Retention</td>
<td>600 (-)</td>
<td>600 (11,25)</td>
<td>600 (-)</td>
<td>600 (0)</td>
<td>600 (204,75)</td>
<td>4,928</td>
</tr>
<tr>
<td>Wilcoxon</td>
<td>Z-value</td>
<td>0.008</td>
<td>0.012</td>
<td>0.012</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>p-value*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Comparison across groups of the time to enter the dark compartment on both trials. *Intergroup analysis for each trial was done by the Non-parametric Kruskal Wallis test. *Series of Wilcoxon Signed Ranks Tests were carried out for within group comparisons. A p-value <0.05 was considered significant. Abbreviations: 6-OHDA (6-hydroxydopamine), IQR (interquartile range).

During the retention trial, although the exercise group took longer to approximate the dark compartment and spent less time exploring it; it was not significantly different from any other group due to high individual variation across groups (Figure 6A, 6B). On average, all groups, except for exercise, were active at least half of the time of the retention trial. Among all, 6-OHDA was the one showing a higher percentage of activity, albeit no significant. Exercise spent around 70% of the time in freezing or minimal motion during this time and was significantly different from the 6-OHDA group (F(4,36)=2.647, p=0.049), but not any other group. No other inter group comparisons were significant by Tukey’s post hoc (Figure 6C).
Figure 6. Retention trial (passive avoidance) supplementary information. A. Percentage of retention trial time spent actively exploring the dark compartment without completely crossing to the other side. Data in Median [IQR]. Exercise showed the lowest value (0[6.96]) but no significant difference in ranks means across groups was shown (Kruskal Wallis Test; H(4) = 4.017, p=0.4). B Latency to explore for the first time the dark compartment. Graphic data in Median [IQR]. Albeit Exercise (600 [309.25]) took longer to approach the conditioned compartment, no significant differences in inter-groups analysis were found (H(4) = 6.114, p=0.19). C Percentage of inactivity (breathing or light movement of whiskers). Graphic data in Mean (SD). The exercise group was significantly more inactive when compared to 6-OHDA ((66.5(17.38) and 33.62(16.16), respectively; p=0.037)). A p-value <0.05 was considered significant in all cases. Abbreviations: DS (difference score), IQR (interquartile range), SD (Standard Deviation), 6-OHDA (6-hydroxydopamine).

4. Discussion

Cognitive effects of 6-OHDA

A neurotoxin-induced model of PD was obtained in our study. Our results indicate that the stereotaxic injection of 6-OHDA injured the nigrostriatal system as evidenced by the
methylphenidate-induced turning behavior and supported by an ipsilateral postural bias observed in all injured animals and the visually asymmetrical immunofluorescence TH staining of the SNc and VTA. The unilateral infusion of 6-OHDA induces rotational activity after the systemic administration of a dopamine (DA) agonist, a behavior believed to be secondary to the unilateral DA depletion and the subsequent supersensitivity of postsynaptic DA receptors (Deumens et al., 2002). In all 6-OHDA-infused groups, the number of ipsilateral rotations was significantly higher than the number of rotations in the sham group, but not significant variation among them was observed. Although we have no quantifiable histological evidence of the exact TH+ cell loss or DA content; as we achieved a distinctive rotational behavior in response to the pharmacological challenge in all groups that received the toxin, a TH+ cell loss in the SN of at least 50% can be assumed (Silvestrin et al., 2009). Likewise, as no significant differences among groups in the number of observed rotations were seen, all groups had averagely similar lesion severity. Roughly, a neuronal loss of at least 30 to 50% in the SNc and, correspondingly, around 80% of DA striatal depletion are needed for motor disturbances to manifest (Cheng et al., 2010). In our model, a poorer motor performance in the foot fault test (a test of motor coordination) was observed in the injured subjects (parallel study - Cucarián J.D. et al., Manuscript in preparation), a fact that adds support to the evidence of an achieved nigral compromise.

The integrity of the mesotelencephalic DAergic system is relevant for the processing and storage of information. The prefrontal cortex (PFC) and its rich interconnections with the basal ganglia and mnemonic areas play important roles in memory and object recognition (Chao et al., 2013; Easton et al., 2001). Considering that prefrontal performance is highly influenced by DA content (both within the striatum and from mesocortical projections), as either over or sub-optimal quantities hinder cognition (Cools and D'Esposito, 2011), the integrity of the DAergic system is essential for the expression of this type of memory. Indeed, patients afflicted by PD demonstrate altered recalling and recognition memory (Whittington et al., 2006). The novel recognition test is a delayed matching to sample-like paradigm, in which the natural tendency to explore new objects is used to assess the capacity to remember a previously explored object (recognition memory)(Antunes and Biala, 2012). As much as 40% of striatal DA depletion has been enough to cause memory deficits (Tissingh et al., 1998). However, in our model, despite the indirectly-measured DA nigral loss obtained, the 6-OHDA group showed an overall preserved recognition memory after both short and long retention times. This lack of gross impairment might be accounted for by two big factors. First, the external validity of the 6-OHDA model is limited. While PD is characterized by a complex physiopathology in which nigrostriatal DA dysfunction,
along with that of several other brain areas and neurotransmitters systems, gradually and pervasively occurs (Gratwicke et al., 2015); 6-OHDA only mimics a non-progressive DAergic-deficient state. Thus, the manifestation of cognitive deficits in PD might additionally be dependent on concurrent pathological mechanisms occurring in the disease but not modeled by this model. Second, compensatory mechanisms might have unfolded. In PD, degeneration of the SN occurs first and is substantially greater than the cell loss of the ventral tegmental area (VTA), and hence the dopamine deficiency is significantly greater in dorsal (DS) compared to ventral striatum (VS). The VTA and its diffuse cortical projections (particularly to the prefrontal, the VS, and insular cortices) play an important role in cognitive flexibility by recruiting additional cognitive circuits and modulating motivational states (Floresco and Magyar, 2006). Evidence suggests that initial malfunction of the frontostriatal network can be compensated for by an hyperactivation of this mesocorticolimbic network, such that only when it is compromised, major impairment ensues (Cools, 2006). In our model, although extensive lesion of both SNc and VTA could have been achieved (Deumens et al., 2002), several experimental factors might have influenced the individual extent of unilateral injury; thus, uneven partial lesions could have been obtained in all groups. It is feasible, then, that spontaneous sprouting of ventral dopaminergic fibers from remaining dopaminergic tissue and the non-injured contralateral side could have compensated for any possible loss of nigrostriatal function, as previously has been shown to occur (Blanchard et al., 1996; Liberatore et al., 1999). Furthermore, with incomplete DA striatal depletions (less than 80%), increased release of DA from remaining neurons and upregulation and sensitization of DA receptors have also been described in the literature as intervening mechanisms of compensation (Zigmond et al., 1990). These two major factors may have brought about high interindividual variation and might explain why our results (obtained from small group samples) differ from other studies showing impairment in recognition memory in more acute 6-OHDA lesions (Goes et al., 2014; Zhou et al., 2017); prefrontal-MFB combined lesions (Chao et al., 2013); bilateral lesions (Bonito-Oliva et al., 2014); or in other toxic and genetic animal models (Ho et al., 2011; Magen et al., 2012; Sardi et al., 2013). Similarly, our model showed preserved instrumental aversive learning as indicated by a significant prolongation of the time to enter the dark compartment. The motor inhibition of an action that previously came out as natural, namely avoiding the dark context to which the rodent is innately drawn but in which an aversive stimulus occurred, is highly dependent on the associative properties of the striatum and the phasic release of DA (Da Cunha et al., 2009). Indeed, others studies previously demonstrated impairments in this paradigm after 6-OHDA (Blurton-Jones et al., 2009; Kopalli et al., 2013; Mansouri et al., 2013; Rajendra Kopalli et al.,
However, as this particular fear memory seems to depend on VTA to a higher level than SNc modulation (Da Cunha et al., 2009; Hefco et al., 2003); the lack of impairment in our study might have been related to the compensatory mechanisms previously explained. Additionally, a heightened pain sensitivity could have reinforced the mechanisms for storing and recalling information as nigrostriatal lesions with 6-OHDA have been related to a hypersensitivity to pain (Chudler and Lu, 2008; Maegawa et al., 2015) and a negativity bias in memory processing has been long known (Bowen et al., 2017).

It is important to remark that granting no major deficit on these tests were seen, subtle interesting differences in the overall performance of our model were observed. In the NOR test, during the acquisition phase, the 6-OHDA group, albeit not statistically significant, explored comparatively less. This drive for exploring seems to have been compensated for once the novel object was introduced one hour later; however, on the next day, this lack of interest reappeared, when they explored significantly less than the sham group. This reduced object interaction could have very well influenced the recognition performance (positive but comparatively lower recognition scores). While a motor contribution to the differences in the time of exploration in our model cannot be ruled out; as no major differences in the numbers of rearings were observed and 6-OHDA models seldom develop full-blown akinesia (Zhou et al., 2017), it might not be the sole main determinant. DA besides participating in encoding and retrieval of object recognition memory (Pezze et al., 2015), has been shown to play a role in the appetitive properties of novel stimuli and thus the rate of exploration (Fink and Smith, 1980). In PD, affective and psychiatric symptoms are prevalent; anxiety, depression and apathy appear conjointly with cognitive symptoms during the motor prodromal stage (Balestrino and Martinez-Martin, 2017). Mood fluctuations and the motivational state are an important issue to bear in mind when assessing cognitive function, as the former has a strong influence on the latter and their neural basis are highly intertwined (Poletti et al., 2012). The NOR task assesses spontaneous behavior, which does not involve any reward. Incentives seem to be important for cognitive performance in PD, as patients performed more efficiently in an antisaccade task when anticipating a monetary reward (Harsay et al., 2010). Additionally, depression, flat affect, lack of motivation, and anxiety have a negative impact, as patients reporting these disorders have performed worse in cognitive tests assessing executive function and memory (Poletti et al., 2012). Dopamine loss within the SNc and VTA have produced similar motivational deficits and affective impairments as those seen in PD patients (Drui et al., 2014; Goes et al., 2014; Ho et al., 2011; Tadaiesky et al., 2008; Winter et al., 2007). What is more, affective regulation seems to be more sensitive to DA levels as a low percentage of DA striatal depletion have caused these alterations, when not even
cognitive or motor symptoms could be detected (Branchi et al., 2008; Goes et al., 2014). Considering that in our model, not just DA, but also noradrenaline (NE) and serotonin (5-HT) homeostasis could have been disrupted. On one hand, since an NE reuptake inhibitor was not administrated before the 6-OHDA injection, an NErgic lesion was not prevented; on the other hand, alternations of the 5-HT system in the PFC following the MFB 6-OHDA injection have been described (Chao et al., 2013). Seeing that in PD these disorders can arise from dysmodulations in these neurotransmitters (Poletti et al., 2012), a plausible affective contribution to the cognitive performance of our model cannot be disregarded. Indeed, during the PA test, our injured group entered the dark compartment comparatively faster than Sham and subsequently presented fewer rearings, which may be interpreted as a higher degree of unconditioned anxiety (Ennaceur, 2014).

Effects of the therapeutic approaches

While all three therapeutic approaches had positive effects in the motor coordination in the foot fault test (parallel study); their effect in the cognitive function of our model was variable and even, surprisingly, detrimental. De facto, the performance on the NOR test was poorer 24 hours after the initial familiarization with the objects in all treatments, but particularly significant for those subjected to the physical training and the combined therapy. Aerobic exercise is a widely known neuroprotector and modulator of neuroplasticity with undeniable benefits for brain health and cognitive function in late ages (Aguiar et al., 2016), and scientific evidence supports its use as a therapeutic tool in neurodegenerative diseases (Campos et al., 2016) and the management of motor symptoms in PD (Petzinger et al., 2013). Having this in mind, we hypothesized that our results, rather than being directly related to a prejudice induced by the exercise practice per se, were a consequence of the context of it. We employed a forced protocol in which our animals were subjected to a treadmill training in an apparatus designed in such a way that every time the subject reached the end of the line, it received a shock to force him to keep on the physical activity. What we observed during the period of training was that some of our subjects were laggars particularly prone to receive foot shocks repeatedly. Unfortunately, we did not take objective, systematic measures of these events, and therefore we could not correlate them directly with our results. Notwithstanding, we speculate that a plausible explanation for the impairment we observed in long-term recognition memory can be the effect of this chronic stressing situation in a rodent with emotional vulnerability, possible increased pain sensitivity and DA depletion on baseline, as afore explained. Forced treadmill training was been shown to be stressful; it has increased anxiety, levels of corticosterone and pro-inflammatory cytokines,
and predisposed to larger neuronal damage in the hippocampus when a global ischemic event was induce post-training (Svensson et al., 2016). Equivalently, a protocol of mild chronic stress (MCS) caused neuroinflammation, accelerated neurodegeneration and strong reduction of dopamine levels in a susceptible genetic model of PD (Wu et al., 2016). Furthermore, MCS has been shown to impair long-term object recognition by interfering with D2 and D3 modulating effects in the PFC and hippocampus (Papp et al., 2017) and decrease hippocampal adult neurogenesis (de Celis et al., 2016). A process which in our model might have been already at risk, as MFB 6-OHDA infusion has demonstrated reduced proliferation within the SGZ (Singh et al., 2017). While parahippocampal structures (e.g., perirhinal cortex (Norman and Eacott, 2004)) seem enough to support object recognition over short periods; the dorsal hippocampus seems to partake of its encoding and, therefore, maintenance over long periods. The temporary inhibition of neuronal activity within CA1 weakened novel object preference in mice after a 24 h but not a 5 min retention interval in a similar protocol to ours (Hammond et al., 2004), in which spatial or contextual cues were constant across all sessions, and therefore possible confounding derived from hippocampal involvement in spatial memory was reduced. This dichotomic participation might explain why short-term memory was unaffected in all treatments groups. The reason why it brought motor improvements despite having being stressful may be because being an active repetitive task, it engages motor and cognitive related processing networks, favoring their plastic remodeling and possibly bringing skill recovery (Petzinger et al., 2013).

Interestingly, during our aversive task, the exercise group was comparatively slower to enter the dark compartment on the first day of the task, when it should be naturally drawn to it, and despite a longer period in the light compartment, it presented fewer rearings than sham (although no significant difference was reached because of high intragroup variation). Similarly, during the retention trial, this group did not approach the dark compartment as much as 6-OHDA or any other group, showed the highest latency to first explore this side and significantly increased inactivity during the whole session. If we consider that this test might create conflict in the rodents by concurrently evoking both approach and avoidance behaviors (approach towards a naturally preferred context but avoidance of it given the precedent occurrence of a negative event), the exploratory behavior of the dark compartment without fully crossing might reflect this. The fact that the exercise group was the least of them to show signals of approaching the dark side at any time, suggest us they had a higher level of anxiety that enhanced their aversive learning. This goes in line with the theorized opposite modulation DA and Acetylcholine (ACh) of behaviors of avoidance and approach, in which ACh is also involved in generating an anxious or depressed state when DA is relatively low (Hoebel et al., 2007). Thus, in an animal with already
low levels of DA possibly not counterbalancing ACh, the chronic stress, which also has been associated with dendritic hypertrophy in the basolateral amygdala and anxiety behavior (Vyas et al., 2004), could have in truth enhanced the encoding of aversive experiences (Rau et al., 2005). Since subtle changes were observed following the toxin administration, the assessment of the therapeutic effect of SCT alone is also restricted to subtle changes in cognitive behavior. We transplanted intrastriatally cells with the characteristic immunophenotype of human MSC (Dominici et al., 2006). This type of SC has previously shown to reduce amphetamine-induced rotations after 6-OHDA toxicity, when transplanted both intrastriatally (Cova et al., 2010; Sadan et al., 2009) and intranigrally (Shetty et al., 2009). A finding that agrees with the motor improvement we saw in motor coordination (parallel study, Cucarian J.D.). Only two studies had assessed cognitive endpoints though, and the results are controversial since one study showed reduced rotational activity but not altered working memory (Berg et al., 2015), and the other, very similar, showed quite the opposite (Schwerk et al., 2015a). In our model, no better NOR discriminative performance and no distinctive difference in the overall performance in the PA task were observed. Much as a significant increase in the exploring behavior after the 24 hrs-delayed object re-exposure, and a (non-significant) increase in the time to enter the dark compartment on training day might hint a beneficial effect; these results are not substantial enough to award the therapy a positive cognitive effect, as opposed to the significant motor improvement parallelly observed. Granting all this, when both therapies were combined, we could observe a somewhat ratification of the individual observations we aforesaided. The treadmill training, rather than being beneficial, seemed to entail a negative influence on recognition memory. The combined group was similarly impaired after the long-term retention time; they comparatively explored the least, had the lowest number of rearings at any NOR phase, and presented a deeper dropping of the DI. Paradoxically, they did not demonstrate the heightened anxiety or fear related behavior the exercise group displayed during the PA task. In fact, while an aversive learning occurred, the subjects seemed more risk taking, exploring the dark compartment in a similar fashion as Sham and even crossing completely before the cutoff time. Stem cells as much as are believed to influence the microenvironment they are immersed into, are also respondents to this environment in positive and negative ways (Oliveira et al., 2016). On the same thought, if one local microenvironment differs from another, so will their nurturing or harmful signaling profile and, in turn, the behavior of the transplanted cells (Kusuma et al., 2017). The ambivalent effect we observed can be analyzed under this perspective. Since we implanted our cells within the striatum and a significant motor recovery was observed, a local positive effect could have indeed ensued, in which as other studies have demonstrated,
migration towards the lesion, trophic secretion, neuronal survival, and reduced DA striatal depletion and inflammation occurred (Berg et al., 2015; Blandini et al., 2010; Cova et al., 2010; Sadan et al., 2009; Schwerk et al., 2015b). While aerobic exercise has systemic effects, the motor component of the training might have caused a higher engagement of related ganglio-basal structures, and a selective increase in blood flow and metabolic activity within these regions, which may have resulted in higher regional positive contribution to cortico-striatal circuits and DA neurotransmission (Petzinger et al., 2015). However, in the hippocampus, being a zone particularly vulnerable to stress (Conrad, 2008), the negative impact it sustained secondary to the forced nature of our protocol could have overrun any beneficial outcome of the exercise per se, or for that matter, the stem cells.

5. Conclusions

While much attention has been paid to the motor component of PD, accompanying cognitive symptoms are also an equally important feature present since early stages of the neurodegenerative process, and at a moment in which patients are still professionally active and with under-control motor symptoms. The 6-OHDA animal model reproduces the nigral neuronal loss and subsequent dopamine depletion observed in clinical PD, and is therefore of value in gaining insight into possible relationships between cognitive dysfunctions, dopaminergic modulation, and the efficacy of strategies of therapeutic potential. Still, controversial results have not permitted to fully understand the extent to which it reproduces the cognitive PD-associated dysfunctions and, given the particular demands of different memory tasks and the cooperative nature of cognitive and affective processing, the endeavor of attributing behavioral effects to the loss of a particular structure becomes very challenging. The more if compensatory changes in the surrounding neural circuitry are computed in. In our model, inter-individual cognitive variability and lack of outright compromise were thus unsurprisingly observed. In future studies larger rat populations should compensate for this bias and even allow to segregate into different percentages of neuronal loss and regional differences in DA, facilitating correlations between concomitant motor, cognitive and affective observations.

The primary goal of this study was to build upon evidence suggesting that, given their shared appointed mechanisms for neuroprotection, exercise and stem cell transplantation could potentially act in synergy to enhance individual pro-cognitive therapeutic effects. While no gross compromise in aversive learning and object recognition memory was observed in untreated 6-
OHDA injured rats, the observed effect of the studied therapies provided conflicting evidence and surprisingly, quite showed us the opposite side of the coin. A 4-week forced treadmill training caused an unexpected significant detriment of long-term object recognition memory and an increased avoidance response. While the handicapping in the recognition memory persisted in the combined therapy, a paradoxically enhanced approaching behavior was identified in this group. This puzzling result may be, in effect, the result of a complex interaction between the different therapeutic approaches at distinct niches of action. Throughout-time evidence has taught us that therapies such as the ones we employed in this study cannot be looked upon as a unity, they are dynamic and highly dependent on particular individualities of the therapy, the disease and the host they are being implemented on. Our results may suggest and even more compartmentalized dependency, in which different brain microenvironment will be acted upon in an individualized fashion. As much was left to presumptions, and few studies have previously addressed this issue, it is paramount to foster research in the area to fill up major gaps in our knowledge.

6. Conflict of interest

The authors declare that there is no conflict of interest.

7. Acknowledgements

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stem cells and exercise on functional recovery following sciatic nerve transection. Funct. Neurol. 25, 33–43.


GENERAL CONCLUSION

In conclusion, our studies showed that in an animal model of a neurotoxic unilateral injury of the mesotelencephalic dopaminergic system, while no major mnemonic deficit was right out observed, the combined use of two neuroprotective therapeutic interventions yielded opposite effects in recognition memory and aversive learning. Exercise alone, despite being largely known as a pro-cognitive intervention, brought about impairment in long-term recognition memory and enhanced aversive learning. When it was combined with mesenchymal stem cell transplantation, the impairment of the recognition memory was still present, but a paradoxical reversion of the enhanced response to an aversive memory occurred. This paradoxical effect might have been due to two parallel factors. On one hand, the stressing effect of a shock-motivated forced training in an already vulnerable subject might have been behind the observed impairments. On the other hand, the activity-induced engagement of cortico-basal circuits might have promoted potentiating effects in a regionalized manner.

We recognize that among the limitations of our study, the small sample and lack of additional histological analysis limit the discussion of our results to a based-on-literature interpretation. Still, we want to highlight that given the small quantity of studies that have assessed the combined use of these two potential disease-modifying strategies, our results are still enlightening to possible interacting effects and are a seed of open questions that warrant proper resolution in future studies in the area. In future studies, larger populations will allow to further characterize cognitive and affective alterations induced by 6-OHDA and to correlate them with motor impairments and objective measures of DA depletion. Additionally, other forms of training would be interesting to test, associating their effect to measured indicators of stress, trophic secretion, inflammatory response, and stem cells survival.
APPENDIX

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Discussion. This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions. The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices. If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.
Essential title page information

- **Title.** Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

- **Author names and affiliations.** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors’ affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author’s name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

- **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**

- **Present/permanent address.** If an author has moved since the work described in the article was done, or was visiting at the time, a ‘Present address’ (or ‘Permanent address’) may be indicated as a footnote to that author’s name. The address at which the author actually did the work must be retained as the main, affiliation address. Superscript Arabic numerals are used for such footnotes.

Abstract

A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. Authors can make use of Elsevier's Illustration Services to ensure the best presentation of their images and in accordance with all technical requirements.

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**Keywords**
Immediately after the abstract, provide a maximum of 6 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, 'and', 'of'). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

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Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

**Acknowledgements**
Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

** Formatting of funding sources.** List funding sources in this standard way to facilitate compliance to funder's requirements:

**Funding:** This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding. If no funding has been provided for the research, please include the following sentence: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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References

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3.2 RELATED ARTICLES

Published


Submitted

3.3 ADDITIONAL GRAPHIC DATA

**Figure 1. Number of rearings during the Novel Object Recognition test.** No statistically significant difference was found across groups at any NOR phase.

**Figure 2. Number of rearings on the training trial of the passive avoidance test.** By Kruskal Wallis test and Dunn’s post hoc, 6-OHDA and Stem Cells are significantly different from Sham. *p<0.05.
3.4 ETHICS AND RESEARCH COMMITTEE’S APPROVAL

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

PARECER CONSUBSTANCIADO DE PROJETO DE PESQUISA E ENSINO

1) PROTOCOLO Nº: 176/15
2) DATA DO PARECER: 11/12/15
3) TÍTULO DO PROJETO:
Transplantes neurais e exercício físico aeróbico: combinação terapêutica em um modelo animal de doença de Parkinson
4) PESQUISADOR RESPONSÁVEL:
Alcyr de Oliveira
5) RESUMO DO PROJETO:
Trata-se de um estudo que tem por objetivo avaliar os efeitos da terapia combinada de transplantes neurais de células tronco e exercício físico aeróbico sobre o comportamento motor, memória, neurogênese e a secreção de BDNF. Para tal, ratos Wistar adultos serão submetidos a um modelo de doença de Parkinson (DP) por meio da injeção unilateral de 6-OHDA e terão implantes de células tronco mesenquimais humanas acompanhado de um regime de exercício aeróbico. Os desfechos avaliados serão memória de reconhecimento (por meio do teste de reconhecimento de objetos), condicionamento aversivo contextual (por meio da esquiva passiva) e atividade motora (por meio do footfault test). Após a realização dos testes comportamentais, os animais serão eutanasiados e os cortes de encéfalo serão submetidos à análise imunoistológica para verificar o estado da lesão, proliferação de células implantadas e expressão de BDNF.
6) OBJETIVOS DO PROJETO:
Investigar os efeitos da adição de exercício físico aeróbico ao tratamento com transplantes de células-tronco mesenquimais humanas derivadas do tecido adiposo sobre a memória e performance motora no modelo animal de DP.
7) FINALIDADE DO PROJETO:
☐ Ensino ☒ Pesquisa
8) **ITENS METODOLÓGICOS E ÉTICOS DO PROJETO:**

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<td>Cronograma para execução da pesquisa</td>
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9) **O PROJETO ESTÁ ADEQUADO À LEGISLAÇÃO VIGENTE:**

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10) **INFORMAÇÕES RELATIVAS AOS ANIMAIS:**

Grau de dor/estresse:  
B | C | D | X | E

*Justifique:* Procedimentos que podem causar dor ou estresse exigindo o emprego de anestésicos, analgésicos ou ansiolíticos.

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<th>Espécie:</th>
<th>Ratos Wistar machos</th>
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Redução Amostral:  
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*Justifique:*

Substituição de Metodologia:  
Se achar necessário, justifique e sugira uma nova metodologia:

Aprimoramento da Metodologia:  
Se achar necessário, justifique e sugira aprimoramentos da metodologia:

Acomodação e manutenção dos animais:  
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Se achar inadequada cite abaixo as melhorias necessárias:
Manipulação dos animais:  

[ ] Adequada  [ ] Inadequada  
Se achar inadequada cite abaixo as melhorias necessárias:

Analgesia dos animais (se aplicável):  

[ ] Adequada  [ ] Inadequada  
Se achar inadequada cite abaixo as melhorias necessárias com analgésico substituto:

Anestesia dos animais (se aplicável):  

[ ] Adequada  [ ] Inadequada  
Se achar inadequada cite abaixo as melhorias necessárias com anestésico substituto:

Eutanásia dos animais (se aplicável):  

[ ] Adequada  [ ] Inadequada  
Se achar inadequada cite abaixo as melhorias necessárias com metodologia substituta:

Local de Realização (Biotério/Labotatório): Laboratório de Fisiologia e Laboratório de Fisiologia Comportamental e Metabólica

Outra instituição. Qual?

11) CRONOGRAMA DE UTILIZAÇÃO DE ANIMAIS

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<td>Ratos</td>
<td>Machos</td>
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12) RECOMENDAÇÃO:

[ ] Aprovado

[ ] Com Pendência

[ ] Não aprovado

Data de início: 02/16  Data de Término: 05/17

Comentários gerais sobre o projeto:

Projeto bem escrito, em tema relevante, bem justificado quanto à necessidade de uso de animais.